Effects of apparently prebiotic doses of Jerusalem artichoke meal on gastrointestinal microbiota as well as glycaemic and insulinaemic responses of adult healthy horses

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For my family

“The role of the infinitely small in nature is infinitely great.”

Louis Pasteur

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I Abbreviations

(used in introduction, background, general discussion)

ABC	ATP-binding cassette
ADL	acid detergent lignin
ADP	adenosine diphosphate
AIA	acid insoluble ash
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BCS	body condition score
bwt	body weight
CAC	citric acid cycle
CAE	caecum
CD	colon dorsale
CFU	colony forming unit
CoA	coenzyme A
CT	colon transversum
CV	colon ventrale
d	lat.: dies, engl.: day
DM	dry matter
DP	degree of polymerization
e.g.	lat.: exempli gratia, engl.: for example
EMS	equine metabolic syndrome
et al.	lat.: et alii, engl.: and others
Fig.	figure
FOS	fructo-oligosaccharide
g	gram
GALT	gut associated lymphoid tissues
GIP	glucose-dependent insulinotrophic peptide
GIT	gastrointestinal tract

GLP-1	glucagon-like peptide 1
GLUT	glucose transporter
GOS	galacto-oligosaccharide
(sc) GOS	(short chain) galacto-oligosaccharide
GPR	G-protein-coupled receptors
h	hour
H^+	hydronium ion
i.a.	lat.: inter alia, engl.: among others
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IR	insulin resistance
ITF	inulin-type fructan
kg	kilogram
L	liter
LM	engl.: body weight; german: Lebendmasse
mg	milligram
min	minute
ml	milliliter
mmol	millimole
MOS	mannan-oligosaccharide
mV	milli volt
Na^+	sodium ion
OF	oligofructose
OS	engl: original matter [OM]; german: Originalsubstanz
PG	*pars glandularis*
PM	post meridiem
PN	*pars nonglandularis*
P_i	inorganic phosphate
PP	postprandial

PSSM	polysaccharide storage myopathy
SCFA	short chain fatty acids
scFOS	short chain fructo-oligosaccharide
SGLT-1	sodium dependent glucose-Co-transporter 1
SI	small intestine
STO	stomach
vs.	lat./engl: versus

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1 Introduction

Horses are hindgut fermenters harbouring a diverse microbial community which is well-adapted to the digestion of fibre-rich, low-energy diets. Due to their usage as sport animals, the kind of rations changed to high-energy diets containing few fibres. In combination with stressors (biotic and/or abiotic ones), the horses often develop gastrointestinal diseases like e.g. colic, diarrhoea or laminitis (DE FOMBELLE et al., 2001; ELLIOT et al., 2006; CRAWFORD et al., 2007; RESPONDEK et al., 2008). This is accompanied by an alteration of the microbial composition and metabolism in the digestive tract (MILINOVICH et al., 2006; DALY et al., 2012a; ONISHI et al., 2012). Moreover, the disturbance of the glucose homeostasis linked to hyperinsulinemia and subsequent development of insulin resistance (IR) often leads to clinical signs of laminitis (ASPLIN et al., 2007; DE LAAT et al., 2010).

The feeding of prebiotics intended to promote the host´ health by providing substrates for the autochthonous microbiota predominantly in the hindgut and therefore counteract the development of gastrointestinal derived diseases. Several health-promoting effects are described for humans and animals e.g. improvement of intestinal epithelial barrier functions, modulation of immune function postnatal, enhancement of mineral absorption and bone health as well as refinement of the gastrointestinal microbial composition and metabolism (CHERBUT et al., 2002; ROLLER et al., 2004; LOH et al., 2006; PATTERSON et al., 2010; ROBERFROID et al., 2010; ŚWIĄTKIEWICZ et al., 2010; DELZENNE et al., 2013). Regarding the nutrition of horses, only limited scientific data are available but there are several products on the market suggesting a prebiotic effect on the gastrointestinal tract. The feeding of prebiotic active compounds to horses, whose diet was changed suddenly, reduced the alteration in the microbial composition (RESPONDEK et al., 2008). Contrasting results were reported concerning the glycaemic and insulinaemic response. Obese horses supplemented with prebiotics showed improved insulin sensitivity (RESPONDEK et al., 2011) whereas ponies, predisposed to laminitis, showed a 5.5-fold increase in the serum insulin concentration (BAILEY et al., 2007). Furthermore, a stimulating effect of prebiotic active compounds on diverse immune parameter could not be proved until now for horses and foals (GUERBUEZ et al., 2010; VENDRIG et al., 2014). The current study aimed to evaluate the effect of feeding a natural prebiotic active compound on the microbial composition and

metabolism as well as the nutrient digestibility in the entire equine gastrointestinal tract. Moreover, the investigation of the impact on the glycaemic and insulinaemic response in healthy, normal-weighed horses was implemented.

2 Background

2.1 Definition and classification of prebiotics

The concept of feeding prebiotics was introduced in 1995 by GIBSON and ROBERFROID. Since then, the idea was applied in human as well as in animal nutrition and continuously more sophisticated. In 2008, the International Scientific Association of Probiotics and Prebiotics (ISAPP, 6th Meeting of the ISAPP, London, Ontario) defined a prebiotic as follows:

“A dietary prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota thus conferring benefit(s) upon host health.” (GIBSON et al., 2010).
ROBERFROID et al. (2010) augmented the definition to the “selective stimulation of growth and/or activity of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host”. The updated definition (GIBSON et al., 2017) describes a prebiotic as “a substrate which is selectively utilized by host microorganisms conferring a health benefit”.

To classifying a feed ingredient as prebiotic, the following 3 criteria must be fulfilled:

1) Resistance to the degradation by mammalian enzymes, gastric acid and intestinal absorption processes,
2) fermentable substrate for the gastrointestinal microbiota,
3) selective stimulation of the autochthonous microflora contributing to the host´ health and well-being (ROBERFROID, 2007).

According to the latest definition, a prebiotic should evoke a net benefit health for the host (human or animal being). The main principle is to affect the microorganisms producing metabolites which cause a health promoting effect (GIBSON et al., 2017). Currently, prebiotics consist of ingredients belonging to two main chemical groups: galacto-oligosaccharides (GOS) and inulin-type fructans (ITF; ROBERFROID et al., 2010; Table 1). Furthermore, several feed compounds are potential candidate

prebiotics or have meanwhile a proven prebiotic effect on the gastrointestinal tract. These include i.a. isomalto-oligosaccharides, xylo-oligosaccharides, soybean oligosaccharides and lactulose (Table 1).

Table 1: Classification of ingredients with a prebiotic effect* (according to KASHIMURA et al., 1996; GUPTA and KAUR, 2000; BLAUT, 2002; FLICKINGER and FAHEY, 2002; ZHANG et al., 2003; SHIBATA at al., 2007; ROBERFROID et al., 2010; SAMANTA et al., 2015)

Biochemical generic term	**Biochemical label and structural characteristics**	**General label and mean degree of polymerization (DP_M)**	**Incidences in feedstuff (extract)**
Inulin-type fructan (ITF)	ITF linear, β (2→1) fructosyl – fructose $g_{py}f_n$ and/or $f_{py}f_n$	inulin	chicory, dahlia, Jerusalem artichoke
	short to long polymers DP 2 – 60 ITF with DP_M 12	inulin DP_M 12	
	short oligomers DP 2 – 8 ITF with DP_M 3 - 4	fructo-oligosaccharides (FOS) short chain FOS (scFOS) DP_M 3.6 oligo-fructose DP_M 4	alfalfa meal, beet pulp, soybean hulls, peanut hulls, wheat middlings
	long polymers DP 10 – 60 ITF with DP_M 25	high-molecular inulin DP_M 25 long-chain FOS (lcFOS)	(chicory, dahlia, Jerusalem artichoke)[#]

Inulin-type fructan (ITF)	mixture DP (2 – 8) + DP (10 – 60) ITF_{mix}	mixture of oligomeric and long polymers	barley, garlic, onion (dried), rye flour, wheat
Phlein-type fructan	linear linked fructosyl units β (2➔6) DP > 30	levan or phlein	timothy grass, orchard grass, red fescue
Galactans (GOS)	mixture of β (1➔6), β (1➔3), β (1➔ 4) galctosyl-galactose gal_n-gal and/or gal_n-glc DP 2 - 8	galacto-oligosaccharides (GOS), *trans*-galactooligosaccharides(TGOS/TOS)	seaweed, beans, lupine
mixture of GOS and ITF	GOS-FOS	galactooligosaccharides and high-molecular inulin known as: GOS-FOS or scGOS-lcFOS	-
Mannan-oligosaccharides (MOS)	β (1➔2) manno-oligosaccharides	mannose, oligomannose	(*Candida albicans*, *Saccharomyces cerevisiae;* cell-wall fragment)
Iso-malto-oligosaccharides (IMO)	transgalactosylation of maltose or glucose α (1➔6) D-glucopyranosyl-D-fructofruranose and glc_n/fru_n DP 2 - 8	contains: isomaltose, panose, isomaltotriose as well as other oligosaccharides (4 to 5 glucose residues)	honey

Xylo-oligosaccharides (XOS)	sugar oligomers β (1→ 4) D-xylose-hexuose DP 2 - 6	xylobiose (DP 2); xylotriose (DP 3); xylotetrose (DP 4); xylopentose (DP 5); xylohexose (DP 6) etc.	honey, fruits, vegetables
soybean oligosaccharides (SOS)	raffinose and stachyose mixtures fru-gal-glc and fru-gal-gal DP 3 – 4	-	soybean
lactulose	galactosyl-fructose β (4→1) DP 3 - 5	semi-synthetic disaccharide, isomerisation product from lactose	(small quantity in heated up milk)

*extract (no claim to completeness)
[#]DP varies according to plant metabolic processes
DP = degree of polymerization; g_{py} =glucopyranosyl; f_n = fructofuranoside; f_{py} = fructofuranosyl; ITF_{mix} = mixture of inulin-type fructans with different DP; gal/gal_n = (n) galactose; glc/glc_n = (n) glucose; fru_n = (n) fructose

In the nutrition of horses, fructans are mostly applied as prebiotic active compounds. The starch as a storage carbohydrate is replaced or supplemented with fructans in some plant taxa. In general, fructans are oligo- or polymeric compounds consisting of several fructose units and one most terminal glucose unit. Depending on the chemical composition, fructans are further declared as ITF or phlein-type fructans (as mentioned above; Fig. 1). Phlein-type fructans are represented predominantly in grasses (*Poales*) which inhabit regions of the temperate latitude. In contrast to this, ITF are predominantly contained in composites (*Compositae*) like chicory, dahlia or Jerusalem artichoke (*Helianthus tuberosus*).

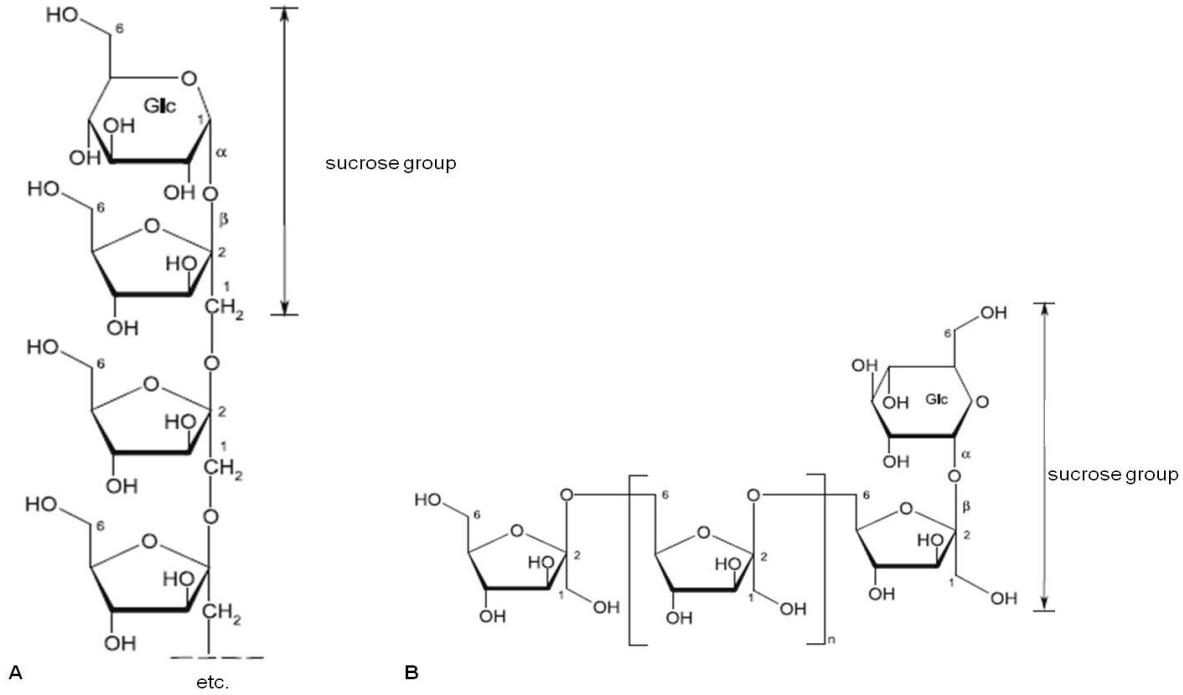

Fig. 1: Structural formula of inulin-type fructans (A) and phlein-type fructans (B) according to HAENSEL, STICHER and STEINEGGER (2010)

High ingestion of ITF as well as phlein-type fructans can evoke laminitis, if the horses are not or not sufficient adapted (LONGLAND and BYRD, 2006; VAN EPS and POLLIT, 2006, 2009). Nevertheless, the two types of fructans can have both positive and negative impacts on the horse. Beside the dosage and adaptation, the effect depends also on the degree of polymerization (DP), the amount of prebiotic active ingredient (predominantly in natural products) and the intramolecular bond. Because of plant metabolic processes, the proportion of fructans and the DP change in the

course of the year as well as circadian (BACON and LOXLEY, 1952; POLLOCK and CAIRNS, 1991). The content of inulin in Jerusalem artichoke increased from 3.5 % 10 d before flowering to 12.2 % 40 d after flowering (LI et al., 2015). The DP varies to the same extent and was the highest on d 40 after flowering. Because of the usage as storage carbohydrate, inulin (or inulin-type fructans) is metabolized in the plants. Fructans are stored in vacuoles (mostly in larger quantities than starch) and are conducive to the osmotic potential by changing their DP from high to low via rapid conversion processes (GUPTA and KAUR, 2000). A prebiotic consists primarily of a mixture from fructans varying in their DP, and in which specific molecule lengths are dominant (Fig. 2).

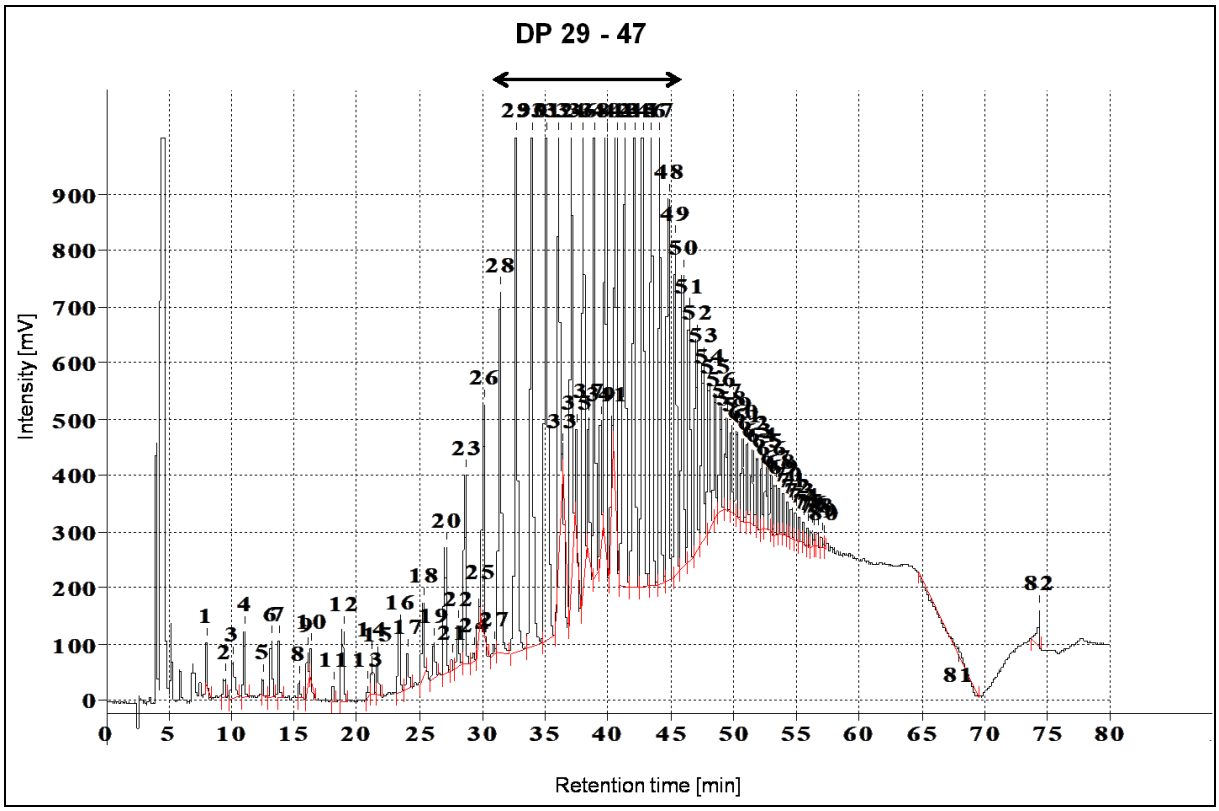

Fig. 2: Fractionation of a commercial available dahlia-extract in their different carbohydrate chains (degree of polymerization, DP) through HPLC (according to HILLEGEIST and GREEF, personal communication)

In the nutrition of horses, the concentration of low-molecular concomitant carbohydrates (e.g. glucose, fructose, sucrose) is likewise essential for the use of prebiotics. The higher the DP or the larger the amount of higher DP in the prebiotic, the more probable ingredients reaches the hindgut and serves as substrates for the autochthonous microbiota (VAN DE WHIELE et al., 2007; AZORÍN- ORTUÑO et al.,

2009; LI et al., 2015). Nevertheless, the ITF are the most applied prebiotic in the nutrition of horses. The recommended prebiotic dosage is 0.2 g/kg bwt x d^{-1} (JULLIAND and ZEYNER, 2013).

2.2 Impact of prebiotics on the organism

The feeding of prebiotics to humans and animals primarily intend to stabilize the autochthonous hindgut microbiota and therefore to counteract possible gastrointestinal derived diseases. However, prebiotics have both direct effects and indirect effects on the organism, which are summarized in the following figure (Fig. 3) as well as further described in the subsequent chapter.

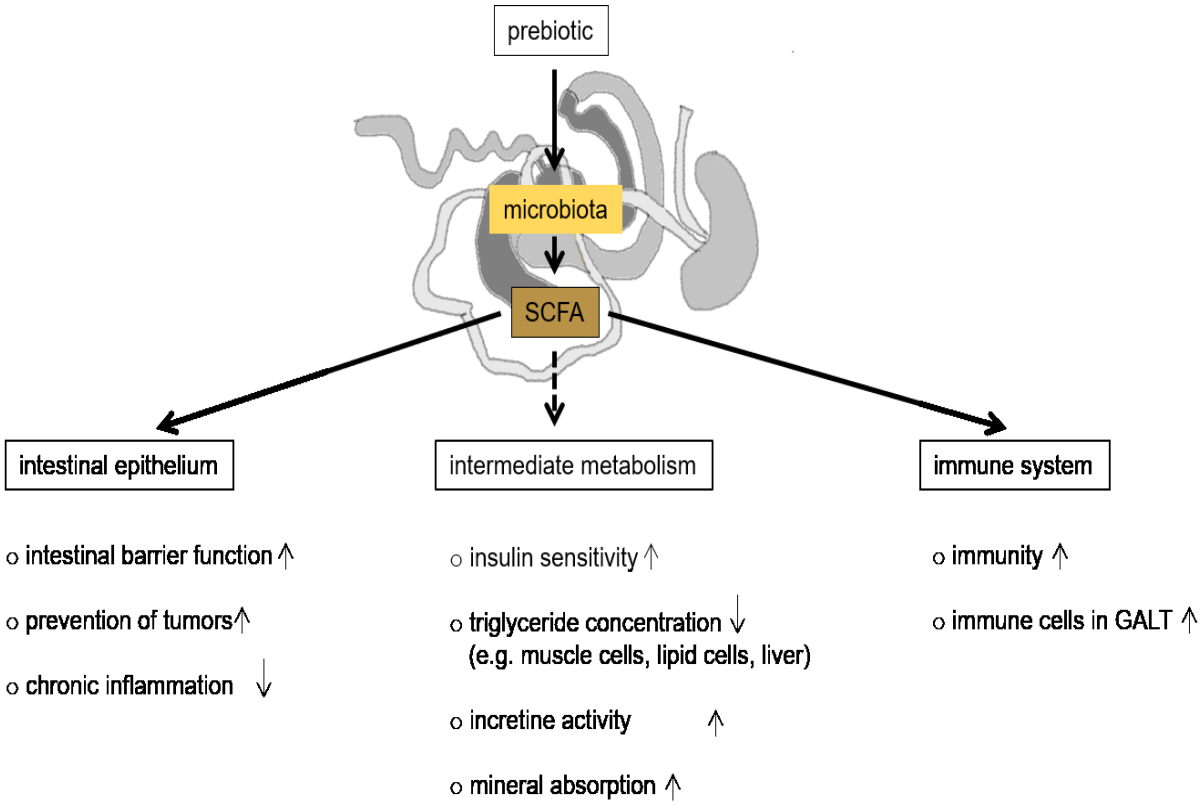

Fig. 3: Assumed impacts of feeding prebiotics to the organism; ↓ = indicates a reduction; ↑ = indicates an enhancement; drawn through lines indicates direct effects; dashed lines indicates indirect effects; SCFA = short chain fatty acids; GALT = gut associated lymphoid tissue (modified according to WÄHLER, 2015)

In the literature, several studies dealt with the impact of prebiotics on the horse´ gastrointestinal health. Nevertheless, the studies do not include all parameters mentioned above. The following table (Table 2) summarizes the available data of prebiotic feeding in horses as well as the major findings.

Table 2: Literature data, in chronological order, on the effect of feeding prebiotics to horses* (modified to BARRY et al., 2009; data according to the given references)

Reference	Animals	Daily ration	Applied prebiotic and dosage	Major outcomes		
				fermentation parameters	microbial counts	intermediate metabolism/immune system
BERG et al. (2005)	9 quarter horses (6 geldings and 3 fillies; bwt: 400 ± 21 kg)	pasture (*ad libitum*) and concentrate supplement (1 % of bwt OM)	8 g FOS/d (~ 0.02 g/kg bwt x d^{-1}) or 24 g FOS/d (~ 0.06 g/kg bwt x d^{-1}) for 10 d	faecal pH ↓ SCFA ↑ lactate ↑	*Escherichia coli* and *Lactobacilli* tendential ↓ (only at 8 g FOS/d)	-
COENEN et al. (2006)	6 trotter geldings (bwt: 473 ± 44 kg)	hay (15 ± 1.4 g/kg bwt * d^{-1})	Jerusalem artichoke; 1.5 g inulin/kg bwt x d^{-1} for 10 d	-	-	breath concentration of: H_2 ↑ CH_4 ↓
RESPONDEK et al. (2007)	6 gelding ponies (bwt: 374 kg)	pelleted concentrate (1.06 kg DM) straw (2 kg OM)	scFOS (Profeed®): 0.06 g/kg bwt x d^{-1} for 21 d	intra gastric pH ↑ SCFA →	- total anaerobes, *Streptococci* and lactate utilizing bacteria ↑ - *Lactobacilli* and cellulolytic bacteria →	-

BAILEY et al. (2007)	11 mixed native-breed ponies (6 LP; 5 control; bwt: 337 ± 36 kg)	hay (*ad libitum*) for 2 weeks than dried grass (1/4) + hay	inulin: 3 g/kg	-	-	- control ponies: serum insulin concentration ↑ (2-fold) - LP ponies: serum insulin concentration ↑ (5.5-fold)
CRAWFORD et al. (2007)	6 control and 6 LP mixed native-breed ponies (bwt: 337 ± 36 kg)	hay (2/3 of daily forage intake by weight) + dried grass (1/4)	inulin (Orafti): 3 g/kg x d^{-1} for 1 week	- faecal pH (control and LP) ↓ - L-lactate (control and LP) ↑ - D-lactate (control and LP) slightly ↓	-	-
RESPONDEK et al. (2008)	4 crossbred geldings (bwt: 425 ± 27 kg)	pelleted feed (P) (1.17 kg/100 kg bwt), wheat straw (0.5 kg/100 kg bwt); after 21 d barley (2.25 kg OM; 0.28 % bwt of starch intake) replace P	scFOS (Profeed® P95): 30 g/d x $horse^{-1}$ (~ 0.07 g/kg bwt x d^{-1}) for 21 d	- pH (cecum, colon) ↓ - L-lactate (cecum, colon) ↓ - D-lactate (cecum ↓; colon ↑) - total SCFA ↑	total anaerobes, *Lactobacilli*, *Streptococci* and lactate utilizing bacteria ↓	-

GUERBUEZ et al. (2010)	4 thorough-bred males (bwt: 471 ± 1.3 kg)	concentrate (0.6 % OM of bwt), alfalfa hay (1.2 % of bwt)	30 g FOS/d (~ 0.06 g/kg bwt x d^{-1}); 30 g MOS/d (~ 0.06 g/kg bwt x d^{-1}); 15 g FOS + 15 g MOS/d (~ each 0.03 g/kg bwt x d^{-1}) for 20 d	- pH (FOS→, MOS↓, FOS + MOS↑) n.s. - total SCFA (FOS/MOS slight↑, FOS + MOS)↓n.s.	-	IgA/IgM (each)↓ IgG (FOS/MOS ↓; FOS + MOS slight ↑) n.s.
RESPONDEK et al. (2011)	8 obese Arabian geldings (bwt: 523 ± 57 kg)	concentrate (3.9 ± 0.6 kg/d), hay (4 kg/d OM)	scFOS (Profeed® P45): 45 g/d * $horse^{-1}$ (~ 0.09 g/kg bwt x d^{-1}) for 6 weeks	-	-	plasma: - insulin↓ - glucose slightly↓ - triglycerides ↓ - leptin nearly→
BORER et al. (2012)	7 control and 5 LP mixed native breed ponies (bwt: 341 ± 71 kg)	adaptation period: pasture, soaked timothy hay (*ad libitum*), 500 g commercially chaff-based feed	inulin (Orafti): 1g/kg x d^{-1} for 3 consecutive d	-	-	hay diet: - glucose (spring: control slight↑, LP↑; fall: control↑, LP nearly→) n.s. - insulin (spring: control and LP↑; fall: control and LP↑)

VENDRIG et al. (2014)	12 warm-blood pony foals	naturally nursed by the mares (free access to hay, grass and straw)	(sc)GOS: 15 g/d for 28 d	-	-	cytokine: interferon-γ and interleukin-6↓ blood concentration of IgG (a)↑

*There are only data included which describe a prebiotic effect. No studies were included whose results deal with gastrointestinal derived diseases.
↑ = indicates an enhancement, ↓= indicates a reduction, → = consistent
bwt = (mean) body weight, DM = dry matter, FOS = fructo-oligosaccharides, GOS = galacto- oligosaccharides, IgA/IgM/IgG(a) = serum immunoglobulin, LP = laminitis prone, MOS = mannan- oligosaccharides, n.s. = not significant, OM = original material, scFOS = short chain FOS

2.2.1 Gastrointestinal tract

The supplementation of prebiotics in human nutrition primarily aims to increase the concentration/relative abundance of *Bifidobacteria* and *Lactobacilli* in the digestive tract. *Bifidobacteria* are known for e.g. to attach themselves to the surface of enterocytes and therefore to inhibit the adhesion of enteropathogens in the intestinal tract (BERNET et al., 1993; KLEESEN et al., 2001; HARMSEN et al., 2002). In other species, the stimulation of *Bifidobacteria* is less intended because they are abundant as residents only in low concentrations. The effect of feeding prebiotics to pigs (as a representative omnivore animal) concerning the stimulation of the above-mentioned bacteria is controversial. Weaned piglets (28 d), which were fed with 1.5 % inulin, exhibited an increased microbial diversity in the small intestine and colon (JANCZYK et al., 2010). Since the role of *Bifidobacteria* in the porcine gastrointestinal tract is of minor importance (LOH et al., 2006), the main intention of the study mentioned above was accounted for the identification of the total *Lactobacilli*. The concentration of these bacterial group was not affected by the supplemental prebiotic. A study with male castrated pigs, fed with 2 % inulin, revealed similar results (BOEHMER et al., 2005). In contrast to this, other investigations indicated a positive effect of feeding inulin (40 mg/kg) to weanling pigs (5 to 6 weeks old) concerning the concentration of *Bifidobacteria* and *Lactobacilli* (PATTERSON et al., 2010).

Research regarding the impact of prebiotics on the microbiota of herbivorous species is sparse. Moreover, a positive effect of prebiotic compounds concerning the intestinal bacterial composition is only described for calves (UYENO et al., 2015). In adult cows, the supplemental prebiotic will already be metabolized in the rumen and therefore, the quantity reaching the hindgut is not sufficient.

In general, the feeding of prebiotics intends to increase the bacterial metabolism which hence elevates the production of SCFA. These can subsequently lead to a decreasing luminal pH which promotes the growth of *Bifidobacteria* and *Lactobacilli* because they are well-adapted to an acid environment (BLAUT, 2002). *Lactobacilli* produce primarily D-lactic acid which can be metabolized by other commensal bacteria in the digestive tract to SCFA (REISCHER, 2012). The combination of high concentrations of SCFA and lactic acid (L- and D- isomer) can decrease the pH thus far, that the viability of several pathogens (e.g. *Clostridia*) is degraded.

The presence or existence of *Bifidobacteria* in the equine gastrointestinal tract is not certainly proven. Nevertheless, the equine GIT harbours a large amount of microorganism (see Table S1 and S2, supplemental material) and from these, i.a. *Streptococcus bovis* has the ability to metabolize inulin (HARLOW et al., 2014). *In vitro* studies with digesta from the stomach and small intestine revealed that phlein-type fructans from *Phleum pratense* (timothy) are partly degraded and that this suggests an inceptive metabolic decomposition already in the equine foregut (INCE et al., 2014). Literature data indicates that a small amount of the fructan might be cleaved by acid hydrolysis (STRAUCH et al., 2017) or metabolized precaecal (COENEN et al., 2006) which is per se critically seen. Additionally, prebiotics which are degraded already in the foregut are not able to reach the hindgut in sufficient concentrations and therefore to induce a health-promoting effect.

Nevertheless, *in vivo* studies which examine the effect of feeding prebiotics to horses, have generated different results (see also Table 2 for summary). Yearlings which were supplemented with different dosages of FOS (8 g/d [~ 0.02 g/kg bwt x d^{-1}] or 24 g/d [~ 0.06 g/kg bwt x d^{-1}]) showed no variation of the *Lactobacilli* concentration in the feces but a reduced concentration of *Escherichia coli* in horses fed with 8 g/d (BERG et al., 2005). Furthermore, scFOS are able to prevent a microbial disorder in the GIT if the horses underwent an abrupt change of the diet (RESPONDEK et al., 2008). The feeding of scFOS (dosage: 30 g/d [~ 0.07 g/kg bwt x d^{-1}]) to 7 year old crossbred geldings for 21 d prevent an increasing concentration of *Lactobacilli* and *Streptococci* after an abrupt incorporation of barley in the diet in comparison to the control group. This aspect is interesting concerning the concentration/relative abundance of *Lactobacilli* in the hindgut because, in contrast to other animals (or human being), an increasing proportion of this genus in that localization is critically seen. Moreover, the feeding of prebiotics intends to enhance the metabolism of the autochthonous microbiota in the GIT and equally to promote the formation of fermentation products like SCFA. In this context, *n*-butyric acid has a key role regarding the barrier function of the intestinal epithelium. This SCFA is quantitatively produced in lower concentrations compared to acetic and propionic acid but has a specific importance for the metabolism of the intestinal epithelium (PLOEGER et al., 2012). *N*-Butyric acid is i.a. the key energy source for colon cells, regulates the cell proliferation, differentiation and apoptosis as well as has anti-inflammatory effects and protect the epithelial barrier (reduce claudin-1/-2, increase zonula occludens

proteins (ZO-1/-2); PLOEGER et al., 2012). However, results from *in vitro* studies with horse gastric tissues assume, that high *n*-butyric acid concentrations in the stomach in combination with a low pH value have negative impacts concerning the intestinal epithelium (NADEAU et al., 2003). The concentration of SCFA (i.a. *n*-butyric acid) increased also in the feces of the yearlings with the feeding of increasing dosages of FOS (BERG et al., 2005). The rising concentration of SCFA was accompanied by a decreased pH in the feces. In contrast to this, the feeding of scFOS (see above-mentioned study; dosage: 30 g/d [~ 0.07 g/kg bwt x d^{-1}]) resulted in no variation of the SCFA concentration in the cecum and colon of adult horses (RESPONDEK et al., 2008).

2.2.2 Immune status

The feeding of prebiotics affects the immune system either directly and/or indirectly (via fermentation products like SCFA). Rats which were fed with inulin and oligofructose (dosage: 100 g/kg) exhibited an enhanced formation of interleukin-10 as well as an increased production of secretory immunoglobulins (class A; ROLLER et al., 2004). The enhanced cytokine production indicated, that either the prebiotic itself or the stimulated autochtonous microbiota (and their metabolites) influenced the process. SCFA, as fermentation end products, are able to affect the immune cells in the GALT (SEIFERT and WATZL, 2007). However, the signal path on which the intraluminal SCFAs are detected from the leucocytes is not known, yet. Furthermore, the assumption is avowed, that specific carbohydrates are capable to interact with the immune cells via specific carbohydrate receptors. The intrinsic receptor for mannose is, e.g., identified (BROWN and GORDON, 2001). Phagocytotic cells as well as i.a. small subgroups of T- and B-lymphocytes contains specific receptors for the identification of a wide range of β (1➔3) and β (1➔6) connected glucans from fungi and plants. Furthermore, the maternal prebiotic supplementation can have a positive impact on the immune status of the offsprings. The feeding of scFOS (dosage: 10 g/d) to sows in the peripartum period increased the colostral immunity (LE BOURGOT et al., 2014). In addition, a stimulating effect on the development and maturation of the mucosal immune system of the piglets is described.

In the nutrition of horses, the impact of prebiotics on the immune system showed no improvement. Adult horses, fed with FOS/MOS (dosage each: 30 g/d [~ 0.06 g/kg bwt x d^{-1}]) or FOS + MOS (each 15 g/d [each ~ 0.03 g/kg bwt x d^{-1}]), depicted no significant variation of the measured serum immunoglobulins IgA, IgM or IgG (GUERBUEZ et al., 2010). The orally supplementation of (sc) GOS to foals (15 g/d) resulted in no significant or health-promoting effects concerning the investigated blood parameters (VENDRIG et al., 2014).

2.2.3 Absorption processes

Feeding of prebiotic active compounds can positively stimulate the absorption processes in the gastrointestinal tract of humans and animals. The fermentation product *n*-butyric acid stimulates the synthesis of mucus (FINNIE et al., 1995) as well as accelerates the formation of tight junctions *in vitro* (PENG et al., 2009) which is accompanied by a reduced paracellular permeability of the enteric barrier. Accordingly, pathogenic bacteria and their toxins are absorbed in a lower amount by the epithelium and hence are distributed in smaller concentrations via the blood stream in the organism. Moreover, prebiotics can have an effect upon the absorption of nutrients. On the one hand, an alteration of the intestinal villi length in the different parts of the digestive tract can influence the absorption processes. Piglets (21 d), fed with lactose (150 g/kg complete feed) and inulin (15 g/kg complete feed), showed a significant enhancement of the villi length in the jejunum (PIERCE et al., 2006). A study with calves, fed with 2 % lactose (added to the milk replacer), revealed similar results (MASANETZ et al., 2010). The intestinal length of the villi in the jejunum and ileum were tendentially increased. In contrast to this, the feeding of 2 % inulin (added to the milk replacer) tendentially decreased the villi length in both gastrointestinal parts. Another *in vitro* study using cell cultures indicated that the supplementation of inulin (1 mg/ml) increases the absorption of glucose by an AMP-activated protein kinase as well as a phosphatidyl-inosityl-3-kinase pathway (YUN et al., 2009).
Furthermore, prebiotics are able to influence the absorption of nutrients on the systemic level. The feeding of inulin (4 % in the complete feed) to pigs increased the expression of genes encoding several iron transporters in the enterocytes (TAKO et al., 2008). In poultry feeding, the supplementation of inulin (~ 0.94 g/d) improved the absorption of calcium, phosphorous and zinc in laying hens (ŚWIĄTKIEWICZ et al.,

2010). The underlying mechanism for an improved availability of macro and trace elements is very complex. Probably, the increased solubility of the nutrients is a result of the decreasing pH in consequence of the enhanced microbial formation of SCFA (SAMANTA et al., 2013).

Hitherto, the number of experimental based evidence on absorption processes in the equine gastrointestinal tract is scarce. DYER et al. (2002) identified a SGLT-1 transporter (sodium dependent glucose-Co-transporter 1) which is responsible for the absorption of glucose and galactose in the small intestine *in vitro*. The results were confirmed by CEHAK et al. (2009) which used tissues from the jejunum. Furthermore, the authors verified a secondary activated H^+-dependent Co-transporter as well as H^+- and Na^+-independent transport mechanism which are responsible for the absorption of peptides in the jejunum *in vitro* (CEHAK et al., 2013). In the equine digestive tract, calcium is absorbed in significant higher concentrations in the duodenum and phosphorous is absorbed in larger quantities in the jejunum compared to other parts *in vitro* (CEHAK et al., 2012). Moreover, the absorption process of SCFA (in particular *n*-butyric acid) is described only in the hindgut (NEDJADI et al., 2014). *N*-Buytric acid is absorbed via a sodium-independent membrane transporter which is driven by a pH gradient. According to the present knowledge, the impact on prebiotics on absorption processes in the equine GIT was not investigated until now.

2.2.4 Intermediate metabolism

The autochthonous microbiota in the digestive tract metabolizes prebiotics to fermentation end products like lactate, ammonia or SCFA. In particular SCFA (acetic, propionic and *n*-butyric acid) influences the organism on several levels and in diverse target areas or tissues/viscera (see Fig. S2, supplementary material). SCFA are partly metabolized in the intestinal epithelium (HERRMANN et al., 2011; BACH KNUDSEN, 2012) or distributed over the bloodstream in the organism and further integrated into the metabolism of different tissues (e.g. fat tissues, muscle cells) and viscera (e.g. liver; BERGMANN, 1990). As mentioned previously, *n*-butyric acid is the key energy source for the colonocytes (SCHEPPACH, 1994) whereas acetic and propionic acid are transported via the portal vein into the liver and are involved in the gluconeogenesis, lipogenesis and fatty acid oxidation (WÄHLER, 2015).

The feeding of prebiotics aims mostly to stimulate the production of SCFA, especially *n*-butyric acid, in the hindgut and therefore is able to contribute positively to the host´ metabolism. Pigs fed with inulin in different dosages (2.1 – 6.3 % pure inulin) revealed significant higher concentrations of SCFA compared to the control animals (LOH et al., 2006; VHILE et al., 2012). Similar results were shown in rats which were incubated with human fecal microbiota. The germ-free rats were fed with fructans (oligofructose, long chain inulin or a mixture of both) in a dosage of 50 g. Total SCFA concentration significantly increased in the caecum and colon (only tendentially for the long chain inulin) irrespective of the supplemental feed (KLEESEN et al., 2001).

In general, investigations of the impact on prebiotic supplementation on the systemic influence resulting from an increased concentration of SCFA are scarce. After feeding of prebiotic compounds, decreasing serum triglyceride concentrations were measured in captive Indian leopards (*Panthera pardus fusca*; 2 % Jerusalem artichoke in the whole diet [DM]; PRADHAN et al., 2015), broiler chicks (one-day old; 5 – 10 g/kg diet inulin; VELASCO et al., 2010) and rats (10 % oligofructose per diet; FIORDALISO et al., 1995). The adding of prebiotics in human nutrition reduced also the serum triglyceride concentration dependent on peculiarity of lipaemia (normo- or moderately hyperlipidaemic persons; DELZENNE and WILLIAMS, 2002). Furthermore, the fermentation end products SCFA are able to influence the formation of peptides (like e.g. glucagon-like peptide 1 [GLP-1], ghrelin) in the endocrine cells (DELZENNE et al., 2005). Feeding prebiotics to rats (100 g/kg oligofructose [OF], synergy or long chain inulin) significantly increased the concentration of GLP-1 in the caecum (only OF) and proximal colon (OF and synergy; CANI et al., 2004). Similar results are reported for humans who consumed once 24 g inulin (added to a high-fructose corn syrup; TARINI and WOLEVER, 2010). The supplemental inulin significantly increased the plasma GLP-1 concentration 30 min postprandial (PP) and reduced the concentration of serum ghrelin 4.5 h and 6 h PP. A nutrigenomic investigation using broiler, which were fed with inulin (5 g/kg diet), revealed an impact of the prebiotic on the gene expression in the hepatic transcriptome (SEVANE et al., 2014). The inclusion of inulin into the diet affected the expression of genes which were involved (generally) in basal processes (like development and maintenance of several tissues), immune system and fatty acid metabolism.

The supplementation of diets with prebiotic active ingredients aims further to improve the glucose and insulin metabolism in the organism. In the nutrition of horses, the

impact of feeding prebiotics or fructans concerning the insulinaemic response is contradictory. Ponies fed with inulin (3 g/kg) included in a forage based diet (hay only) exhibited a 2.0-fold higher PP serum insulin concentration compared to the basal diet (BAILEY et al., 2007). Interestingly, ponies which were prone to laminitis showed a 5.5-fold higher PP serum insulin concentration. The PP plasma glucose and triglyceride concentrations were not affected by the supplemental prebiotic. In contrast to this, the supplementation of scFOS (45 g/d [~ 0.09 g/kg bwt x d^{-1}]) to obese horses (BCS 8/9) decreased the plasma insulin concentration (RESPONDEK et al., 2011). The plasma glucose, serum triglyceride and leptin concentrations were equally not affected by the treatment.

In the gastrointestinal tract, fructans are fermented by microbial specific enzymes like fructosidases (GAENZLE and FOLLADOR, 2012). Especially the families *Lactobacillaceae, Bacillaceae* and *Bifidobacteriaceae* are predestinated for the metabolism of oligosaccharides and to be in possession of these enzymes. In the genus *Lactobacilli,* glucansucrase and fructansucrase catalyzes the hydrolysis as well as the synthesis of oligo- and polysaccharides (VAN HIJUM et al., 2006). The synthesis is essential to form a biofilm consisting of exopolysaccharides which enhances the bacterial resistance to several stressors (chemical or physical ones; WALTER et al., 2008). FOS are transported into the cell by a specific four-component ATP-binding cassette (ABC) transport system (BARRANGOU et al., 2003) and are further hydrolyzed by intra-cellular fructo-furanosidases (WALTER et al., 2008). The main release product is fructose which can be either metabolized further to SCFA or absorbed directly in the GIT. In comparison to glucose, the induced insulinaemic response is lesser (BORER et al., 2012). A part of the absorbed fructose is transported via the bloodstream into the liver and swiftly converted to fat which will be further deposited in the body (JOHNSON et al., 2013). This is accompanied by a reduced responsiveness of the tissue to leptin and contributed to the development of obesity which in turn decreases the insulin sensitivity. However, the absorption of fructose in the gastrointestinal tract seems to have a minor role. Instead, the microbial fermentation and production of SCFA is the preferred metabolic pathway.

Equally, in horse´ nutrition, the postprandial secretion of incretins (e.g. GLP-1) plays a key role regarding the regulation of the glycaemic and insulinaemic response. Unfortunately, scientific based evidences about the impact of diet and particularly prebiotics are very rare and not fully investigated, yet. BAMFORD et al. (2015)

examined the impact of micronized maize (1.7 g/kg bwt x meal^{-1}) on the PP insulin and GLP-1 concentration in different breeds. Ponies and Andalusian horses showed significant higher PP plasma insulin and GLP-1 concentrations compared to Standardbred horses which indicated a breed related correlation. Moreover, the plasma GLP-1 concentration during an oral sugar test revealed no significant differences between normal horses and those with equine metabolic syndrome (CHAMEROY et al., 2016). Further investigations might determine the effect of prebiotics on the PP incretin secretion depending on i.a. breeds, predisposition (for several gastrointestinal derived diseases) and basal diets.

Annotation: The main features of the background part are published in the German-language journal "Tierärztliche Umschau" (3/2016, pp. 65-71), which is printed in the original language in the appendix.

2.3 Glycaemic and insulinaemic response in horses

The glycaemic and insulinaemic response in horses is a sensitive regulatory system which is always anxious to establish a homeostatic situation in the organism. Several factors, like e.g. diet and/or training, influence the availability of glucose and insulin for metabolic processes. Therefore, the equilibrium can be easily unbalanced and resulted perhaps in metabolic disorders. The following paragraph introduces to the glycaemic and insulinaemic response in horses and describes the role of prebiotics as possible intervention regarding the development of metabolic imbalances.

2.3.1 Glucose metabolism

2.3.1.1 Chemical composition and function in the organism

Glucose is a monosaccharide belonging to the hexoses. Further, glucose is described as aldose because of the aldehyde group at the first carbon atom in the molecule structure (Fig. 4).

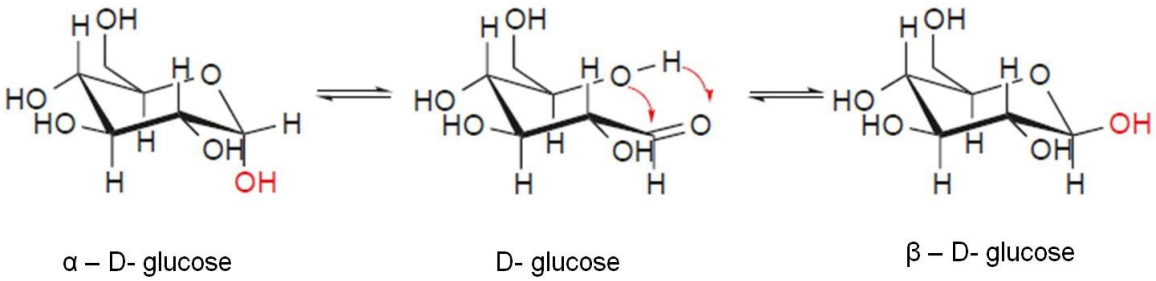

Fig. 4: Mutarotation of the spatial chemical structure of glucose in the chair conformation (according to EBNER, 2017)

Horses obtain their glucose requirement from the ingested amount of starch-rich feeds. Moreover, equines obtain glucose using a combination of two ways (according to D´MELLO, 2000):

1) The direct enzymatic digestion and further absorption of the dietary, non-structural carbohydrates in the gastrointestinal tract or
2) via the fermentation of the ingested carbohydrates by the intestinal microbiota.

Regarding the second pathway, glucose is thus synthesized by the horse itself using fermentation end-products (here: propionat) of the microbial metabolism in the gut (D´MELLO, 2000). In the intestine, glucose is absorbed either via transcellular or paracellular pathways. Thereby the second mentioned way depends on the transcellular absorption. Due to the impermeability of the lipophilic biomembrane of eucaroytic cells for hydrophilic molecules, glucose can only pass the plasma membrane by specific membrane associated carrier proteins (SCHEEPERS et al., 2004). Two different types of membrane associated carrier proteins are responsible for the glucose uptake into the cell: the Na^+-dependent-Co-transport system (SGLT-1) and the glucose transporter (GLUT; D´MELLO, 2000; SCHEEPERS et al., 2004). In horses, the SGLT-1 transporter is mostly located (in descending order) in the duodenum, jejunum and ileum (DYER et al., 2002). The equine intestinal Na^+-dependent-Co-transport system can be modified by the diet. DYER et al. (2009) induced a 2-fold (duodenum) as well as a 3.3-fold (ileum) enhanced expression of SGLT-1 proteins after increasing the amount of carbohydrates in the diet (from < 1.0 g/kg bwt x d^{-1} to 6.0 g/kg bwt x d^{-1}). DALY et al. (2012b) detected the sweet receptor T1R2 and T1R3 (activated by natural sugars and artificial sweeteners) in the equine small intestine which are able to stimulate the GLP-2 (and GLP-1) production. The authors assume, that especially the enhanced GLP-2 secretion might lead to an upregulated SGLT1 expression and therefore to an enhanced glucose absorption. Nonetheless, the glucose transport capacity might not be a limiting factor in the equine small intestine. DENGLER et al. (2018) described *in vitro*, that the transepithelial glucose uptake is not altered under hypoxia and the energy-independent transport over the intestinal epithelium is secured. The GLUT sugar transporter family consist of several members, of these each is expressed in specific target tissues or viscera (SCHEEPERS et al., 2004). For instance, GLUT 2 transporters are primarily located in the liver, small intestine, islet cells or the kidney whereas the GLUT 4 carriers are mostly expressed in the adipocytes and muscles. Furthermore, some carrier proteins are specific for the uptake of fructose, e.g. the GLUT 5 transporter (SCHEEPERS, et al., 2004). In the gastrointestinal tract of horses, GLUT 5 is expressed (in descending order) in the duodenum, jejunum and ileum (MEREDIZ et al., 2004).

Concerning the intermediate metabolism, glucose plays a central role and is the main energy source for several conversion processes (Fig. 5). Glucose can be

metabolized via aerobic or anaerobic glycolysis to pyruvate in each cell (KIRCHGEßNER et al., 2014; RASSOW, 2016). Furthermore, pyruvate is introduced into the citric acid cycle to obtain ATP via several steps. For maintenance of a homeostatic situation in the organism, glucose can be stored as glycogen in the liver or be synthesized from pyruvate via gluconeogenesis. Moreover, the absorption and metabolism (catabolic and anabolic pathways) of glucose is essential in the synthesis of e.g. fatty acids, nucleotides and amino acids as well as proteins.

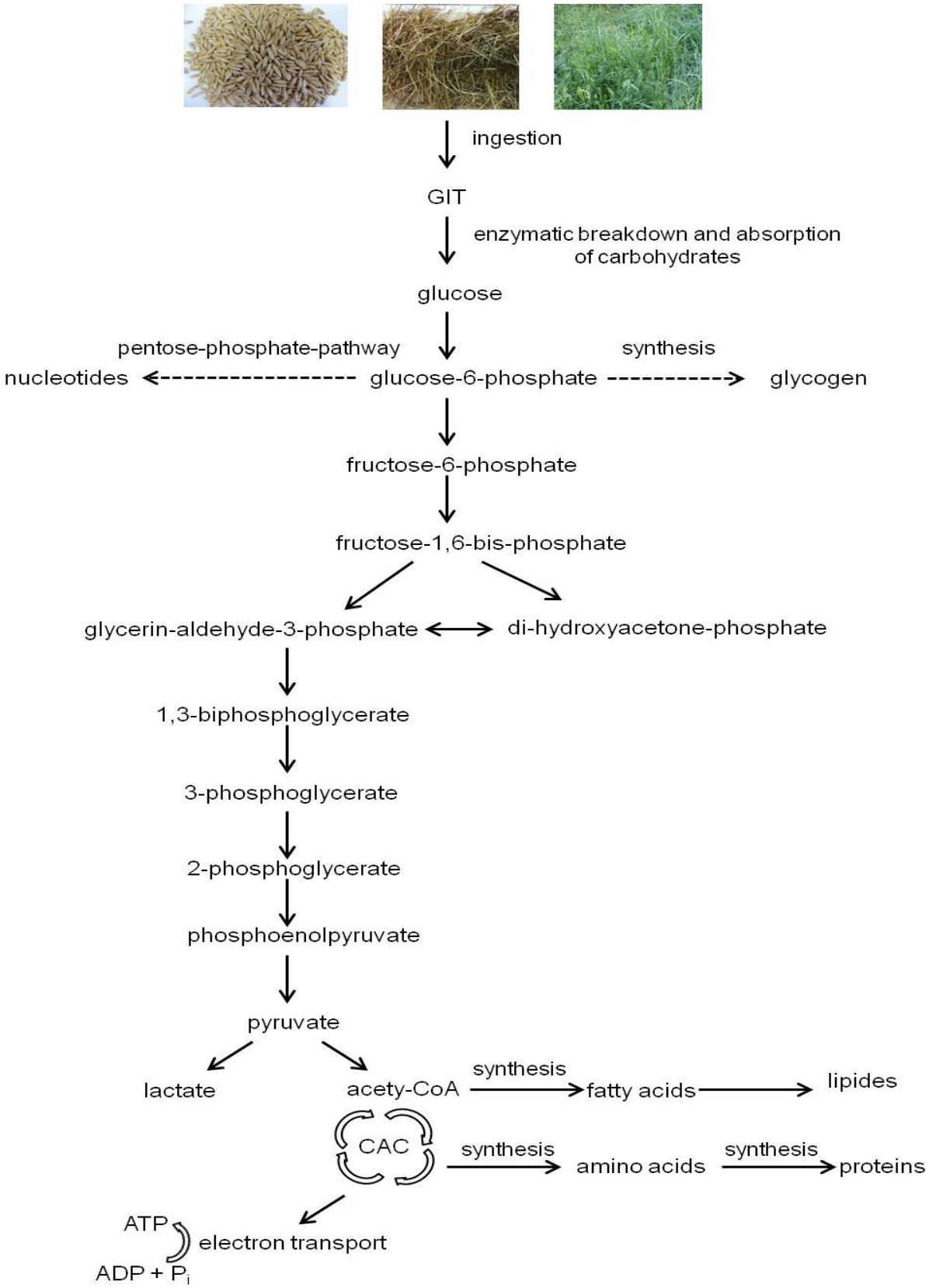

Fig. 5: Metabolism of glucose in the equine organism (modified according to HORTON et al., 2008; KIRCHGEßNER et al., 2014; RASSOW, 2016); CAC = citric acid cycle; P_i = inorganic phosphate; photos by BOCHNIA, CZETOE and GLATTER

2.3.1.2 Variability of the equine blood glucose concentration

The fasting blood glucose concentration in horses is in the range of 4.5 – 6.0 mmol/L (HEPPES, 2003). In relation to several influencing factors e.g., diet composition, body weight and breed of the horses as well as amount of meals per day, the concentration of glucose changes in the bloodstream. Fibre rich diets induce a minor glycaemic response in horses compared to concentrate meals which have a high concentration of easily available carbohydrates. Ponies fed with a diet high in fat and fibre showed significant lower PP plasma glucose concentration compared to an oat grain diet (6.1 mmol/L versus 7.3 mmol/L; BOCHNIA et al., 2017). Equally, the feeding of hay (only) to coldblooded trotters did not change the PP glucose concentration (~ 5.2 mmol/L after 3 h and 9 h PP; BRØKNER et al., 2016) whereas the feeding of haylage induced a high PP glycaemic response dependent on the non-structural carbohydrate content (LINDÅSE et al., 2018). The feeding of low-starch and high-fat diets likewise resulted in a minor PP glycaemic response compared to cereals only (ZEYNER et al., 2006). In contrast to this, the inclusion of starchy-rich diets lead to a considerable increase of the PP blood glucose concentration (VERVUERT et al., 2003, 2009b; ZEYNER et al., 2017). Besides the diet composition, the weight of the horses as well as the breed similarly influences the PP variation of the glucose concentration in the bloodstream. BAMFORD et al. (2014) revealed a slight breed variability concerning the PP glycaemic response of Standardbred horses, mixed-breed ponies and Andalusian-cross horses which were fed long-stem grass hay *ad libitum* and underwent an oral glucose tolerance test. Obesity per se might not be responsible for an alteration of the PP glucose release but, in combination with cereal-rich diets, this might have a greater influence regarding the insulin sensitivity of several insulin-dependent tissues (BAMFORD et al., 2016 a, b). Furthermore, the amount of meals per day affects the PP blood glucose concentration. Generally, feeding of a starch-rich compound feed significantly increased the glycaemic response in comparison to a fibre-rich compound (KARASU et al., 2015). Moreover, the glucose peak of the second meal was significantly lower compared to the first meal, even for the starch-rich compound feed (KARASU et al., 2015). The elevation of the number of meals per day from two to three tendentially reduces the PP glucose release (PRATT-PHILLIPS et al., 2014). In this context, the basal diet (high or low in non-structural carbohydrates) might be of

minor importance. In addition, the order of feeding fibre and cereals also varied the PP glucose response with a tendentially higher blood glucose level when e.g. chopped alfalfa was fed before or together with unprocessed oats (VERVUERT et al., 2009a).

2.3.2 Insulin metabolism

2.3.2.1 Chemical composition and function

Insulin is a pleiotropic peptide-hormone which is synthesized in the pancreatic β-cells of the islet of Langerhans (SUAGEE et al., 2011; KLEINE and ROSSMANITH, 2014). The formation of insulin is glucose-stimulated and begins with the translation of the preproinsulin mRNA (WICKSTEED et al., 2001, 2003). The preproinsulin mRNA is mobilized from an inert cytosolic pool and linked to membrane-bound polysomes on the rough endoplasmatic reticulum. Furthermore, the interaction of signal peptides and signal recognition particle are responsible for the translation of the preproinsulin mRNA (WICKSTEED et al., 2001). The newly synthesized proinsulin acts as a precursor for the actually insulin (SOENKSEN et al., 1973). Proinsulin consists of the polypeptide chain A and B which are connected via the C-peptide (Fig. 6; SOENKSEN et al., 1973; KLEINE and ROSSMANITH, 2014). The C-peptide itself is built from a specific concentration of amino acids (e.g. 31 for humans, 33 for porcines; SOENKSEN et al. 1973). Afterwards, the C-peptide chain is cleaved by peptidases (e.g. carboxypeptidase-H) from the remaining insulin molecule (KLEINE and ROSSMANITH, 2014). Furthermore, the enzymes separate the N-terminal end. Finally, the insulin consists of the A- and B-chain which are connected via disulfide bonds (Fig. 6; SOENKSEN et al., 1973; KLEINE and ROSSMANITH, 2014). The amino acid sequence of the B-chain in equines is absolutely equal to the human sequence whereas the A-chain differs only in one amino acid (HARRIS et al., 1956; KOELLER et al., 2016).

Insulin plays a key role in the glucose homeostasis of the organism (HEPPES, 2003) and primarily stimulates the uptake of glucose from the bloodstream into e.g. skeletal muscles and adipocytes (SALTIEL and KAHN, 2001; GEOR, 2008). Furthermore, insulin reduces the glycogenolysis and gluconeogenesis in the liver as well as the lipolysis in adipose tissues (BESSESEN, 2001). Insulin promotes lipogenesis and

protein synthesis and likewise the retention of several substrates in the fat and muscle tissue as well as in the liver (SALTIEL and KAHN, 2001). Moreover, insulin stimulates the cell growth and differentiation, functioned as regulator of vascular endothelial functions and has anti-inflammatory effects (GEOR, 2008).

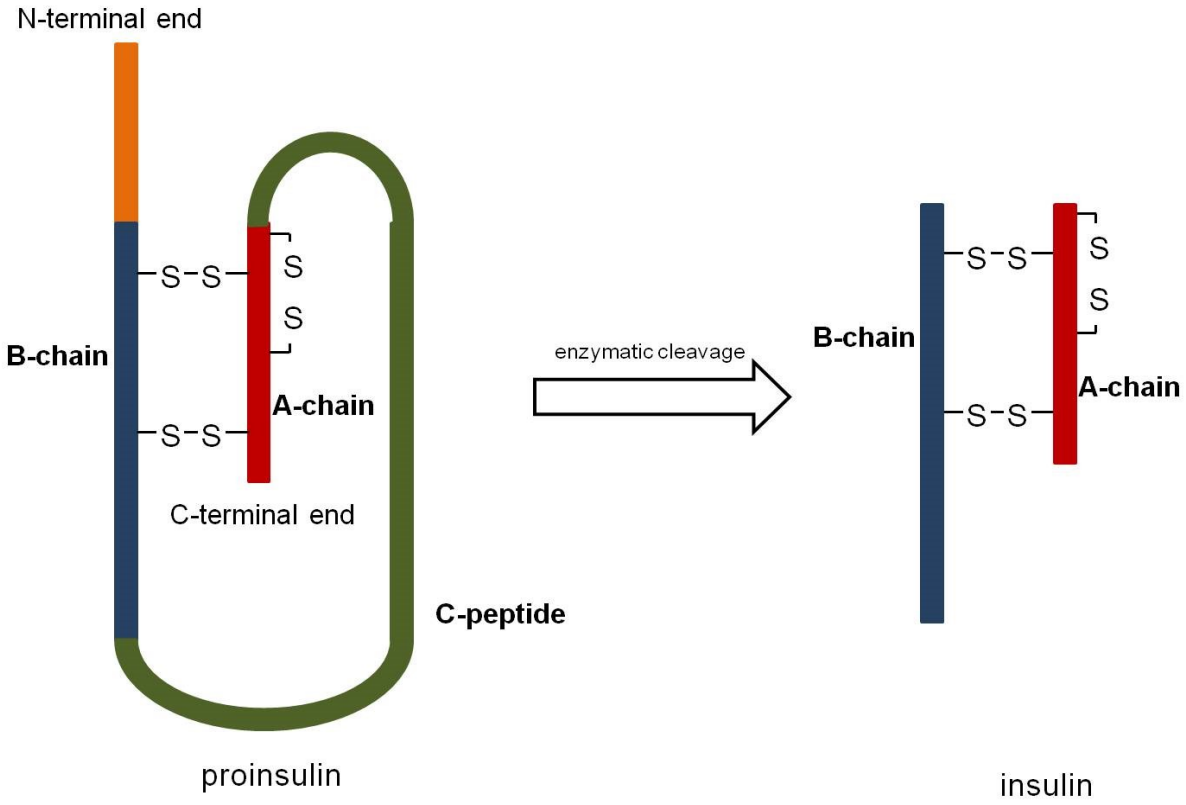

Fig. 6: Synthesis and structure of insulin (according to KLEINE and ROSSMANITH, 2014)

The glucose uptake into the cells (fat and muscle) is stimulated by insulin via the recruitment of the glucose-transporter GLUT 4 (SALTIEL and KAHN, 2001). The GLUT 4 transporter is localized in vesicles which permanently move from the intracellular stores to the biomembrane (SALTIEL and KAHN, 2001). Insulin stimulates the exocytosis of the GLUT 4 vesicles and therefore increases the transport of glucose into the cell.

2.3.2.2 Postprandial state of the equine blood insulin concentration

The insulin secretion is predominantly determined by the glucose concentration in the blood stream and varies especially PP (the PP state) but also circadian. Variation in the circadian secretion rate is contrary discussed in the literature (STULL and RODIEK, 1988; HEPPES, 2003). Reference borders are difficult to describe but for the definition of reference ranges, a measurement of the fasting blood insulin values is intended. KOELLER et al. (2016) defined such a reference range for ponies, which had daily access to pasture for maximal 10 h, at 2 – 21.1 mU/L (≙ 0.012 – 0.126 nmol/L). The measured concentration of the insulin hormone mostly depends on the handling of the samples (e.g. storage time and temperature) but also on the collection time in relation to the last meal as well as the amount and type of feed (KOELLER et al., 2016). Furthermore, the insulin secretion (fasting and PP) is subjected to the body condition (moderate or obese), the predisposition for gastrointestinal derived disease (like laminitis) and the breed. Feeding of fibre-rich diets (here: hay) induced nearly no variation of the PP insulin secretion in comparison to the basal level (basal fibre-rich diet: 5.7 ± 1.1 µU/mL vs. PP: 5.7 ± 0.4 µU/mL; STULL and RODIEK, 1988) whereas feeding a high content of non-structural carbohydrates (4.2 – 18.2 % DM) via haylage induced a PP insulinaemic response in the range of concentrates (LINDÅSE et al., 2018). Generally, the inclusion of concentrates increased the PP insulinaemic response (STULL and RODIEK, 1988). The insulin secretion to maintain a fasting level depends further on the processing of the concentrate. VERVUERT et al. (2003) revealed the highest basal insulin level if the horses fed with finely ground oats (7.4 µU/mL) in comparison to untreated oats (4.4 µU/mL). Moreover, the body condition of the horses influences the insulin secretion. The basal insulin concentration was not significantly different between non-obese, moderately obese or obese horses but the PP insulinaemic response was significantly higher in obese horses compared to the other ones (HOFFMAN et al., 2003). A predisposition for (i.a. reduced insulin sensitivity, obesity) specific diseases (e.g. laminitis, PSSM) tendentially increased the basal insulin secretion (BORER et al., 2012). Furthermore, the variable PP insulinaemic response of predisposed horses was addicted to the season (highest in fall compared to spring). The comparison of different breeds revealed a variable PP insulin response but an equal

basal insulin level (BAMFORD et al., 2014, 2015). Measuring the insulinaemic response after a second meal indicated a minor PP insulin secretion whereas the basal level was nearly the same (KARASU et al., 2015).

2.3.2.3 Disturbances of the insulin metabolism

The horse is susceptible to metabolic disturbances mostly induced by the feeding. Especially not or not sufficient adapted, but clinically inconspicuously equines are prone for disturbances, e.g. by an abrupt change of feedstuff or an increasing amount of carbohydrate-rich (including high concentrations of starch) and high in fat diets.

Besides the diet and a predisposition, certain factors are able to influence the insulin sensitivity in horses e.g. age, breed, pregnancy and lactation, training, BCS/obesity and supplements (like chromium, metformin, levothyroxine, corticosteroids or xylazine; FIRSHMAN and VALBERG, 2007). Insulin dysregulation in the horse is associated with insulin resistance (IR), and hyperinsulinaemia which in turn might have a potential relevance for the equine metabolic syndrome (EMS) or laminitis (DE LAAT et al., 2016). Insulin resistance is the minimized sensitivity of target tissues (muscle, adipose or liver) to insulin mediated glucose uptake into the cell or the perturbation of the hepatic glucose production (KRONFELD et al., 2005; GEOR, 2008). The IR can either occur before binding to the cell receptor by interference in the insulin-signaling mediators or at the cell surface by changes in the postbinding signal transduction or intracellular by malfunctions in the glucose metabolism (KRONFELD et al., 2005; TREIBER et al., 2006). Formation of insulin resistance is secondarily mostly induced by prolonged stimulation (pancreatic overstimulation via permanent high PP blood glucose levels) with subsequent downregulation of the insulin receptors (FIRSHMAN and VALBERG, 2007; DE LAAT et al., 2016). On the opposite, an increased insulin sensitivity in the target tissues enhance the glucose disposal from the bloodstream and therefore might lead to hypoglycaemia (DE LA CORTE et al., 1999; FIRSHMAN and VALBERG, 2007).

Obese Thoroughbred geldings (BCS 7-8/9) showed significantly lower insulin sensitivity in comparison to moderately obese (6/9) or non-obese (5/9) horses (HOFFMAN et al., 2003). Moreover, the non-obese horses revealed a tendentially reduced insulin sensitivity when fed with a starch- and sugar-rich supplement

compared to a fat- and fibre-supplement whereas the initial obese level had no further influence. Furthermore, the dietary impact on the development of insulin resistance was already proven for young horses. Weanlings (age 199 ± 5 d; BCS 5-6/9), which were fed either with a starch and sugar feed or a fat and fibre diet (twice daily), indicated a significantly reduced insulin sensitivity if the horses ingested the high glycaemic meal (TREIBER et al., 2005a). Interestingly, the starch and sugar fed group showed an alike disposition index in comparison to the fat and fibre group which assumed a compensatory effect. Nevertheless, the authors emphasize that both the compensatory mechanism and the increased blood insulin level might be precursors for the development of metabolic diseases like e.g. laminitis. In particular, ponies (pre-laminitic genotype; TREIBER et al., 2005b) have a predisposed risk to develop, e.g., pasture-associated laminitis (BAILEY et al., 2007). By implication, the ponies which underwent a laminitis disorder were insulin resistant and showed decreased insulin sensitivity compared to the control ponies (BAILEY et al., 2007). The laminitis-prone ponies were able to maintain the plasma glucose concentration in the same level as the control ponies by an increased secretion of insulin derived from the pancreas but the overall insulin clearance from the bloodstream was reduced. Postprandial hyperinsulinaemia, which is mostly the consequence of grain-based, high-energy diets, is involved in the development of insulin resistance. SUAGEE et al. (2011) investigated the impact of a 6 h insulin-infusion on normal, lean horses (mean age: 14 years, mean BCS: 6.3). The authors demonstrated a decreased abundance of glucose transporter (GLUT 4 and GLUT 1) and insulin receptors in adipose tissues whereas the GLUT 1 abundance increased in skeletal muscles. The second mentioned stimulatory effect of hyperinsulinaemia related to the GLUT 1 transporter supports the compensatory mechanism whereby the non-insulin stimulated glucose uptake into the cells is promoted (SUAGEE et al., 2011). Furthermore, laminitis was induced in healthy ponies without predisposition by sustained hyperinsulinaemia and euglycaemia (ASPLIN et al., 2007). This supports the assumption, that insulin toxicity is a key factor regarding the development of laminitis.

2.3.3 Regulation of the glycaemic and insulinaemic response

The maintenance of the glucose homeostasis in the organism depends on the interaction of glucose and insulin in the target tissues as well as the secretion areas. High circulating blood glucose concentrations induce a glucose-dependent insulin secretion in the β-cells of the pancreas (Fig. 7; KLEINE and ROSSMANTIH, 2014). The insulin secretion is a two-phase process: the first and fast PP release resulted from ready insulin vesicles at the membrane of the cell and the second secretion phase depends on the additionally insulin synthesis in the cell (GRODSKY, 1972). The glucose uptake into the pancreatic cells is possible via GLUT 2 transporter. Glucose is phosphorylated to glucose-6-phosphate and metabolized to carbon dioxide in the mitochondria with subsequent production of ATP which is released into the cell (KLEINE and ROSSMANITH, 2014). Thus, the ATP to ADP relation is increased and causes the liberation of potassium ions through specific channels out of the cells. As a consequence, the membrane tension is changed and opens subsequently a calcium channel. The calcium ions in the cell induce the fusion of the insulin vesicles with the membrane and thus release the insulin into the organism.

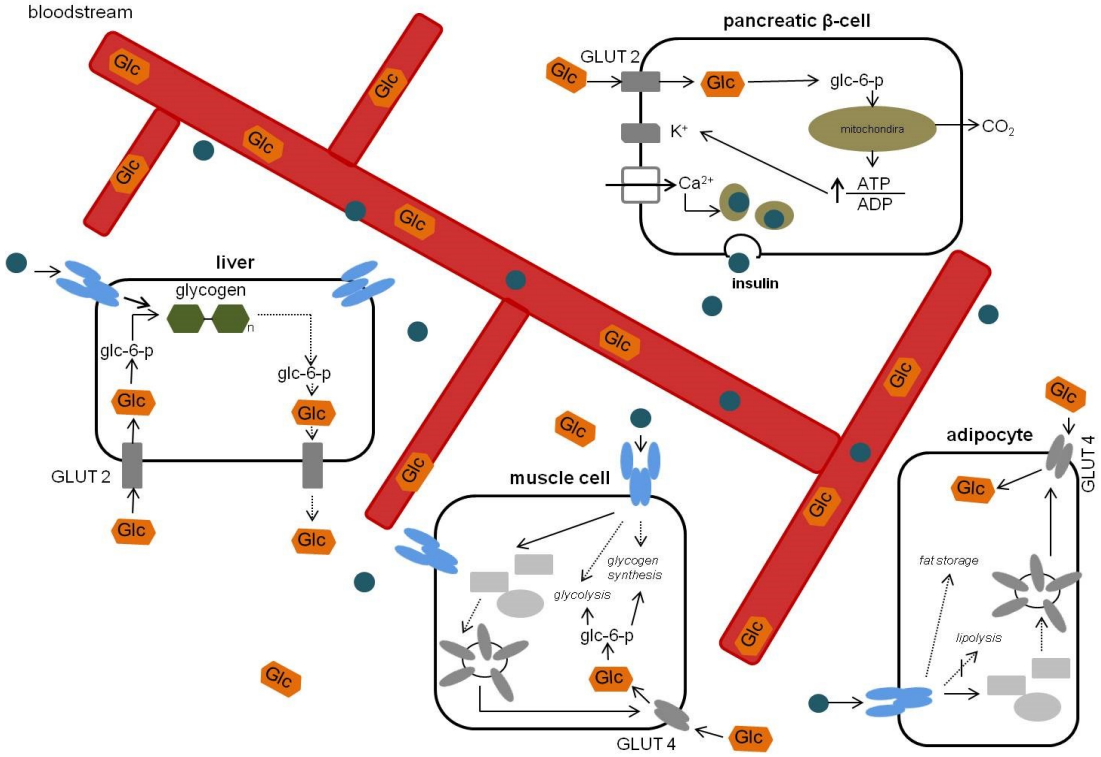

Fig. 7: Regulation of the glucose and insulin uptake in target tissues and viscera (according to Mc COWEN and ABEL, 2000; SCHEEPERS et al., 2004; KLEINE and ROSSMANITH, 2014); Glc = glucose; glc-6-p = glucose-6-phosphate

Furthermore, insulin stimulates the recruitment of GLUT 4 transporter in the muscle and fat cells so that glucose can be absorbed into the cells (Fig. 7; SCHEEPERS et al., 2004). The binding of insulin to the transmembrane receptor induces an autophosphorylation through several tyrosine residues which hence stimulates the receptor kinase activity (Mc COWEN and ABEL, 2000; BESSESEN, 2001). Subsequently, insulin receptor substrates (IRS) interact with the insulin receptor and certain tyrosine residues phosphorylate on the IRS. The phosphorylated IRS proteins participate via activation on several intracellular processes like the glucose metabolism and the mitogenesis (Mc COWEN and ABEL, 2000). Therefore, the regulatory subunit (p85), which derived from the phosphatidylinositol-3-kinase (p-3-kinase), binds to the phosphorylated IRS and activates the catalytic subunit of p-3-kinase. Further stimulation of downstream proteins resulted in a translocation of intracellular GLUT 4 vesicles to the cell membrane which thus increases the glucose uptake from the bloodstream (BESSESEN, 2001; KLEINE and ROSSMANITH, 2014). Additionally, insulin increases the storage of glucose via synthesis of glycogen as well as stimulates the degradation of glucose via glycolysis to i.a. ATP in the muscle cells whereas in the adipocytes, insulin has a key role in the storage of fat and avoidance of lipolysis (Fig. 7; Mc COWEN and ABEL, 2000). In the liver, the glucose homeostasis is regulated via the synergetic shift between glucose degradation and glucose synthesis (Fig. 7; KLEINE and ROSSMANITH, 2014). Specific sensors in combination with the key enzyme glucokinase, whose expression depends on the glucose concentration, measure the blood glucose concentration and introduce either the catabolic or anabolic pathway (KLEINE and ROSSMANITH, 2014). High blood glucose concentrations cause a glucose uptake into the liver via insulin-independent GLUT 2 transporter. Equally, the enzyme glucokinase is expressed in higher concentrations which therefore stimulates the production of glucose-6-phosphate from glucose (catabolic pathway) or the synthesis of glycogen as storage carbohydrate (KLEINE and ROSSMANITH, 2014). If the blood glucose concentration is low, the metabolism switches to the anabolic pathway which either results in the gluconeogenesis via glucose-6-phosphate or the degradation of glycogen. Insulin participates at the hepatic metabolism via the stimulation of the glycogen synthesis (KLEINE and ROSSMANITH, 2014).

The insulin secretion in the homeostatic process is further regulated respectively influenced by the release of incretin hormones. In human but also in equine

physiology, two incretin hormones are well-known: the glucagon-like peptide 1 (GLP-1) and the glucose-dependent insulinotrophic peptide (GIP; DEACON and AHRÉN, 2011; KAZAKOS, 2011). The major role of both incretins is the enhancement of the postprandial glucose-dependent insulin secretion in the pancreatic β-cells but both hormones can have several effects on the viscera, target tissues and on neuronal and cardiovascular systems (Table 3).

Table 3: Impacts of the incretin hormones GLP-1 and GIP on the organism (according to DUEHLMEIER et al., 2001; DEACON and AHRÉN, 2011; KAZAKOS, 2011)

Target	GLP-1	GIP
pancreas	stimulate glucose-dependent insulin release in β-cells increase insulin biosynthesis via activation of protein kinase A enhance proliferation and differentiation of β-cells reduce β-cell apoptosis	
	suppress glucagon secretion from α-cells (glucose-dependent)	stimulate glucagon secretion from α-cells
gastrointestinal tract	delay gastric empty (transient of nutrients into small intestine)	minimal effects on gastric empty time known
nervous system (central and peripheral)	neuroprotective via activation of anti-apoptotic neuronal pathways, physiological role in regulation of appetite/satiety and body weight, reduce amyloid β-peptide level in brain, protect hippocampal neurons improve learning and memory (in rats)	-
cardiovascular system	increase heart rate and blood pressure, modulate function and survival of cardiomyocytes (cardioprotective), improve myocardial function, improve endothelial dysfunction, anti-atherosclerotic effect	anti-atherosclerotic effect
bone	regulate bone resorption and bone formation, increase osteocalcin level and bone mineral density (in rats)	regulate bone resorption and bone formation, anti-osteoporotic effects (in rodents)
adipose tissue	-	lipogenic and lipolytic effects (in rodents), stimulate triglyceride clearence

The GIP is synthesized in the enteroendocrine K-cells which are primarily located in the proximal regions of the small intestine, especially the duodenum (DEACON and AHRÉN, 2011; KAZAKOS, 2011). The synthesis of GLP-1, which derives from

proglucagon, takes place in the enteroendocrine L-cells. In human, these cells are mostly located in the distal part of the small intestine (ileum) as well as in the colon (DEACON and AHRÉN, 2011; KAZAKOS, 2011). K- and L-cells are stimulated by the ingestion of food (mainly carbohydrates) and secreted the intact hormones (stored in secretory granules) via the direct contact with the nutrients (DEACON and AHRÉN, 2011). The secreted incretin hormones are rapidly degraded by the enzyme dipeptidyl-peptidase 4 (DPP-4), so that the circulation time only amounts to several minutes (1 – 2 min for GLP-1; 2 – 3 min for GIP; DEACON and AHRÉN, 2011).

In horse nutrition, the existence of an enteroinsular axis with a relevant impact of incretin hormones on the digestion is a relatively new aspect. DUEHLMEIER et al. (2001) revealed no significant differences between large Standardbred horses (bwt: 520 – 635 kg) and Shetland ponies (bwt: 146 – 219 kg) concerning the PP GIP secretion after an oral or intravenous glucose tolerance test. Further investigations mostly concentrate on the incretin GLP-1 instead of the above-mentioned GIP. Different breeds (ponies, bwt: 300 ± 19 kg, BCS: 5.3 ± 0.3; Standardbred horses, bwt: 458 ± 17 kg, BCS: 5.2 ± 0.2; Andalusian cross horses, bwt: 509 ± 23 kg, BCS: 5.7 ± 0.3) showed varying PP GLP-1 concentrations which were further strongly correlated with the PP blood insulin concentration (BAMFORD et al., 2015). The ponies and Andalusian cross horses had significantly higher PP GLP-1 concentrations and equally higher blood insulin levels compared to Standardbred horses. Another study examined the PP incretin release in the context with a disturbed insulin regulation. A mixed pony group (consisting of Shetland, Welsh, Connemara, Australian and mixed breeds), divided into normoinsulinemic (NI; bwt: 284 ± 24 kg, BCS: 6.5 ± 1.0) or hyperinsulinemic (HI; bwt: 172 ± 7 kg, BCS: 7.4 ± 0.5) responders showed a significant different PP blood GLP-1 level (DE LAAT et al., 2016). Here, the HI ponies showed significant higher PP glucose, insulin as well as GLP-1 concentrations. CHAMEROY et al. (2016) investigated the PP GLP-1 secretion of horses with EMS (bwt: 475 ± 59 kg, BCS: 7 – 9/9) in comparison to control horses (bwt: 469 ± 25 kg, BCS: 4 – 6/9). The authors revealed a tendentially (P = 0.097) higher percentage of the GLP-1 concentration in the EMS versus the control horses when the equines were fed a high-grain diet. Differences in the PP insulin secretion regarding IR ponies were also investigated on pasture where the ponies with severe insulin dysregulation exhibited the highest PP concentration of GLP-1 and GIP (FITZGERALD et al., 2019). In general, the impact of incretins on the

PP insulin secretion in horses might be not as high as in other species (e.g. humans: responsible for 70 % of the PP insulin release; DE LAAT et al., 2016) but this needs to be investigated further.

Prebiotics are able to influence the intermediate metabolism, as aforementioned. Furthermore, the feeding of prebiotic active ingredients aims to interact positively in the glucose homeostasis. In rats, the supplementation of inulin-type fructans with different degrees of polymerization significantly increased the mRNA proglucagon and GLP-1 secretion chiefly in the proximal colon (DELZENNE et al., 2005; 2007; DELZENNE and CANI, 2010). Equally, the enzymatic activity of dipeptidyl-peptidase-4 is reduced after feeding of oligofructose which thus lessens the fast degradation and cleavage of incretins from the bloodstream (DELZENNE et al., 2005, 2007). Accordingly, rats fed with oligofructose ingested less energy per day which suggests a satiogenic effect of the prebiotic (DELZENNE et al., 2007). Subsequent studies confirm (as tendency) the impact of prebiotic active ingredients on the satiety in humans (CANI et al., 2009). Moreover, supplemental prebiotics are able to reduce the feed intake, the consequent weight gain and fat mass development in obese humans and therefore counteract the formation and/or progression of obesity and metabolic syndrome (PARNELL and REIMER, 2009). Horses have an affinity for grasses with a high concentration of non-structural carbohydrates (e.g. glucose, sucrose, levan-type fructan; LONGLAND and BYRD, 2006). Therefore, there is a reason to believe that the ingestion of prebiotic levans by horses might not reduce the feed intake in contrast to e.g. humans. Beside the direct effect of prebiotics on the glucose homeostasis (concerning the increment of GLP-1 and GIP), indirect impacts related to the bacterial fermentation (and their fermentation products) are known. The feeding of prebiotics aims i.a. to enhance the microbial metabolism in the digestive tract (ROBERFROID, 2007). Fermentation products like SCFA are absorbed in the gastrointestinal tract and transported via the bloodstream e.g. in the liver. Especially propionate is known to interact in the hepatic glucose metabolism by reducing the gluconeogenesis or enhancing the glycolysis. In consequence, the circulating glucose concentration is reduced (ROBERFROID and DELZENNE, 1998). In cats, the feeding of a mixture of oligofructose and inulin significantly reduced the methylmalonylcarnitine and aspartate aminotransferase concentration which indicated an inhibition of the amino acid catabolism and a similar enhancement of the gluconeogenesis from propionate in the liver (VERBRUGGHE et al., 2009).

Furthermore, SCFA (in physiological concentrations) have the ability to modify the size and differentiation of adipocytes (ROBERTSON, 2007). Mainly acetate and propionate act as ligands for specific adipocyte G-protein-coupled receptors (GPR 41 and 43). The SCFA elevate the leptin expression of the adipocytes whereas propionate is more effective compared to acetate. In general, the size of the adipocytes correlates well with the insulin sensitivity in the organism but the increase in size is limited (ROBERTSON, 2007). Large adipocytes shift i.a. to a higher secretion rate of pro-inflammatory cytokines and to a lesser expression of adipokines (like leptin or adiponectin). In the nutrition of horses, the impact of prebiotics on the overall glucose homeostasis (in combination with feed intake) is not sufficient known until now. RESPONDEK et al. (2011) described a positive impact of the supplementation with scFOS to obese horses. Arabian geldings (bwt: 523 ± 57 kg, BCS: 8 ± 1/9) were fed with 45 g scFOS/d (~ 0.09 g/kg bwt x d^{-1}) over 6 weeks. The plasma insulin concentration decreased after the treatment with scFOS whereas neither no variation was noticed concerning the plasma glucose and leptin concentration nor the body weight or body condition score. Moreover, the insulin sensitivity was improved. The authors merely speculate about the possible direct or indirect effects of the prebiotic supplementation which leads to the improvement. Nevertheless, a positive impact of prebiotic active compounds on the equine glycaemic and insulinaemic responses was firstly described and needs to be critically investigated further.

2.4 Compartmental and total tract digestibility of prebiotics

As aforementioned, prebiotics are not hydrolysable by mammalian enzymes (ROBERFROID, 2007) but are a possible substrate for the intestinal microbiota. In human, the supplementation of prebiotic active compounds to the diet promoted satiety and reduced the appetite leading to an overall lower food intake (ROBERFROID et al., 2010). Related to the regulation of the feed intake in humans, the enhanced production of incretins (here: GLP-1) by prebiotics might be the influencing factor (study with rats; CANI, DEWEVER and DELZENNE, 2004). Furthermore, prebiotics are able to delay the gastric emptying time and equally the transit time in the small intestine which might influence the absorption capacity of macronutrients (especially carbohydrates; ROBERFROID and DELZENNE, 1998).

Studies with Carnivores (Leopard, dogs), fed with prebiotics, revealed a reduced digestibility of crude protein but had no effect on the dry matter digestibility (HESTA et al., 2003; PRADHAN et al., 2015). In pigs, the supplementation of inulin showed different results, either with an enhanced digestibility of crude fibre, dry matter and crude protein (BOEHMER et al., 2005) or crude fat (HEDEMANN and KNUDSEN, 2010). Cows showed, by a supplementation of 0.5 - 1.0 % FOS to a total mixed ration, a significant improved digestibility of dry and organic matter (SAMANTA et al., 2013). In equine, the literature data concerning the impact of prebiotics on nutrient digestibility are very scarce. In general, it is believed that the autochthonous microbiota metabolizes the prebiotic compound. Consequently, the nutrient digestibility might be enhanced due to several distinct and/or coherent factors:

a) elevated concentration/abundance of specific, autochthonous bacteria (human: e.g. saccharolytic; GIBSON et al., 2010),
b) enhanced metabolism of the health-promoting bacteria with elevated concentrations of fermentation products (i.a. SCFA) and consequently improved solubility of nutrients (laying hens; ŚWIĄTKIEWICZ and ARCZEWSKA-WŁOSEK, 2010),
c) upregulated expression of transporter genes (pigs: TAKO et al., 2008),
d) impact of fermentation products on intestinal epithelium (here: *n*-butyrate; PLOEGER et al., 2012).

In horses, the feeding of scFOS (0.06 g scFOS/kg BW x d^{-1}) for 21 d had no impact on the dry matter, organic matter, crude protein and crude fat digestibility (RESPONDEK et al., 2007).

The knowledge of the compartmental tract digestibility of specific feed ingredients (especially prebiotic active compounds) in horses is scarce. As previously mentioned, *in vitro* and *in vivo* results lead to the assumption that fructans itself might already be degraded in the equine foregut (COENEN et al., 2006; INCE et al., 2014; STRAUCH et al., 2017). Unfortunately, the compartmental tract digestibility of these prebiotic active compounds is not known, until yet. Furthermore, the selection of the marker to define the total tract/compartmental digestibility is crucial for estimating the nutrient digestibility in the GIT. In horses, the usage of internal markers (acid insoluble ash, AIA; lipid markers/plant alkanes) might be preferred in contrast to external applied markers (chromdioxide, titandioxide, synthetic alkanes; BACHMANN et al., 2016a, b).

Fructans are not dispersible in water and have a water holding capacity which could increase the volume and dry weight of the digesta (SCHNEEMANN, 1999). In horses, a higher digesta weight leads to a prolonged mean retention time in the GIT respectively the colon which might be associated with an increased microbial activity and finally to an enhanced digestibility of nutrients (VAN WEYENBERG et al., 2006; MIJAYI et al., 2008). Furthermore, the ingestion of the prebiotic compound intends to promote the autochthonous microbiota (specifically: elevated relative abundance/CFU) which an accompanied increased production of fermentation products (as mentioned above). In horses, prebiotic feeding might intend to increase fibrolytic/saccharolytic bacterial families like *Lachnospiraceae*, *Ruminococcaceae* or *Prevotellaceae* which in turn might produce higher concentrations of SCFA, especially *n*-butyrate. In accordance to the literature, the fermentation products per se as well as in particular *n*-butyrate might led to an increased digestibility of nutrients due to an improved solubility in the digesta (e.g. because of pH decrease) and/or absorption capacity of the intestinal epithelium (PLOEGER et al., 2012).

Unfortunately, to the author´s knowledge, there are no scientific based investigations concerning the passage rate of the digesta in the GIT after the supplementation of prebiotic active compounds. Furthermore, the literature data regarding to the effect of prebiotic feeding on nutrient digestibility in horses is scarce.

3 Scope of the thesis

The horse is a widely used sport partner mostly fed with components which might counteract the high susceptibility for gastrointestinal derived disturbances. Feeding of prebiotics or prebiotic active compounds to horses is common because of the well-described positive effects regarding the intestinal microbiota and their metabolism in human beings. Unfortunately, the scientific based evidence of a health-promoting effect in equines is scarce, like the outcomes of the above-mentioned studies implies. Furthermore, the results concerning the impact of prebiotics on the microbiota in the digestive tract usually focussed on the hindgut whereas a degradation of prebiotic active substances in the foregut and equally an effect on the microbial composition in this region might be suggested. Moreover, the influence of prebiotics on the glycaemic and insulinaemic response mostly considered horses with a known history of metabolic imbalances like laminitis prone or obese animals. Furthermore, the impact on the nutrient digestibility by supplemental feeding of prebiotics is only limited known and besides the usage of internal or external markers needs to be discussed. The current state of research in equine nutrition regarding the application of prebiotics encloses heterogenic results which are high in inter-individual variability. Therefore, the current work deals with several distinct and withal coherent aspects of feeding prebiotics to adult, healthy horses with no known history of gastrointestinal derived diseases. Regarding the recent literature the following hypothesis were constructed:

1) The fermentative degradation of prebiotic substances by horses already starts in the stomach.
2) Water-soluble carbohydrates, including fructans, are degraded to a large extent in the foregut.
3) The feeding of FOS + inulin as prebiotic active compounds has an impact on the microbiota of the entire gastrointestinal tract.
4) The concentration of *n*-butyric acid will be elevated in a particular degree, formerly in the hindgut.
5) Due to the disappearance of prebiotic active substances in the foregut, the impact on the microbiota in the hindgut dependent on the dosage is comparatively low.

6) Daily prebiotic doses of FOS + inulin improves the insulin sensitivity and thus postprandial glucose clearance in several animals and the benefit is also assumed for clinically normal, non-obese horses following a hay-concentrate meal.

The hypothesis 1 and 4 were investigated in **paper I**. This study deals with the impact of feeding a naturally prebiotic substance (Jerusalem artichoke meal) to adult, healthy horses regarding the fermentative parameters in the entire gastrointestinal tract. The alteration of the metabolic indicators is a consequence or an outcome of the bacterial composition in the digestive tract. Due to this, **paper II** analysed the microbial structure in the overall gastrointestinal tract after feeding a naturally prebiotic and examined hypothesis 3 and 5. The impact of prebiotic supplementation on nutrient digestibility is investigated in **paper IV**. This study deals with hypothesis 2 and used the same animal material like in **paper I and II**. The effect of feeding a recommended prebiotic dosage of Jerusalem artichoke meal on the glycaemic and insulinaemic response in healthy horses was investigated in **paper III**. This study deals with the last hypothesis (6) and used adult, healthy, clinically normal and non-obese horses to clarify the impact of feeding a naturally prebiotic compound on the glucose and insulin homeostasis.
The results of the above-mentioned investigations are listed and discussed in the following chapter.

4 Original articles

4.1 Paper I

Fermentation characteristics along the gastrointestinal tract after feeding of Jerusalem artichoke meal to adult healthy warmblood horses

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Abstract

Objective

Prebiotics are used to support the gastrointestinal health *via* stimulating particular beneficial parts of the autochthonous microflora and enhancing their metabolism. Horses often suffer from gastrointestinal disturbances after feed changes or behavioral stress in response to transport. Therefore, the supplementation with prebiotic compounds might reduce the risk for intestinal dysregulation. The aim of this study was to investigate the influence of supplementation with Jerusalem artichoke meal (JAM) in a recommended prebiotic dosage on fermentation characteristics in the equine gastrointestinal tract.

Methods

Twelve adult healthy horses received crushed oat grains (1 g starch/kg BW x d^{-1}) and meadow hay (as fed basis; 1.5 kg/100 kg BW x d^{-1}). Additionally, they were fed either an apparently prebiotic quantity of fructo-oligosaccharides (FOS) and inulin (0.2 g/kg BW x d^{-1}) *via* Jerusalem artichoke meal (JAM) or an equal amount of maize cob meal without grains as control (CON) over 3 weeks. On d21, horses were euthanized, gastrointestinal contents were removed from 7 different regions of the gastrointestinal tract, the dry matter (DM) content, pH value and concentrations of short chain fatty acids (SCFA), L- and D-lactate and ammonia were measured.

Results

JAM did not had a significant ($P < 0.05$) effect on any of the measured fermentation products and the pH value as well. Numerically, JAM increased the concentrations of SCFA ($P > 0.05$), lactate (both isomers, $P > 0.05$) and ammonia ($P < 0.05$) predominantly in the stomach but had no impact on the pH value overall. In the hindgut, the stimulation of the microbial activity was limited to the ventral colon only indicated by slightly higher SCFA ($P > 0.05$) and ammonia ($P > 0.05$) but lower L-lactate ($P > 0.05$) concentrations compared to control.

Conclusion

FOS and inulin from JAM seem extensively be fermented already in the stomach of horses. The resulting organic acids might elevate the risk for gastric ulcers. Recently

the gastric pH was buffered by concomitantly elevated ammonia, which requires a careful delineation of the influences of either individual acids or low pH levels or both together on the mucosa health in both distinct parts of the equine stomach. In the hindgut, the effect of JAM on the microbial activity seems to be much less pronounced than expected or advertised.

Key words: gastrointestinal tract, horse, microbial fermentation, prebiotic, Jerusalem artichoke

1. Introduction

The equine digestive tract is prone to health disturbances, predominantly after feed changes and overload with easily fermentable carbohydrates (DE FOMBELLE et al., 2001; LONGLAND and BYRD, 2006). This is usually accompanied by alterations in the diversity and activity of the intestinal microbiota (MILINOVICH et al., 2006; MUHONEN et al., 2009). Prebiotics are supposed to have the potency to promote the guts' health by providing substrates selectively for particular beneficial indigenous microorganisms. In horse feeding, inulin-type fructans with different chain length such as short chain fructo-oligosaccharides (scFOS) or inulin itself are implemented as prebiotic substances. Depending on the amount of intake, both inulin- and phlein-type fructans (the latter deriving from vegetative parts of temperate grasses) have either critical or beneficial properties (LONGLAND and BYRD, 2006; VAN EPS and POLLIT, 2006; RESPONDEK et al., 2007; VAN EPS and POLLIT, 2009; JULLIAND and ZEYNER, 2013). Moreover, the quantity of fermentation products such as short chain fatty acids (SCFA) and lactate varies with the dosage and the composition of the supplemental feed (VAN LOO, 2004). Feeding scFOS in low (8 g/d) or high (24 g/d) dosages increased the concentration of total and individual SCFA in the feces of yearling horses (BERG et al., 2005). Contrary, the addition of 30 g scFOS/d to a diet for adult horses failed to influence the concentration of SCFA in both caecum and colon content (RESPONDEK et al., 2008). Except for these studies, there is a lack of knowledge dealing with the impact of prebiotics on fermentative parameters in the entire gastrointestinal tract. The passed down assumption, however, that prebiotics will exclusively be fermented in the hindgut (SAMANTA et al., 2013) might not be transferred to horse nutrition. Fermentation gasses in the breath following inulin

ingestion indicate that microbial fermentation already starts in the lower tract (COENEN et al., 2006).

From this we hypothesized that following apparently prebiotic intakes of inulin-type fructans by horses i) fermentative degradation starts already in the stomach and ii) due to this disappearance of prebiotic substances the impact of the microbiota in the hindgut is comparatively low. As indicated by the literature (PLOEGER et al., 2014) we iii) further hypothesized the concentration of *n*-butyrate will be elevated in a particular degree.

Thus, the aim of the study was to investigate the microbial metabolic responses following apparently prebiotic doses of FOS and inulin originating from Jerusalem artichoke meal to adult healthy warmblood horses in different parts of the gastrointestinal tract.

2. Materials and methods

The experimental procedures were approved by the Animal Welfare Commissioner of the University of Veterinary Medicine Hannover in accordance with the German Animal Welfare Law.

Animals, diets and experimental protocol

Twelve adult, healthy warmblood horses (10 mares, 1 stallion, 1 gelding) were included in the study. The mean bodyweight (BW) and age were 534 ± 64.5 kg and 14 ± 7.5 years, respectively. The horses were maintained in individual boxes bedded with wood shavings and had free access to tap water as well as to a salt block. During a 3 weeks adaptation period, they received crushed oat grains (1 g starch/ kg BW x d^{-1}) and meadow hay (as fed basis; 1.5 kg/100 kg BW x d^{-1}) in quantities to meet their energy requirements for maintenance (SOCIETY FOR NUTRITIONAL PHYSIOLOGY, 2014). The horses were randomly allocated to either the treatment group (JAM) or the control group (CON). The treatment group received additionally inulin-type fructans in a doses currently supposed prebiotic (JULLIAND and ZEYNER, 2013; 0.2 g FOS + inulin/kg BW x d^{-1}) *via* Jerusalem artichoke meal (LIVEN GmbH, Zossen, Germany), and the control group alternatively an equivalent amount of maize cob meal without grains. The dose of prebiotic active substances (fructans including inulin) was calculated according to what was declared for the commercially available JAM. From that, the content of water-soluble carbohydrates

was as follows (as fed basis): glucose 1.1 %, fructose 5.8 %, sucrose 11.5 %, fructans 62.7 % (containing 6.3 % inulin in absolute terms). Horses were offered the diet in two equal meals per day (09:00 h and 15:00 h). Because both the Jerusalem artichoke meal and the placebo had the consistency of finely ground material the entire concentrate was moistened to bind fine particles better to coarser ones. Furthermore, selective feed intake and aspiration was avoided as well. Subsamples of all feedstuffs were taken throughout the entire study for later analyses. The analyzed chemical composition of all feedstuffs is given in Table 1.

Table 1: Analyzed chemical composition of the feedstuffs and calculated contents of metabolizable energy and pre-caecal digestible crude protein and selected amino acids

item	oat	hay	placebo[1]	prebiotic[2]
dry matter (DM), g/kg	912	944	927	939
crude ash, g/kg DM	29	52	26	136
crude protein, g/kg DM	124	90	36	63
acid ether extract, g/kg DM	49	9	8	6
crude fiber, g/kg DM	124	349	343	14
NDF, g/kg DM	316	651	799	3
ADF, g/kg DM	178	391	415	11
ADL, g/kg DM	31	47	39	4
glucose, g/kg DM	0	30	9	14
fructose, g/kg DM	0	39	10	63
saccharose, g/kg DM	11	6	16	122
fructan, g/kg DM	0	40	14	466
starch, g/kg DM	498	0	0	0
ME[3], MJ/kg DM	12.4	6.6	7.5	11.9
pcdCP[4], g/kg DM	75	19	13	56
pcd (lysine)[4], g/kg DM	2.92	0.80	0.05	0.36
pcd (cysteine + methionine)[4], g/kg DM	3.41	0.56	0.06	0.20
pcd (threonine)[4], g/kg DM	3.99	0.82	0.06	0.50

[1]maize cob meal without grains
[2] Jerusalem artichoke meal
[3]calculation according to SOCIETY OF NUTRITIONAL PHYSIOLOGY (2014) and KIENZLE and ZEYNER (2010)
[4]calculation according to SOCIETY OF NUTRITIONAL PHYSIOLOGY (2014) and ZEYNER at al. (2015a)

Digesta sampling and preparation

On d21 of the adaptation period, the horses were euthanatized by a veterinarian. Horses were euthanized approximately 1 hour after they received the half of the daily concentrate ration. This served thereto to assure that the foregut (especially the stomach) contains a sufficient quantity of digesta for sampling. The horses were sedated with romifidine (0.12 mg/kg BW). The anesthesia was induced with a combination of diazepam (0.05 mg/kg BW) and ketamine (2.2 mg/kg BW). Finally the horses were euthanatized with pentobarbital (60 mg/kg BW). Digesta samples were collected immediately after the death from 7 different regions of the gastrointestinal tract: Stomach (*Pars nonglandularis* [PN]*, Pars glandularis* [PG], and mixed stomach content [MC]), small intestine (SI), caecum (CAE), and colon: ventral (CV), dorsal (CD) as well as transversum (CT). Because of its extremely watery consistency, small intestinal content was used as native material. Digesta samples from the other parts of the digestive tract were prepared for pH measurement and further analyses as described formerly for equine faeces samples (ZEYNER et al., 2015b). For this, 50 grams of digesta were mixed with 50 mL distilled water immediately post mortem. The suspension was covered with aluminum foil to avoid interchange of gases and incubated for 60 min. After that, the pH value was measured, the suspension centrifuged (4,000 x *g*) for 10 min at room temperature (RT), the supernatant collected in plastic tubes (2 mL) and stored at – 20 °C until analysis of organic acids and ammonia. Samples from the particularly watery digesta from the small intestine was centrifuged and stored without any previous dilution.

Analyses

Feedstuffs and digesta: The feedstuffs were analyzed for dry matter (DM), proximate nutrients including starch and amino acids according to official methods in which for starch the enzymatic procedure was applied (PAVIS et al., 2001; ASSOCIATION OF GERMAN AGRICULTURAL STUDY AND RESEARCH INSTITUTES [VDLUFA], 2015). In the digesta, the DM content only was determined with the same method as for feedstuffs. The method from PAVIS et al. (2001) modified by HILLEGEIST and GREEF was used for detection of water-soluble carbohydrate contents including fructans in the feedstuffs, and the characterization of the structure of fructan isomers as separation according to the degree of polymerization (DP).

pH value: The pH value in the digesta-water-mixtures and native digesta (small intestine), respectively, was measured potentiometrically by use of a pH meter (Mettler – Toledo, Ohio, USA; precision: ± 0.01, temperature compensation: 0 °C – 100 °C).

SCFA: The liquid phase of digesta samples (supernatant of the digesta-water-mixture or native liquid phase) were analyzed for SCFA concentrations according to the method from GEIßLER as described by ZEYNER et al. (2004). The analysis was performed using a gas chromatograph (Shimadzu 17A GC, Jena, Germany) with a flame ionization detector and a separation column FFAP 30 m x 0.25 mm (Supelco, Pennsylvania, USA) but without use of a water cooling sample rack. Helium was taken as carrier gas (3.99 L/h, split 40:1). The temperature program started at an initial temperature of 80 °C and heated up at a rate of 10 °C up to 110 °C, then at 15 °C up to the end temperature of 175 °C. The temperature was held for 4 min. The total cycle time for one measurement was 11:33 min. For analysis, the supernatant of the digesta-water-mixture was thawed at room temperature and centrifuged (2,000 x *g*) for 5 min. Five hundred microliter of the supernatant were transferred into a separate tube (1.5 mL Eppendorf tube). The supernatant was mixed with 50 µL of the internal standard solution and centrifuged (2,000 x *g*) for 5 min to segregate the protein precipitation. Then the clear solution was transferred into a micro vial and immediately injected into the GC. The concentrations of the individual SCFA (acetic acid, propionic acid, iso-valeric acid, iso-butyric acid, *n*-valeric acid, 3-methyl-butyric acid [*n*-butyric acid], *n*-caproic acid) in the supernatant of the digesta-water-mixture were calculated upon the internal standard.

L- and D-lactate: L- and D-lactate were analyzed according to the method of SCHEIJEN et al. (2012) by using HPLC-MS/MS after sample preparation according to HENRY et al. (2012). As the internal standard (IST) sodium DL-lactate-3, 3, 3-d_3 (CDN Isotopes, Quebec, Canada) was taken. First, the samples were thawed at room temperature, mixed and centrifuged for 5 min (18,000 x g) at 4 °C. Assuming that the foregut contains higher concentrations of lactate, the samples were treated differently according to their origin in the gastrointestinal tract. After centrifugation, the supernatant (25 µL from samples of the stomach and the small intestines; 1000 µL from samples belonging to the hindgut) were transferred in a separate Eppendorf

tube (1.5 mL). The supernatants of the foregut samples were mixed with 200 µL of the IST (3 mol/L, respectively) and 825 µL distilled water, the supernatants of the hindgut samples were instead mixed with 50 µL of the IST (0.3 mmol/L, respectively). Both types of samples were mixed with 25 µL of hydrochloride acid 27 % (w/w) and then loaded to the Extrelut NT1 columns (Merck, Darmstadt, Germany). The samples were eluted with 6 mL 2-methyl-2-butanol: chloroform (ratio 11:9), then extracted by 1 mL ammonium hydroxide (0.1 mol/L; solved in water). After centrifugation at 800 x *g* for 3 min the aqueous phase was transferred in a new 2 mL Eppendorf tube (100 µL for the foregut samples; and the entire aqueous phase for the hindgut samples) and evaporated at 65 °C under a stream of nitrogen. After that, the residue was dissolved in 400 µL methanol, mixed thoroughly and was then centrifuged for 5 min (18,000 x *g*) at room temperature. The supernatant was transferred to an opaque HPLC vial and evaporated at 50 °C under a stream of nitrogen. Residues were mixed with 100 µL of diacetyl-L-tartric anhydride (50 mg/mL in dichloromethane: acetic acid, ratio 4:1) and incubated for 30 min at 75 °C in closed vials for derivatization. Subsequently, the vials were opened and the samples were evaporated at 50 °C under a stream of nitrogen. Conclusively, the residues were dissolved in 100 µL acetonitrile: water (ratio 1:2) and transferred in vials with inserts. Samples were measured using an API 2000 (Applied Biosystems, Darmstadt, Germany) in combination with an HPLC 1100 (Agilent, Waldbronn, Germany) using an Hypersil ODS column (150 mm x 2 mm, 5 µm; VDS optilab, Berlin, Germany) at 25 °C. The temperature of the ESI-source was set to 300 °C. The eluent was 1.5 mmol/L ammonium formate (pH 3.6) in water: acetonitrile (99:1) at a flow rate of 150 µL/min. After a run of 13 min, the column was washed for 4 min with acetonitrile at 250 µL/min and re-equilibrated. Lactic acids were measured using the mass transition of 305/89 m/z and the IST at 308/89 m/z.

Ammonia: Ammonia was analyzed according to CONWAY et al. (1933), modified by VOIGT and STEGER (1967). Therefore, the samples were thawed at room temperature. Afterwards, 1 mL of the digesta-water mixture was transferred into a micro-diffusion vessel and incubated at room temperature for 24 h. Ammonia diffused from the sample into the boric acid und changed the indicator from red to green. After the incubation period, hydrochloride acid (0.01 mol/L) was used for titration until the indicator reaches the initial color. The amount of ammonia in the sample can be

calculated by the depleted amount of hydrochloride acid using the following equitation:

$$c_A = \frac{(V_{HCL} \times 0.17)}{M_{NH3}} \times 1{,}000\,,$$

where c_A is the concentration of ammonia in the sample (in mmol/L), V_{HCL} is the volume (in mL) of hydrochloride acid, which was used to reach the initial indicator color and M_{NH3} is the molar mass (in g/mol) of ammonia. Assuming that 1 mL hydrochloride acid neutralizes 0.17 mg/mL ammonia, the factor 0.17 is used to relate the depleted volume of hydrochloride acid with the amount of ammonia in the sample.

Calculations

Basing on the analyzed chemical composition of the feed, the contents of metabolizable energy (ME) and pre-caecal digestible (pcd) crude protein (pcdCP), pcd methionine and cysteine (as sum of sulfur-containing amino acids) and pcd threonine were calculated according to KIENZLE and ZEYNER (2010) and ZEYNER et al. (2015a), respectively. With subject to digesta samples, the concentrations of fermentation products (total and individual organic acids, ammonia) in the native liquid phase of the digesta from the respective parts of the digestive tract were calculated basing on the analyzed DM content and the measured concentration of the fermentation product in the supernatant of the digesta-water-mixture using the equation given by ZEYNER et al. (2004). For digesta samples from the small intestine this kind of calculation was dispensable because all analyses were performed directly in the liquid digesta phase.

Statistical analyses

Statistical analysis was performed using SAS (version 9.4, SAS Inst. Inc., Cary, NC, USA). Results were reported as means ± SD or least square means (Lsmeans) ± SE. Data were analyzed by PROC MIXED (two-way ANOVA with repeated measures) using a model with the fixed factors treatment (CON and JAM), part of the gastrointestinal tract (PN, PG, MC, SI, CAE, CV, CD and CT), and the interaction between treatment and part. The simultaneous analysis including all parts of the

gastrointestinal tract was not possible because (a) no or small variation of the traits was observed in some parts (PN, PG, MC or SI), (b) the assumption of normality of the residual effects was rejected following Shapiro-Wilk test, and (c) including of the complete covariance matrix of the eight observations per animal lead to convergence problems on the estimation of the model parameters. Therefore, parts of the digestive tract were clustered in “foregut” (including PN, PG, MC and SI), and “hindgut” (including CAE, CV, CD and CT). In order to take into account the repeated measures, the residual effects corresponding to the four observations of one animal within “foregut” and “hindgut” were considered as dependent with residual covariance matrix R. The following competing covariance structures of R were tested using the repeated statement of the MIXED procedure: unstructured (type = UN), exchangeable (type = CSH), Toeplitz (type = TOEPH (4), and autoregressive (type = ARH(1)). In all structures inhomogeneous residual variances were assumed. The final covariance structure was selected using AIC-values based on the log-likelihood function of the REML-method. Comparison between the different parts of the gastrointestinal tract was made using the *post hoc* Tukey-Kramer test. The multiple t-test implemented in PROC MIXED (two-way repeated measures ANOVA with Kenward-Roger approximation to the degrees of freedom) was used to compare the two groups (CON and JAM) within the parts of the two tracts (“foregut” and “hindgut”). The Shapiro-Wilk test was applied to check the normality of the studentized residuals. In the case of significant differences and of rejecting the normal distribution, the Wilcoxon rank sum test was additionally taken to compare the two groups within the parts. Data were statistical significant at $P < 0.05$.

3. Results

Chemical composition of the Jerusalem artichoke meal

The contents of water-soluble carbohydrates determined in the pooled material sampled throughout the entire study was largely different from the declared ones. The analyzed contents were as follows (as fed basis): glucose 1.4 %, fructose 6.3 %, sucrose 12.2 %, fructans 46.6 % (content of inulin depending from what the DP range is defined being inulin). The fructan of the JAM product was represented by particularly high and largely similar percentages of isomers up to DP 22.

General observations

In general, the horses consumed the moistened concentrates quite well and did not exhibit obvious signs of gastrointestinal disturbances. In the stomach of one horse of the placebo group, however, mucosal lesions particularly alongside the *margo plicatus* were observed post mortem.

Digesta characteristics

Dry matter: Irrespective of the diet ($P > 0.05$), the DM content of the digesta (Table 2) was highest in the stomach (≈ 21 %), particularly low in the small intestine (≈ 6 %) with further gradual increase until the colon transversum (≈ 15 %). Along the majority of the digestive tract the digesta DM was numerically higher after feeding of JAM *vs.* CON, with inverse relation in the colon transversum ($P > 0.05$).

pH value: The pH values did not show any differences between the control and the treatment group (Figure 1; $P > 0.05$) in any part of the gastrointestinal tract. In the stomach, the pH value reached 4.5 ± 1.1 on average in the *Pars nonglandularis* and 3.4 ± 0.8 on average in the *Pars glandularis* (approximately 1 hour postprandial) in both groups. The pH value in the small intestine was higher than in the stomach with 8.0 ± 0.1 (SI) *vs.* 3.9 ± 0.7 (MC), but was similar within both groups. In the further gastrointestinal tract, the pH value declined from 6.7 ± 0.2 in the caecum to 6.4 ± 0.3 in the colon transversum, irrespective of the treatment ($P > 0.05$).

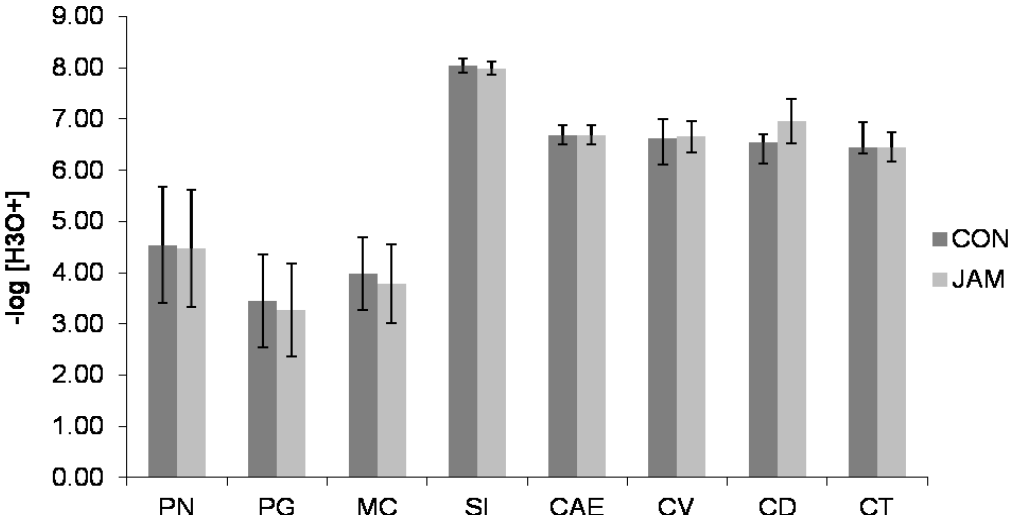

Fig. 1: Lsmeans (± SE) of the pH value ($-\log_{[H3O+]}$) along the gastrointestinal tract after feeding of JAM (n = 6) or CON (n = 6)

SCFA: Irrespective of the diet, concentrations of total SCFA were particularly high in the hindgut and low in the small intestine with the following order along the digestive tract: CV > CD > CAE > CT > PN > PG > SI. In the non-glandular region of the stomach, the content of total SCFA was about 1.7-fold as high as in the glandular part. In general, the treatment group (JAM) showed numerically higher concentrations of total SCFA in almost all parts of the gastrointestinal tract, except for caecum and small intestine (Table 2; $P > 0.05$). The difference between diets was particularly high in the non-glandular region of the stomach. Total SCFA in the PN amounted to two-thirds only in CON *vs.* JAM group horses. The supplemental feeding of JAM resulted in a tendential higher concentration of acetic acid in the mixed content of the stomach ($P > 0.05$) as well as in higher concentrations in the *Pars nonglandularis* ($P > 0.05$) and *Pars glandularis* ($P > 0.05$; Table 2). Except for the caecum, the concentration of acetic acid was higher in all parts of the hindgut in the JAM feeding group in comparison to the control group (Table 2; $P > 0.05$). The concentration of propionic acid was elevated in the mixed content of the stomach ($P > 0.05$) as well as in the colon (CV, CD and CT; Table 2; $P > 0.05$) but lower in the *Pars nonglandularis*, *Pars glandularis*, small intestine and caecum (Table 2; $P > 0.05$) with the addition of JAM in the diet. Iso-butyric acid concentration increased only in the *Pars glandularis*, colon dorsal and colon transversum (Table 2; $P > 0.05$) but was reduced in the other parts of the gastrointestinal tract. The concentration of *n*-butyric acid raised numerically in almost all parts of the digestive tract with exception of the caecum and the dorsal colon (Table 2; $P > 0.05$). Feeding of JAM resulted in an elevated concentration of iso-valeric acid in the stomach (PN and PG) as well as in the colon (CV, CD and CT; Table 2; $P > 0.05$). The concentration of the isomer *n*-valeric acid increased in the *Pars glandularis*, mixed content of the stomach and also in the colon (ventral and transversum; Table 2; $P > 0.05$). *N*-caproic acid was measured only in low concentrations and in a few samples, predominantly in the JAM group (Table 2; $P > 0.05$). The percentage distribution of the SCFA was affected by the addition of JAM in the diet, mainly in the foregut (Figure 2). In the *Pars nonglandularis* of the JAM group, the percentage of *n*-butyric acid increased with coincident reduction of propionic acid but with no impact on the percentage of acetic acid (Figure 2a; $P > 0.05$). The same effect was noticed in the *Pars glandularis* but with a significant lower proportion of propionic acid in the JAM group (Figure 2b; $P < 0.05$). In the further gastrointestinal tract, the individual SCFA were distributed almost

similarly in the control and the treatment group except for the ventral colon. Therein, the percentage of *n*-butyric acid was significant higher in the JAM group compared to the control group (CON: 19.5 ± 1.66 %; JAM: 22.5 ± 1.37 %; $P < 0.05$) with a slightly coincident reduction of the percentage of acetic acid (CON: 39.1 ± 3.80 %; JAM: 38.8 ± 2.62 %; $P > 0.05$) and propionic acid (CON: 28.2 ± 3.82 %; JAM: 26.8 ± 1.64 %: $P > 0.05$).

Table 2: Dry matter content (in %; mean ± SD) and concentrations (Lsmeans ± SE) of individual short chain fatty acids (SCFA) along the gastrointestinal tract of 6 control horses (CON) and 6 horses fed with Jerusalem artichoke meal (JAM)

Item		**part of the gastrointestinal tract**[1]							
		PN	**PG**	**MC**	**SI**	**CAE**	**CV**	**CD**	**CT**
DM	CON[2]	21 ± 4	19 ± 4	21 ± 5	6 ± 1	7 ± 2	10 ± 2	10 ± 2	16 ± 4
	JAM[3]	24 ± 6	20 ± 3	21 ± 3	6 ± 1	8 ± 2	10 ± 2	11 ± 2	13 ± 5
acetic acid	CON[2]	22.7 ± 4.5	14.7 ± 5.7	15.7 ± 2.6	3.9 ± 1.2	70.4 ± 7.3	76.7 ± 7.5	70.4 ± 8.1	29.7 ± 4.7
	JAM[3]	30.9 ± 4.5	22.8 ± 5.7	24.8 ± 2.6	2.8 ± 1.2	54.5 ± 7.3	84.0 ± 7.5	70.7 ± 8.1	35.4 ± 4.7
propionic acid	CON[2]	1.8 ± 1.2	0.5 ± 0.3	0.3 ± 0.4	0.0 ± 0.1	50.9 ± 5.9	55.2 ± 5.6	41.9 ± 5.0	19.7 ± 3.2
	JAM[3]	1.4 ± 1.1	0.3 ± 0.3	0.7 ± 0.4	0.1 ± 0.1	41.5 ± 5.2	58.2 ± 5.6	45.4 ± 5.0	23.7 ± 3.2
iso-butyric acid	CON[2]	0.7 ± 0.4	0.0 ± 0.0	0.9 ± 0.4	0.0 ± 0.0	2.5 ± 0.3	9.4 ± 0.8	8.2 ± 1.0	3.3 ± 0.7
	JAM[3]	0.0 ± 0.0	0.3 ± 0.4	0.1 ± 0.4	0.0 ± 0.0	2.0 ± 0.3	8.7 ± 0.8	9.6 ± 1.0	4.6 ± 0.7
n-butyric acid	CON[2]	11.5 ± 8.3	4.9 ± 2.7	7.6 ± 4.5	0.3 ± 0.1	31.3 ± 3.0	38.1 ± 4.8	28.9 ± 5.3	11.1 ± 2.2
	JAM[3]	28.3 ± 8.3	8.3 ± 2.7	18.8 ± 4.5	0.3 ± 0.1	25.8 ± 3.0	49.3 ± 4.8	27.4 ± 5.3	11.9 ± 2.2
iso-valeric acid	CON[2]	1.6 ± 1.0	2.1 ± 2.5	6.4 ± 3.4	0.3 ± 0.2	2.5 ± 0.4	11.2 ± 1.4	9.9 ± 1.2	3.7 ± 0.8
	JAM[3]	1.5 ± 1.0	3.4 ± 2.5	2.1 ± 3.4	0.1 ± 0.2	1.8 ± 0.4	12.1 ± 1.4	10.6 ± 1.2	4.3 ± 0.8
n-valeric acid	CON[2]	0.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	2.4 ± 0.3	4.8 ± 0.6	4.0 ± 0.4	1.9 ± 0.3
	JAM[3]	0.0 ± 0.0	0.3 ± 0.3	0.9 ± 0.3	0.1 ± 0.3	2.2 ± 0.3	5.2 ± 0.6	3.9 ± 0.4	2.2 ± 0.3

n-caproic acid	CON[2]	0.0 ± 1.0	0.0 ± 0.0	2.8 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	JAM[3]	0.0 ± 0.0	0.2 ± 1.0	1.0 ± 1.0	0.4 ± 1.0	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

[1]part of the gastrointestinal tract: PN = *Pars nonglandularis*; PG = *Pars glandularis*; MC = mixed content (stomach); SI = small intestine; CAE = caecum; CV = colon ventral; CD = colon dorsal; CT = colon transversum;
[2]CON = control group fed with maize cob meal without grains;
[3]JAM = treatment group supplemented with Jerusalem artichoke meal;
Statistical analysis did not indicate any significant differences between CON and JAM group horses for a given segment of the digestive tract ($P > 0.05$)

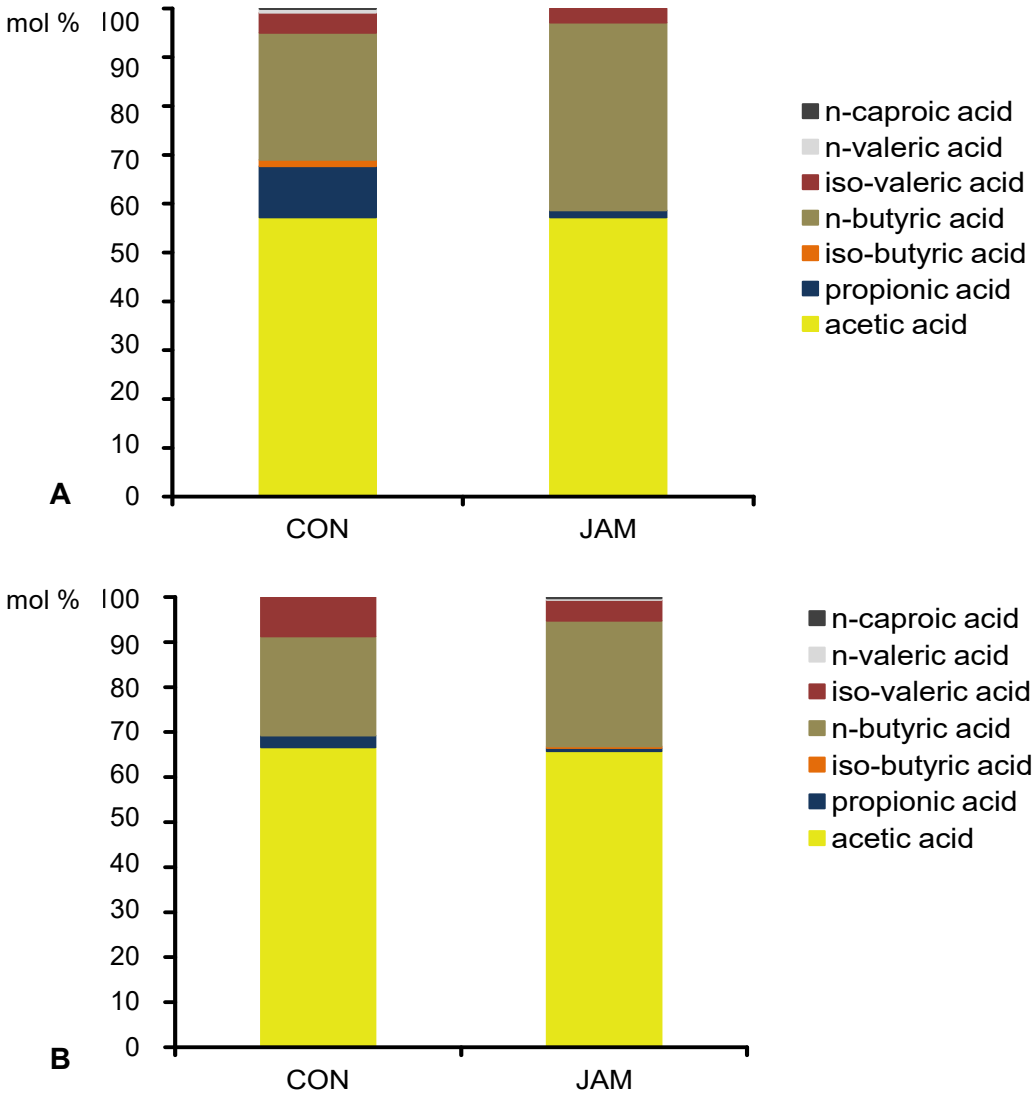

Fig. 2: Mean percentage distribution of different short chain fatty acids in the *Pars nonglandularis* (A) and *Pars glandularis* (B) of 6 horses fed the control diet (CON) and 6 horses supplemented with a Jerusalem artichoke meal (JAM)

L- and D-lactate: The concentration of both L- and D-lactate was tendentially higher in the JAM group in both parts of the stomach ($P > 0.05$). In the small intestine, the concentration of L-lactate was slightly elevated in the CON group compared with the treatment group and *vice versa* regarding the D-lactate concentration (Table 3; $P > 0.05$). Furthermore, the concentration of L-lactate was significantly lower after feeding of JAM in comparison to the control group, exclusively in the ventral colon (Table 3; $P < 0.05$). In the colon transversum, the concentrations of L-lactate as well as D-lactate were tendentially lower in the treatment group (Table 3; $P > 0.05$).

Ammonia: Feeding of JAM elevated the ammonia concentration predominantly in the stomach (PN, PG and MC) as well as in the ventral colon (Table 3). Particularly in the *Pars glandularis*, the concentration of ammonia was significantly higher in the JAM group in comparison to the control group (Table 3; $P < 0.05$). The concentration of ammonia was tendentially lower in the small intestine, caecum, colon dorsal and colon transversum when JAM was fed (Table 3; $P > 0.05$).

Table 3**:** Concentrations (Lsmeans ± SE) of ammonia and lactate (L-lactate as well as D-lactate) along the gastrointestinal tract of 6 control horses (CON) and 6 horses fed with Jerusalem artichoke meal (JAM)

Item		part of the gastrointestinal tract[1]							
		PN	**PG**	**MC**	**SI**	**CAE**	**CV**	**CD**	**CT**
ammonia	CON[2]	9.7 ± 1.4	6.5[a] ± 0.5	8.5 ± 0.7	8.6 ± 1.3	2.7 ± 0.6	7.6 ± 2.5	10.2 ± 2.1	9.9 ± 1.1
	JAM[3]	12.7 ± 1.4	8.2[b] ± 0.5	9.4 ± 0.7	7.1 ± 1.3	2.4 ± 0.6	13.4 ± 2.5	8.8 ± 2.1	9.7 ± 1.1
L-lactate	CON[2]	17.2 ± 7.9	19.2 ± 6.9	37.2 ± 19.3	4.1 ± 4.2	0.0 ± 0.0	0.0[c] ± 0.0	0.1 ± 0.0	0.2 ± 0.1
	JAM[3]	34.9 ± 9.5	24.9 ± 6.9	13.5 ± 15.7	10.2 ± 3.9	0.0 ± 0.0	0.0[d] ± 0.0	0.0 ± 0.0	0.2 ± 0.1
D-lactate	CON[2]	19.7 ± 6.9	8.8 ± 6.9	22.6 ± 11.6	4.0 ± 7.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1
	JAM[3]	23.6 ± 6.9	27.7 ± 6.9	15.9 ± 9.5	16.0 ± 6.9	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1

[1]part of the gastrointestinal tract: PN = *Pars nonglandularis*; PG = *Pars glandularis*; MC = mixed content (stomach); SI = small intestine; CAE = caecum; CV = colon ventral; CD = colon dorsal; CT = colon transversum

[2]CON = control group fed with maize cob meal without grains

[3]JAM = treatment group supplemented with Jerusalem artichoke meal

[a-d]Different superscripts indicate with $P < 0.05$ different means between CON and JAM group horses for a given segment of the digestive tract.

4. Discussion

Feed analysis indicate that the horsed received 199 g of mono- and dimeric sugars but only 466 g of fructans *via* 1 kg of the commercial JAM product (as fed basis) being 161 g/kg lower than declared. From that, the horses did not have ingested as much prebiotic active substances as it is recommended to be prebiotic (JULLIAND and ZEYNER, 2013) and thus was aimed in the study. On the one hand, this has probably influenced the outcome of the study. On the other hand, differences between assumed and realized contents of inulin-type fructans in the additive in question may have biased former studies too. The authors of the resent study are not aware of analytical control of the fructan content in the majority of studies from the literature. To overcome the problem analytical control might further be an issue for both science and practical use. The amount of starch intake was calculated based on feedstuff analysis before the study. During the experiment, the pooled sample (oat grains) was analyzed once again and a higher starch content was measured. Therefore, the amount of starch intake was 1.19 g/ kg BW x d^{-1} instead of 1 g starch per kg BW and day.

Nevertheless, the horses consumed the moistened concentrates quite well during the entire study. The stomach was full of digesta after euthanasia and the stomachs' DM content measured in the recent study is in the range of what is reported from the literature (COENEN, 1992). Despite no significant differences were noticed the DM content in nearly all parts of the gastrointestinal tract was numerically higher when JAM was fed which coincided with also higher total concentrations of SCFA. Elevated SCFA might have triggered water absorption probably at least in the terminal gut. An argument against this explanation is the DM content in the colon transversum being just there higher in CON vs. JAM horses.

Feeding of prebiotics aimed to elevate the microbial activity associated with an increase of microbial fermentation products predominantly in the hindgut (SAMANTA et al., 2013). Contrary, the results of the present study indicate that an elevated microbial metabolism already exists in the foregut following JAM intake. Feeding of JAM increased the total amounts of SCFA in both parts of the stomach. Interestingly, the percentages of acetic acid of total SCFA were almost similar in both parts of the stomach (*Pars nonglandularis* and *Pars glandularis*) after feeding of JAM, but both the total amount and the percentage of *n*-butyric acid as well tended to increase with coincident reduction of the percentage of propionic acid. *N*-butyric acid was reported

to have several beneficial impacts on the gut epithelium e.g. energy source for the colonic epithelial cells, activation of mucin release, regulation of cell proliferation and apoptosis (PLOEGER et al., 2012) primarily in the hindgut. In the equine large intestine, butyrate transport can be mediated by the monocarboxylate transporter 1 (MCT 1) and inhibited through luminal acetate, propionate and lactate concentrations of about 20 mmol/L (NEDJADI et al., 2014). As opposed to this, the impact of *n*-butyric acid in the stomach seems to be detrimental. In an *in vitro* study with tissues from the nonglandular part of the equine stomach, *n*-butyric acid significantly decreased the mucosal barrier function at a concentration of 60 mmol/L and in combination with a low pH of 4.0 or even 1.5 (NADEAU et al., 2003a). At least pH values around 4 are normally reached in the stomach content of horses even rather on hay- than concentrate-based diets (COENEN, 1992) as it was provided here. The pH levels measured in the recent study were actually between 4.4 and 3.4 in the nonglandular and glandular region, respectively, of the stomach. Therefore, higher amounts of *n*-butyric acid together with a low pH might have been an issue in the pathogenesis of gastric ulcers related to the nonglandular part of the equine stomach. The horses in the recent study did however not exhibit gastrointestinal disturbances or any other health discomfort during the study period. Post mortem observation of the stomach wall, however, did also not indicate that 3 weeks feeding of JAM in a dose chosen here causes mucosal lesions in any part of the organ. The only horse with mucosal lesions in the stomach belonged to the control group. It needs, however, be taken into account that the measurements were performed in digesta sampled approximately 1 hour after concentrate intake. This means that the availability of rapidly fermentable carbohydrates was rather high at this time point, and thus the concentration of organic acids might be as well. The subsequent time course over the day has probable mitigate this. It is further imaginable that the 3 weeks lasting adaptation period was not long enough for a clear impact of the diet on the gastric mucosa health. The higher concentrations of *n*-butyric acid in the stomach might be a result of two potential mechanisms. Firstly, the supplementation of prebiotic compounds can have increased the amount of butyric acid producing bacteria like *Sarcina spp.* or *Clostridium spp*. (COSTA et al., 2015). Consequently, the elevated microbial metabolism due to the increased availability of specific substrates would lead to an overall increased content of fermentation products, particularly *n*-butyric acid. Secondly, the increased concentration of this particular

SCFA might be a result of interconversion reactions. Results from an *in vitro* study revealed that there is a considerable contribution of interconversion reactions from extracellular acetate as well as lactate to butyrate after adding oligo-fructose as substrate (MORRISON et al., 2006). The present results indicate a tendency for higher contents of L-lactate as well as of D-lactate especially in both parts of the stomach. There was also a tendency for higher contents of *n*-butyric acid which was notably noticed in the *Pars nonglandularis*, but only a slight increase in the *Pars glandularis.* The dominant bacteria in the horse stomach were found to be *Lactobacilli spp.*, *Sarcina spp.* and *Streptococci spp.* (COSTA et al., 2015). Consequently, the elevated metabolism of the dominant *Lactobacilli spp.* might result in a higher concentration of lactate in the stomach, particularly in the *Pars nonglandularis*. This might enhance other bacteria (like *Sarcina spp.*) to convert lactate to *n*-butyric acid leading to an overall increased amount of this intrinsic SCFA.
Moreover, the results indicated a tendential increase of the content of iso-valeric acid in the *Pars glandularis* and only a slight increase in the *Pars nonglandularis*. *In vitro* studies with nonglandular tissue samples revealed a decrease in the barrier function after the incubation with *iso*-valeric acid (60 mmol/L) at pH ≤ 7.0 (NADEAU et al., 2003b). *Iso*-types of SCFA indicate microbial proteolysis which is in good increment with the higher ammonia contents in the stomach digesta following JAM feeding in this study. Further, branched-chained SCFA are not rapidly absorbable in the nonglandular part of the stomach (NADEAU et al., 2000), which might result in a further accumulation in the glandular part of this organ.
Prebiotics are used to stimulate predominantly the autochthonous microflora as well as their metabolism in the hindgut (SAMANTA et al., 2013). In contrast to studies with scFOS fed in different dosages (BERG et al., 2005; RESPONDEK et al., 2008), the present results indicated a much lesser impact of JAM on the microbial fermentation products in the hindgut than expected. Already in a former study fermentation gases in the exhaled air indicated that inulin started to be fermented in more distal parts of the horses' gut (COENEN et al., 2006). In the current study, feeding of JAM was accompanied by lower concentrations of SCFA and lactate (L- and D- isomer) in the caecum. An increased content of L-lactate was noticed after feeding of scFOS (30 g/d x horse^{-1}, over a 21 d period), but there was no impact on the concentration of total SCFA (RESPONDEK et al., 2008). In the ventral colon, the content of *n*-butyric acid as well as acetic acid were elevated and the concentration of L-lactate was

significantly reduced in the recent study which needs to be assessed rather fortunately in this part of the digestive tract. In contrast to this, the supplementation of scFOS had no impact on the concentration of SCFA and decreased the amount of D-lactate but not L-lactate in the colon. In the study of Respondek et al. (2008), horses (7 years old) supplemented with scFOS were fed a roughage to concentrate ratio of approximately 30:70. The horses in the current study received a *vice versa* ratio of approximately 85:15. Therefore, apart from the dosage of the prebiotic, the composition of the basal ration might be relevant for the effects of the prebiotic especially in the hindgut. Diets containing more than 50 % of concentrate can lead to an augmented concentration of SCFA in the hindgut, so that the prebiotic effect might be less pronounced (JULLIAND et al., 2001).

Furthermore, feeding of JAM resulted in a tendential increased concentration of ammonia in the *Pars nonglandularis* and a significantly elevated content in the *Pars glandularis*. This might be a result of an increased metabolic activity of several proteolytic and/or ureolytic microorganisms in the stomach as mentioned above. Some studies indicated the highest proteolytic activity primarily in the small intestine of horses (KERN et al., 1974) but more recent results revealed the presence of proteolytic active bacteria like *Pseudomonas spp.* equally in the stomach (COSTA et al., 2015). Due to the low pH in the stomach, urease seems to be inactive but the increased amount of ammonia might be a result of the contribution of intracellular gastric urease originated from bacteria (HECKER, 1971; KORNBERG and DAVIES, 1955). Elevated amounts of ammonia were also noticed in the hindgut particularly in the ventral colon after feeding of JAM. Redundant amounts of ammonia could either be excreted *via* feces or be absorbed and converted to non-proteinogenic amino acids or urea in the liver (HECKER, 1971; MARTIN et al., 1996).

Although the supplementation of JAM increased the concentration of SCFA and lactate predominantly in the stomach, no pH differences between both groups were recorded. This might be based upon the somewhat higher elevated concentration of ammonia in the stomach following JAM feeding, which acts as puffer therefore counteracts a pH decrease. Particularly in human being and may be other target animals such as pigs feeding of prebiotics aims, among others, to decrease the pH predominantly in the hindgut and thus to reduce the chance of pathogens to colonize the digestive tract (VAN LOO, 2004). The present results indicated no decreasing pH in the hindgut related to only a slight increase of organic acids as acidifying

fermentation products and in some anatomical regions a concomitant ammonia increase.

In the recent study, the amount of prebiotics that actually might have reached the hindgut seemed to be much lesser than required and did not lead to a significant elevation of the microbial activity. An elevation of the dose, however, may increase the risk for pronounced microbial fermentation although in the stomach with subsequent negative impact on the mucosal health and can therefore not unconditionally be recommended. This issue needs to be investigated further as well as probable impacts of the chain length of individual fructans in the prebiotic in question, which are partly responsible for the rapidness of degradation. At last but not least, future galenic treatment might ensure prebiotics to be fermented first in the hindgut where they actually should be decomposed to unfold their positive effects. When working on such a strategy one should consider that apparently prebiotic doses recommended for practical use (JULLIAND and ZEYNER, 2013) unavoidably included that part of prebiotic substances that have been decomposed by both microbial fermentation and acidic hydrolysis particularly in the stomach of horses. Thus, the situation when the full prebiotic dose as recommended by JULLIAND and ZEYNER (2013) would actually reach the caecum needs to be investigated carefully.

5. Conclusions

Fructo-oligosaccharides + inulin originating from Jerusalem artichoke meal fed to horses in an apparently prebiotic dosage might cause an increased microbial activity and equally elevated amounts of fermentation products predominantly in the stomach. Although increased acidity represents a risk factor for gastric ulcers this effect needs to be discussed carefully with respect to particular SCFA, the elevated ammonia concentration and the fairly stable pH value as overall result. The impact of feeding the prebiotic compound in the dosage applied here on the hindgut might be much lesser pronounced than expected.

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References

Berg, E. L.; Fu, C. J.; Porter, J. H.; Kerley, M. S. (2005): Fructooligosaccharide supplementation in the yearling horse: Effects on fecal pH, microbial content and volatile fatty acid concentration. *Journal of Animal Science* 83: 1549–1553.

Coenen M. (1992): Beobachtungen zum Vorkommen von Magenulcera beim Pferd. *Pferdeheilkunde* (Sonderausgabe): 188-191.

Coenen, M.; Mößeler, A.; Vervuert, I. (2006): Fermentative gases in breath indicate the Inulin and starch start to be degraded by microbial fermentation in the stomach and small intestine of the horse in contrast to Pectin and Cellulose. *The Journal of Nutrition* 136: 2108S–2110.

Conway, E. J.; Byrne, A. (1933): An absorption apparatus for the micro-determination of certain volatile substances: the micro-determination of ammonia. *Biochemical Journal* 27: 419–429.

Costa, M. C.; Silva, G.; Ramos, R. V.; Staempfli, H. R.; Arroyo, L. G.; Kim, P.; Weese, J. S. (2015): Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. *Veterinary journal* 205: 74–80.

De Fombelle, A.; Julliand, V.; Drogoul, C.; Jacotot, E. (2001): Feeding and microbial disorders in horses: 1-effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities. *Journal of Equine Veterinary Science* 21: 439–445.

Gesellschaft für Ernährungsphysiologie (Eds.; 2014): Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere. Nr. 11,Empfehlungen zur Energie- und Nährstoffversorgung von Pferden. DLG-Verlag, Frankfurt (Main).

Hecker, J. F. (1971): Ammonia in the large intestine of herbivores. *British Journal of Nutrition* 26: 135-145.

Henry, H.; Marmy Conus, N.; Steenhout, P.; Béguin, A.; Boulat, O. (2012): Sensitive determination of D-lactic acid and L-lactic acid in urine by high-performance liquid chromatography-tandem mass spectrometry. *Biomedical chromatography: BMC* 26: 425–428.

Julliand, V.; Fombelle, A. de; Drogoul, C.; Jacotot, E. (2001): Feeding and microbial disorders in horses: Part 3—Effects of three hay: grain ratios on microbial profile and activities. *Journal of Equine Veterinary Science* 21: 543–546.

Julliand, V.; Zeyner, A. (2013): Dietary strategies for optimizing gastro intestinal health: an update on pre- and probiotics. In: Horse Health Nutrition, European Equine Health & Nutrition Congress; 75–84.

Kern, D. L.; Slyter, L. L.; Leffel, E. C.; Weaver, J. M.; Oltjen, R. R. (1974): Ponies vs. Steers: Microbial and chemical characteristics of intestinal. *Journal of Animal Science* 38: 559–564.

Kienzle E.; Zeyner A. (2010): The development of a metabolizable energy system for horses. *Journal of Animal Physiology and Animal Nutrition* 94: e231-e240.

Kornberg, H. L.; Davies, R. E. (1955): Gastric urease. *Physiological Reviews* 35: 169–177.

Longland, A. C.; Byrd, B. M. (2006): Pasture nonstructural carbohydrates and equine laminitis. *Journal of Nutrition* 136: 2099S–2102.

Martin, R. G.; McMeniman, N. P.; Norton, B. W.; Dowsett, K. F. (1996): Utilization of endogenous and dietary urea in the large intestine of the mature horse. *British Journal of Nutrition* 76: 373–386.

Milinovich, G. J.; Trott, D. J.; Burrell, P. C.; van Eps, A. W.; Thoefner, M. B.; Blackall, L. L.; Al Jassim, R. A. M.; Morton, J. M.; Pollit, C. C. (2006): Changes in equine hindgut bacterial populations during oligofructose-induced laminitis. *Environmental Microbiology* 8: 885–898.

Morrison, D. J.; Mackay, W. G.; Edwards, C. A.; Preston, T.; Dodson, B., Weaver, L. T. (2006): Butyrate production from oligofructose fermentation by the human faecal flora: what is the contribution of extracellular acetate and lactate? *British Journal of Nutrition* 96: 570–577.

Muhonen, S.; Julliand, V.; Lindberg, J. E.; Bertilsson, J.; Jansson, A. (2009): Effects on the equine colon ecosystem of grass silage and haylage diets after an abrupt change from hay. *Journal of Animal Science* 87: 2291–2298.

Nadeau, J. A.; Andrews, F. M.; Mathew, A. G.; Argenzio, R. A.; Blackford, J. T.; Sohtell, M.; Saxton, A. M. (2000): Evaluation of diet as cause of gastric ulcers in horses. *American Journal of Veterinary Research* 61: 784–790.

Nadeau, J. A.; Andrews, F. M.; Patton, C. S.; Argenzio, R. A.; Mathew, A. G.; Saxton, A. M. (2003a): Effects of hydrochloric, acetic, butyric, and propionic acids on pathogenesis of ulcers in the nonglandular portion of the stomach of horses. *American Journal of Veterinary Research* 64: 404–412.

Nadeau, J. A.; Andrews, F. M.; Patton, C. S.; Argenzio, R.A.; Mathew, A.G., Saxton, A. M. (2003b): Effects of hydrochloric, valeric and other volatile fatty acids on pathogenesis of ulcers in the nonglandular portion of the stomach of horses. *American Journal of Veterinary Research* 64: 413–417.

Nedjadi, T.; Moran, A. W.; Al-Rammahi, M. A.; Shirazi-Beechey, S. P. (2014): Characterization of butyrate transport across the luminal membranes of equine large intestine. *Experimental Physiology* 99: 1335–1347.

Pavis, N.; Chatterton N. J.; Harrison, P. A.; Baumgartner, S.; Praznik; Boucard, J.; Prud´homme, M. B. (2001): Structure of fructans in roots and leaf issues of Lolium perenne. *New Phytologist* 150 (1): 83-95.

Ploeger, S.; Stumpff, F.; Penner, G. B.; Schulzke, J.-D.; Gäbel, G.; Martens, H.; Shen, Z.; Günzel, D.; Aschenbach, J. R. (2012): Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Annals of the New York Academy of Sciences* 1258: 52–59.

Respondek, F.; Goachet, A.– G.; Rudeaux, F.; Julliand, V. (2007): Effects of short-chain fructo-oligosaccharides on the microbial and biochemical profile of different segments of the gastro-intestinal tract in horses. *Pferdeheilkunde* 23: 146-150.

Respondek, F.; Goachet, A. G.; Julliand, V. (2008): Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. *Journal of Animal Science* 86: 316–323.

Samanta, A. K.; Jayapal, N.; Senani, S.; Kolte, A. P.; Sridhar, M. (2013): Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. *Sociedade Brasileira de Microbiologia* 44: 1–14.

Scheijen, J. L. J. M.; Hanssen, N. M. J.; van de Waarenburg, M. P. H.; Jonkers, D. M. A. E.; Stehouwer, C. D. A.; Schalkwijk, C. G. (2012): L(+) and D(-) lactate are increased in plasma and urine samples of type 2 diabetes as measured by a simultaneous quantification of L(+) and D(-) lactate by reversed-phase liquid chromatography tandem mass spectrometry. *Experimental diabetes research*: doi:10.1155/2012/234812.

Van Eps, A. W.; Pollit, C. C. (2006): Equine laminitis induced with oligofructose. *Equine Veterinary Journal* 38: 203–208.

Van Eps, A. W.; Pollit, C. C. (2009): Equine laminitis model: Lamellar histopathology seven days after induction with oligofructose. *Equine Veterinary Journal* 41: 735–740.

Van Loo, J. (2004): The specificity of the interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy. *Nutrition Research Reviews* 17: 89–98.

Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA; 2015): Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch). Band III: Die chemische Untersuchung von Futtermitteln (Ergänzungslieferungen), VDLUFA Verlag, Darmstadt.

Voigt, J.; Steger, H. (1967): Zur quantitativen Bestimmung von Ammoniak, Harnstoff und Ketokörpern in biologischem Material mit Hilfe eines modifizierten Mikrodiffusionsgefäßes. *Archiv für Tierernährung* 17: 289–293.

Zeyner, A.; Geißler, C.; Dittrich, A. (2004): Effects of hay intake and feeding sequence on variables in faeces and faecal water (dry matter, pH value, organic acids, ammonia, buffering capacity) of horses. *Journal of Animal Physiology and Animal Nutrition* 88: 7–19.

Zeyner A.; Kirchhof S.; Susenbeth A.; Südekum K.-H.; Kienzle E. (2015a): A new protein evaluation system for horse feed from literature data. *Journal of Nutritional Sciences:* doi: 10.1017/jns.2014.66.

Zeyner, A.; Gefrom, A.; Hillegeist, D.; Sommer, M.; Greef, J. M. (2015b): Contribution to the method of sugar analysis in legume grains for ensiling- A pilot study. *International Journal of Scientific Research in Science and Technology* 1 (2): 1–22.

4.2 Paper II

Modification of the equine gastrointestinal microbiota by Jerusalem artichoke meal supplementation

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Abstract

The objective of this study was to investigate the impact of natural prebiotic active compounds on the microbial composition in different regions of the equine gastrointestinal tract. Twelve adult horses (body weight [bwt] 534 ± 64.5 kg; age 14 ± 7.5 years) were randomly divided into two feeding groups. Six horses received a basal diet consisting of 1.5 kg hay/100 kg bwt x d^{-1} and oat grains equal to 1.19 g starch/kg bwt x d^{-1}, supplemented with Jerusalem artichoke meal providing prebiotic fructooligosaccharides + inulin in a quantity of 0.15 g/kg bwt x d^{-1}. The remaining horses received a placebo added to the basal diet. The horses were fed for 21 d and euthanized at the end of the feeding period. Digesta samples from different parts of the gastrointestinal tract were taken, DNA extracted and the V1-V2 region of the 16S rRNA gene amplified. Supplementation with the prebiotic increased the relative abundance of *Lactobacillus* ($P < 0.05$), with a concurrent reduction of the relative abundance of *Streptococcus* mainly in the stomach ($P < 0.05$). In the hindgut, the supplemental prebiotic also increased the relative abundance of *Lactobacillus* but further reduced the relative abundance of fibrolytic bacteria, specifically the unclassified members of the families *Lachnospiraceae* ($P < 0.05$) and *Ruminococcaceae*. The relative abundance of the genus *Ruminococcus* increased solely in the caecum and colon transversum. Overall, the addition of the prebiotic significantly increased the diversity in nearly all parts of the gastrointestinal tract ($P < 0.05$). The feeding of this natural prebiotic compound to horses had an impact on the microbial community in the entire gastrointestinal tract. Furthermore, the effect on the bacterial community in the foregut (especially the stomach) was more pronounced in comparison to the effect in the hindgut. Therefore, the impact on stomach health should be carefully considered.

Key words: prebiotic; horse; nutrition; gastrointestinal tract; microbiota

1. Introduction

The gastrointestinal tract (GIT) of horses is a large and complex ecosystem containing a broad range of different microorganisms (DICKS et al., 2014; JULLIAND and GRIMM, 2016). Particularly in the large intestine, horses harbor a specialized microbial community (e.g.; *Ruminococcus* spp.*, Streptococcus* spp.*, Lactobacillus* spp.*,* and *Enterococcus* spp.) which is responsible for fermentation and the provision of fermentative products, e.g. short chain fatty acids (SCFAs; SANTOS et al., 2011). The stomach also harbors a specific mucosal, as well as a luminal microbial community mainly consisting of *Firmicutes* (e.g., *Lactobacillus* spp., 10^8-10^9 CFU/mL), *Bacteroidetes* (e.g. *Prevotella* spp.) and *Proteobacteria* (e.g., *Actinobacillus* spp.) (AL JASSIM and ANDREWS, 2009; PERKINS et al., 2012; DICKS et al., 2014). The microbiota of the small intestine (duodenum, jejunum and ileum) is inhabited by microbial groups (10^6-10^7 CFU/mL) (DICKS et al., 2014), which are known for their proteolytic activity (MACKIE and WILKINS, 1988), mainly species that belong to classes *Bacilli*, *Clostridia* and *Gammaproteobacteria* (COSTA et al., 2015).

The abundance and large diversity of microorganisms in the GIT is essential for efficient fermentation, particularly of nutrients not degradable by endogenous enzymes. Because of its structural complexity and size, the equine digestive tract is prone to disturbances caused by biotic and/or abiotic stressors (JULLIAND and GRIMM, 2017), which ultimately may lead to the development of gastrointestinal-derived diseases (e.g., colic or laminitis) (MILINOVICH et al., 2010; DESTREZ et al., 2015; SCHOSTER et al., 2016). Prebiotics can help to stabilize the predominantly autochthonous large intestine microbiota by providing substrates for their metabolism and therefore might counteract the development of gastrointestinal-derived diseases (ROBERFROID et al., 2010). In the nutrition of horses, inulin-type fructans, such as fructo-oligosaccharides (FOS), or inulin itself, are predominantly used as prebiotic substances (JULLIAND and ZEYNER, 2013). The impact on the animal depends mostly on the level of intake of the prebiotic compound and can have either negative or health-promoting effects (LONGLAND and BYRD, 2006; MILINOVICH et al., 2006; VAN EPS and POLLIT, 2006; RESPONDEK et al., 2008). Fecal samples are used in most studies to assess the impact of prebiotics on the large intestine microbiota. Because of the need for invasive sampling and/or sacrifice, other parts of the equine GIT are not routinely taken into consideration, and hence, the overall impact on the

digestive tract is often not described. Furthermore, literature (COSTA and WEESE, 2012a; SCHOSTER et al., 2013) showed that fecal samples are inadequate to draw conclusions about the microbial composition in the proximal parts of the equine digestive tract. Hitherto, some authors speculated from in vivo or ex vivo results that the fermentation of inulin-type fructans, as well as inulin itself, begins in the foregut (COENEN et al., 2006; GLATTER et al., 2016) and particularly the stomach (GLATTER et al., 2016), which might cause negative effects concerning stomach health. In vitro studies support the suggestion that the digestion of fructans in particular (in terms of acid hydrolysis) might not occur in the large intestine alone (as previously assumed; VOSMER et al., 2012; INCE et al., 2014; STRAUCH et al., 2017). Consequently, we hypothesized that i) the feeding of FOS + inulin as active prebiotic ingredients originating from Jerusalem artichoke meal has an impact on the microbiota of the entire GIT and that ii) prebiotic compounds act as possible substrates for the microbiota in the stomach. The objective of the study was to evaluate the impact of supplementation with prebiotic FOS + inulin derived from Jerusalem artichoke meal to a hay-based diet on the microbial composition in different regions of the equine digestive tract.

2. Material and Methods

The Animal Welfare Commissioner of the University of Veterinary Medicine Hannover approved the experimental procedure in accordance with the German Animal Welfare law (permit number: DFG CE186/2-1).

Animals, Diet and Sample Collection

Twelve adult, healthy warmblood horses (body weight [bwt] 534 ± 64.5 kg; age 14 ± 7.5 years) consisting of 10 mares, one stallion and one gelding with no known history of gastrointestinal diseases were used in this study (Table 1). The horses were slaughter animals (killed for reasons other than gastrointestinal-derived diseases) and purchased for this study. The sample size (6 animals per group) was chosen to guarantee, in accordance with the power of analysis of the superior DFG project, a statistically assured analysis. Horses were housed in individual boxes (bedding: wood shavings) with ad libitum access to tap water and a salt block consisting of sodium chloride. The horses received meadow hay (1.5 kg/100 kg bwt x d^{-1}; Table 2) and crushed oat grains (1.2 g starch/kg bwt x d^{-1}; Table 2) in two equal meals per day

(9 a.m. and 3 p.m.) during a 3-week adaptation period to cover the energy needs according to GfE (2014) as quote in advance by COENEN et al. (2011). Additionally, horses were supplemented either with FOS + inulin (originating from Jerusalem artichoke meal [JAM], LIVEN GmbH, Zossen, Germany; Table 2) with the intention to reach the recommended prebiotic dosage of 0.2 g/kg bwt x d^{-1} (JULLIAND and ZEYNER, 2013) or with an equal amount of maize cob meal without grains as placebo (CON; Table 2). To avoid selective feed intake and the aspiration of fine particles, water was added (6 % of the final volume) to the concentrated meal (w/w) prior to feeding. The horses were randomly distributed to either the treatment (n = 6; JAM) or the control group (n = 6; CON).

Table 1: Population characteristics of the horses

group	gender	age [years]	body weight [kg]
CON	female	3	395
	male	5	560
	female	21	461
	female	12	528
	female	18	570
	female	23	582
JAM	female	11	500
	male castrated	5	590
	female	19	548
	female	20	624
	female	11	570
	female	24	480

CON = placebo group; JAM = Jerusalem artichoke meal

The feed samples were ground to pass through a 1 mm sieve of a standard laboratory sample mill. For starch analysis, the cereal grains were pulverized using a ball mill. The dry matter (DM) content, crude ash (CA), crude protein (CP), acid ether extract (AEE), crude fiber (CF), the Van Soest detergent fibers and sugar were determined according to the official German key book for feed analysis (NAUMANN and BASSLER, 1976; methods no. 3.1, 4.1.1, 5.1.1 B, 6.1.1, 6.5.1, 6.5.2, 6.5.3, 7.1.2 and 8.1). The content of nitrogen-free extracts (NFE) was calculated (NFE = OM –

CP – AEE − CF). Starch was enzymatically determined by the amyloglucosidase method (NAUMANN and BASSLER, 1976; method no. 7.2.5). The contents of individual water-soluble carbohydrates (WSC; glucose, fructose, sucrose, fructans) and the degree of polymerization (DP) of FOS and fructopolysaccharides in feedstuffs and digesta were investigated using HPLC as described by (PAVIS et al., 2001; ZEYNER et al., 2015).

The horses were euthanized at d21 of the feeding period (approximately 1 hour after the morning meal) by a veterinarian using 0.12 mg/kg bwt romifidine as a sedative. A combination of diazepam (0.05 mg/kg bwt) and ketamine (2.2 mg/kg bwt) was used for introducing the anesthesia. The horses were euthanized with pentobarbital (60 mg/kg bwt). Immediately post mortem, luminal digesta were collected from 7 different parts of the GIT (stomach: *pars nonglandularis*, PN; *pars glandularis*, PG; small intestine, SI; caecum, CAE; colon: ventrale, CV; dorsale, CD; transversum, CT) and stored at - 20 °C until further analysis for proximate nutrients (e. g., starch, WSC, DP) and the microbial composition.

DNA Extraction

Samples (0.25 g) from the different parts of the GIT were weighed and homogenized using sterile 2.0 ml screw-cap tubes containing 0.5 g of 0.1 mm zirconia beads, 4 glass beads (3 mm), and 1.0 ml lysis buffer (500 mM sodium chloride, 50 mM Tris-hydrochloric acid at pH 8, 50 mM EDTA, 4 % SDS and double-distilled water (ddH_2O)). Samples were homogenized by double bead beating (5.5 ms for 3 x 1 min) at room temperature (RT) using a bead beater (Precellys 24, Bertin Technologies, France). After the homogenization steps, samples were heated at 95 °C for 15 min and centrifuged at 4 °C for 5 min (full speed; 15,000 rpm). After cell lysis, nucleic acids were precipitated by adding 260 µl of 10 M ammonium acetate, isopropanol (1 mL) and 500 µl ethanol (70 % v/v). Subsequently, the nucleic acid pellet was dissolved in 200 µl TE buffer (containing 10 mM Tris-hydrochloric acid, 1 mM EDTA and ddH_2O). Finally, DNA was extracted using the commercial QIAmp DNA stool Mini Kit (Qiagen Ltd., UK) according to the manufacturer`s manual. The DNA content was measured using a NanoDrop 2000 (Thermo Fisher Scientific Inc., USA)..

PCR Amplification

Samples were amplified in two steps as described previously (TIAN et al., 2016). Specifically, the first PCR was used to amplify the V1-V2 variable region of the bacterial 16S ribosomal RNA (rRNA) gene using specific linker primers (forward primer: UniTag 1–27F-DegS: GAGCCGTAGCCAGTCTGCGTTYGATYMTGGC TCAG; reverse primer: UniTag 2-338R–I: GCCGTGACCGTGACATCGGCWGCCTC CCGTAGGAGT, UniTag 2–338R–II: GCCGTGACCGTGACACGGCWGCCACCCGT AGGTGT). For each sample, the PCR master mix with a final volume of 20 µl contained 4 µl 5x HF buffer (Fermentas, Thermo Fisher Scientific Inc., USA), 1 µl forward linker primer (UniTag1-27R-DegS, 10 µM), 1 µl reverse linker primer (UniTag2–338R-I + II, 10 µM), 0.4 µl dNTPs (Roche Nederland B.V.), 0.2 µL Phusion Hot start II DNA polymerase (Finnzymes, Thermo Fisher Scientific Inc., USA; 2 U/µl) and 12.4 µl nuclease free water (Promega Corporation, Madison, Wisconsin, USA). Each reaction tube contained 1 µl of DNA template at 10–20 ng/µl. The PCR was performed under the following conditions: hot start at 98 °C for 30 s, followed by 25 cycles of 98 °C for 10 s, 56 °C for 20 s, 72 °C for 20 s and a final extension at 72 °C for 10 min using a Labcycler (Sensoquest, Göttingen, Germany). The PCR product was analyzed using the Lonza Flash Gel™ system (Lonza Group Ltd., USA). In the second PCR, specific barcoded primers (see S1 Table) were used. The master mix (final volume 85 µl consisting of 20 µl 5 x HF buffer (Fermentas, Thermo Fisher Scientific Inc., USA), 2 µl dNTPs (Roche Nederland B.V.), 1 µl Phusion Hot start II DNA polymerase (Finnzymes, Thermo Fisher Scientific Inc., USA; 2 U/µl) and 62 µl nuclease free water) was mixed with 5 µl of the PCR product from the first step PCR and run under the following conditions: heating at 98 °C for 30 s followed by 5 cycles of 98 °C for 10 s, 52 °C for 20 s, 72 °C for 20 s and a final extension at 72 °C for 10 min using also a Labcycler (Sensoquest, Göttingen, Germany).

PCR Purification and Sequencing

PCR products were purified using a HighPrep PCR paramagnetic beads solution (MagBio Genomics Inc., USA). In brief, the PCR tube was mixed with the paramagnetic bead solution, placed on a magnetic separation device and cleaned up using 70 % (v/v) ethanol several times. Finally, the sample was eluted with nuclease-free water, and the DNA content was measured with a NanoDrop 2000 (Thermo Fisher Scientific Inc., USA).

For library preparation, the DNA content of each sample was measured with a fluorometer (Qubit® 2.0 fluorometer, Thermo Fisher Scientific Inc., USA) using a dsDNA BR assay kit. Each library consisted of 46 samples and 2 mock communities serving as internal controls (S1 Table). Library pools were prepared to contain equimolar amounts of sample DNA at 100 ng of each sample per library pool. If the final volume of the library was above 100 µl, the library was concentrated to 20 µl using the paramagnetic beads solution as before. DNA content was measured again with the Qubit® 2.0 fluorometer and set to 100 ng/µl. If necessary, the library was diluted with nuclease-free water to reach a DNA concentration of 100 ng/µl in a final volume of 20 µl. Finally, the samples were sequenced on an Illumina MiSeq sequencer by GATC Biotech AG (Konstanz, Germany).

Bioinformatic and Statistical Analysis

The sequencing output data were analyzed using QIIME software (CAPORASO et al., 2010). Barcode and primer sequences were removed, and the data were checked to identify and remove chimeras by using the chimera slayer (HAAS et al., 2011). The resulting sequences were clustered into OTUs at a discrimination level of 97 % identity by using the SILVA database version 119 (PRUESSE et al., 2007). Each sample was rarefied at a cut-off of 2500 reads, alpha diversity indices (Simpson and Shannon-Wiener), species richness (Menhinick) and beta diversity (Whittaker) were calculated in PAST (version 3.1; HAMMER et al., 2001), and the results were imported into Microsoft Excel for further analysis. Statistical analyses were conducted using SAS 9.4 (SAS Inc., Cary, NC, USA). The nonparametric Wilcoxon rank-sum test was used to compare the relative abundance of microbial taxa in the different parts of the GIT between the two feeding groups. Diversity indices in the several tracts and the population characteristics of both feeding groups were compared with the t-test implemented in PROC mixed. The principal component analysis was conducted in PAST. Differences in the specific parts of the GIT and between the two feeding groups were tested *via* one-way ANOSIM of ranked Bray-Curtis similarity indices. The unweighted UniFrac distances (LOZUPONE and KNIGHT, 2005) were calculated in QIIME (CAPORASO et al., 2010), imported in PAST (HAMMER et al., 2001) and analyzed *via* one-way ANOSIM. Furthermore, the Bonferroni correction was applied on the PCoA data for the different regions of the digestive tract. The

significance level was set at $P < 0.05$. The sequencing data are available at the European Nucleotide Archive (ENA) under the accession number PRJEB31758.

3. Results

General Observations

The feeding group composition was not significantly different between CON and JAM regarding to age and body weight distribution ($P > 0.05$).

All horses accepted the provided feed well and showed no clinical signs of gastrointestinal disturbances throughout the duration of the study. One young mare (age 3 years) belonging to the placebo group showed mucosal lesions alongside the *margo plicatus* in the stomach post mortem.

Diet Components

The chemical analysis of the commercially available JAM meal revealed a much lower content of FOS and inulin than declared (62.7 % declared *vs*. 46.6 % analysed). Therefore, the intake of the prebiotic active compound achieved was lower than calculated (0.15 *vs.* 0.2 g/kg bwt x d^{-1}). Furthermore, the DP was not as high as expected for FOS and inulin (Fig S1).

The chemical composition of the diet components are presented in Table 2. The placebo contained higher concentrations of fibre (CF: 343 g/kg DM, NDF: 799 g/kg DM, ADF: 415 g/kg DM, ADL: 39 g/kg DM) whereas the prebiotic compound contained higher concentrations of water-soluble carbohydrates (e.g. fructose: 63 g/kg DM, sucrose: 122 g/kg DM, fructan: 466 g/kg DM).

Table 2: The analyzed chemical composition of the feedstuffs

nutrient		oat	hay	placebo[1]	prebiotic[2]	diet
DM	g/kg	912	944	927	939	940
CA	g/kg DM	29	52	26	136	50
CP	g/kg DM	124	90	36	63	93
AEE	g/kg DM	49	9	8	6	14
CF	g/kg DM	124	349	343	14	318
NDF	g/kg DM	316	651	799	3[3]	605
ADF	g/kg DM	178	391	415	11[3]	361
ADL	g/kg DM	31	47	39	4[3]	44
glucose	g/kg DM	0	30	9	14	26
fructose	g/kg DM	0	39	10	63	34
sucrose	g/kg DM	11	6	16	122	8
fructan	g/kg DM	0	40	14	466	38
starch	g/kg DM	498	0	0	0	65
ME[4]	MJ/kg DM	12.4	6.6	7.5	11.9	7.5

The analyzed proximate nutrients are ADF (acid detergent fiber), ADL (acid detergent lignin), AEE (acid ether extract), CA (crude ash), CF (crude fiber), CP (crude protein), DM (dry matter) and NDF (neutral detergent fiber). The ME (metabolizable energy) was calculated according to [4].

[1]maize cob meal without grains.

[2]Jerusalem artichoke meal.

[3]The uncommon carbohydrate composition of Jerusalem artichoke meal causes partly paradoxical results in detergent fiber analysis, which is, however, not worthy of note in the diet calculations because of the small quantities of both the prebiotic supplement in the diet and cell wall carbohydrates in the supplement.

[4]Calculated according to KIENZLE and ZEYNER (2010) as recommended by GfE (2014)

Digesta composition of water-soluble carbohydrates including starch

The composition of the digesta from the different parts of the GIT regarding WSC, including starch, is presented in S2 Table. No significant differences were observed between the feeding groups or between the different parts of the gastrointestinal tract. The DP of the digesta content in the stomach is presented in Fig 1. The PN contained 61 % carbohydrates with a DP of 1 - 5 units in the treatment group and 20 % of molecules had a DP of 6 - 10 in the CON group. Longer chains were presented by < 10 % of the entire composition. In the PG, the quantity of the chains with a DP of

1 – 5 decreased slightly to 59 % and the chains with a DP of 6 - 10 increased to 29 %. Equally, in the JAM group, the largest amount of chains contained 1 – 5, as well as 6 – 10, molecules in both parts of the stomach. In contrast to the CON group, the percentage of the shorter chains (DP 1 - 5) in the stomach content of the JAM group increased from 51 % (PN) to 68 % (PG).

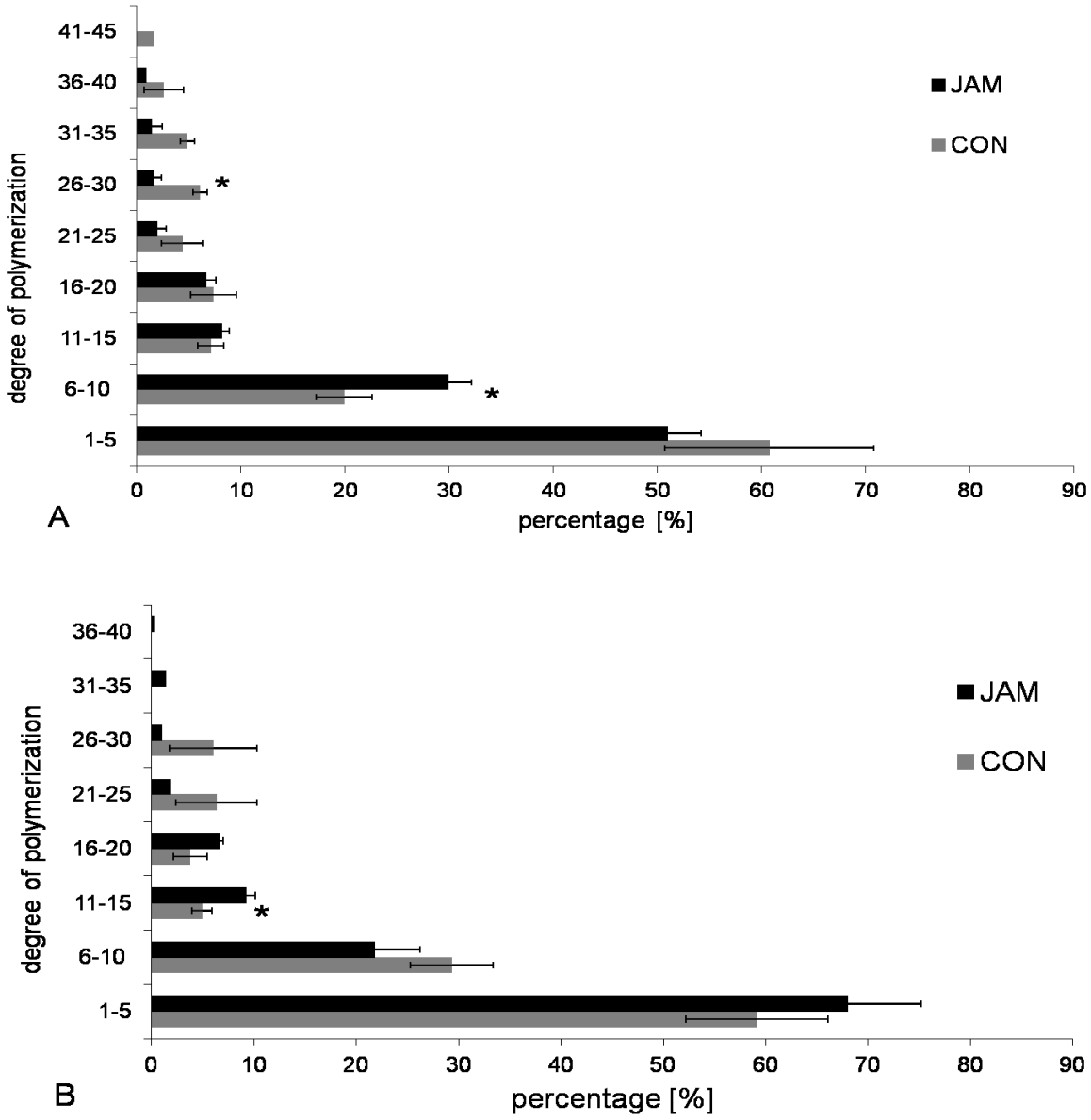


Fig. 1 A/B: Distribution of the degree of polymerization (%, mean ± SE) in the stomach.

The figure presents the degree of polymerization in the *pars nonglandularis* (A) and *pars glandularis* (B) for the CON (placebo) and JAM (Jerusalem artichoke meal) groups. The asterik (*) indicates significant differences ($P < 0.05$).

16S rRNA gene MiSeq sequencing data

The sequencing of the samples generated 2,933.868 reads with a mean total number of bases sequenced per sample of 27,419.327 (min–max: 249–204,888). During downstream analysis in the QIIME pipeline, eight samples with fewer than 2,500 reads per sample were excluded (see S1 Table and S3 Table). Therefore, 2,920.961 reads were retained for further analysis. The plateau of the rarefraction curve (Fig. S2) indicated an adequate sub-sampling size.

Diversity

The calculated diversity indices are presented in Tables 3 and 4. The alpha diversity (Simpson and Shannon-Wiener) indicated a higher diversity in all parts of the GIT for the JAM group in comparison to the CON group ($P < 0.05$). Particularly, in the large intestine (CAE, CV, CD and CT), the mean diversity (Simpson 1-D) was higher in the JAM group than in the CON group ($P < 0.05$). Furthermore, the Simpson evenness index showed a more evenly distributed microbial species in the JAM group compared to the CON group in the large and small intestine (Table 3, $P < 0.05$) but not in the stomach ($P > 0.05$). The species richness (Menhinick) was higher in each part of the digestive tract in the JAM group compared to the CON group. The beta diversity is summarized in Table 4 (for detailed information, see S4 Table). Both feeding groups showed nearly identical low similarity between the digestive compartments. In the JAM group, the species similarity between the PN and CAE/CT as well as CAE and CT were lower compared to the CON group.

Table 3: LSmeans (± SE) of the calculated diversity indices (Simpson and Shannon-Wiener), Simpson´s evenness and species richness (Menhinick) of the two feeding groups in the different parts of the gastrointestinal tract.

item		PN	PG	SI	CAE	CV	CD	CT
Simpson 1-D	CON	0.495 ± 0.005	0.487 ± 0.017	0.514 ± 0.017	0.533 ± 0.003	0.535 ± 0.008	0.523 ± 0.009	0.535 ± 0.009
	JAM	0.589 ± 0.005	0.578 ± 0.016	0.646 ± 0.019	0.722 ± 0.003	0.707 ± 0.008	0.719 ± 0.009	0.713 ± 0.009
	p value	< 0.001	0.0039	0.0006	< 0.001	< 0.001	< 0.001	< 0.001
Shannon-Wiener	CON	0.969 ± 0.022	0.934 ± 0.062	1.125 ± 0.092	1.434 ± 0.043	1.430 ± 0.067	1.328 ± 0.084	1.449 ± 0.076
	JAM	1.089 ± 0.022	1.055 ± 0.057	1.355 ± 0.101	2.012 ± 0.055	1.868 ± 0.067	1.975 ± 0.084	1.941 ± 0.076
	p value	0.0052	0.1822	0.1274	0.0002	0.0010	0.0003	0.0010
Simpsons evenness	CON	0.290 ± 0.045	0.285 ± 0.024	0.278 ± 0.021	0.164 ± 0.011	0.177 ± 0.009	0.176 ± 0.009	0.185 ± 0.010
	JAM	0.403 ± 0.045	0.334 ± 0.022	0.400 ± 0.023	0.320 ± 0.014	0.299 ± 0.009	0.282 ± 0.009	0.290 ± 0.010
	p value	0.1096	0.1671	0.0040	0.0001	< 0.001	< 0.001	< 0.001
Menhinick	CON	5.539 ± 0.594	5.201 ± 0.258	6.437 ± 0.661	14.926 ± 0.924	13.767 ± 0.816	12.900 ± 1.542	13.493 ± 0.603
	JAM	5.667 ± 0.866	6.253 ± 0.430	7.083 ± 0.585	16.797 ± 1.514	15.695 ± 1.352	18.238 ± 0.924	17.333 ± 1.282
	p value	0.9055	0.0780	0.7825	0.3015	0.2500	0.0141	0.0219

The following parts of the GIT were examined: CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG (*pars glandularis*) PN (*pars nonglandularis*) and SI (small intestine) in the CON (placebo group fed with maize cob meal without grains) and in the JAM group (Jerusalem artichoke meal). The p value refers to the difference of means between both feeding groups within the calculated indices.

Table 4: LSmeans (± SE) of the calculated beta diversity index (Whittaker) of the two feeding groups in relation to the different regions of the gastrointestinal tract.

compared GIT	CON	JAM	p value
PN - PG	0.273 ± 0.073	0.302 ± 0.049	0.7427
PN - SI	0.469 ± 0.038	0.387 ± 0.093	0.4381
PN - CAE	0.626 ± 0.058	0.661 ± 0.075	0.7247
PN - CV	0.616 ± 0.066	0.618 ± 0.042	0.9845
PN - CD	0.568 ± 0.081	0.681 ± 0.062	0.2998
PN - CT	0.620 ± 0.101	0.698 ± 0.043	0.4927
PG - SI	0.425 ± 0.034	0.454 ± 0.069	0.7077
PG - CAE	0.677 ± 0.031	0.670 ± 0.044	0.8915
PG - CV	0.674 ± 0.018	0.641 ± 0.021	0.2628
PG - CD	0.644 ± 0.056	0.708 ± 0.038	0.3565
PG - CT	0.720 ± 0.017	0.711 ± 0.039	0.8442
SI - CAE	0.667 ± 0.019	0.653 ± 0.022	0.6494
SI - CV	0.671 ± 0.012	0.596 ± 0.047	0.1275
SI - CD	0.672 ± 0.038	0.674 ± 0.036	0.9817
SI - CT	0.688 ± 0.023	0.695 ± 0.039	0.8756
CAE - CV	0.195 ± 0.047	0.238 ± 0.055	0.5863
CAE - CD	0.218 ± 0.047	0.233 ± 0.054	0.8374
CAE - CT	0.324 ± 0.019	0.313 ± 0.027	0.7361
CV - CD	0.274 ± 0.055	0.237 ± 0.027	0.5542
CV - CT	0.274 ± 0.028	0.305 ± 0.025	0.4339
CD - CT	0.283 ± 0.050	0.234 ± 0.038	0.4547

The following GIT regions were examined: CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG (*pars glandularis*) PN (*pars nonglandularis*) and SI (small intestine) in the CON (placebo group fed with maize cob meal without grains) and in the JAM group (Jerusalem artichoke meal). Each part of the GIT was compared with the other parts of the GIT. The p value refers to the differences of means within the individual parts of the gut.

Microbial composition in relation to the different intestinal sections examined

Fig 2 illustrates the within-compartment similarity along the equine gastrointestinal tract. Axis 1 describes 46.6 % of the total variation and axis two 9.3 % of the variation. The principal component analysis indicates a differentiation of the specific microbial composition in the distinct parts of the GIT ($P < 0.05$; Table 5). Nonetheless, the analysis revealed no obvious separation of the microbial community in the JAM group in comparison to the CON group ($P > 0.05$).

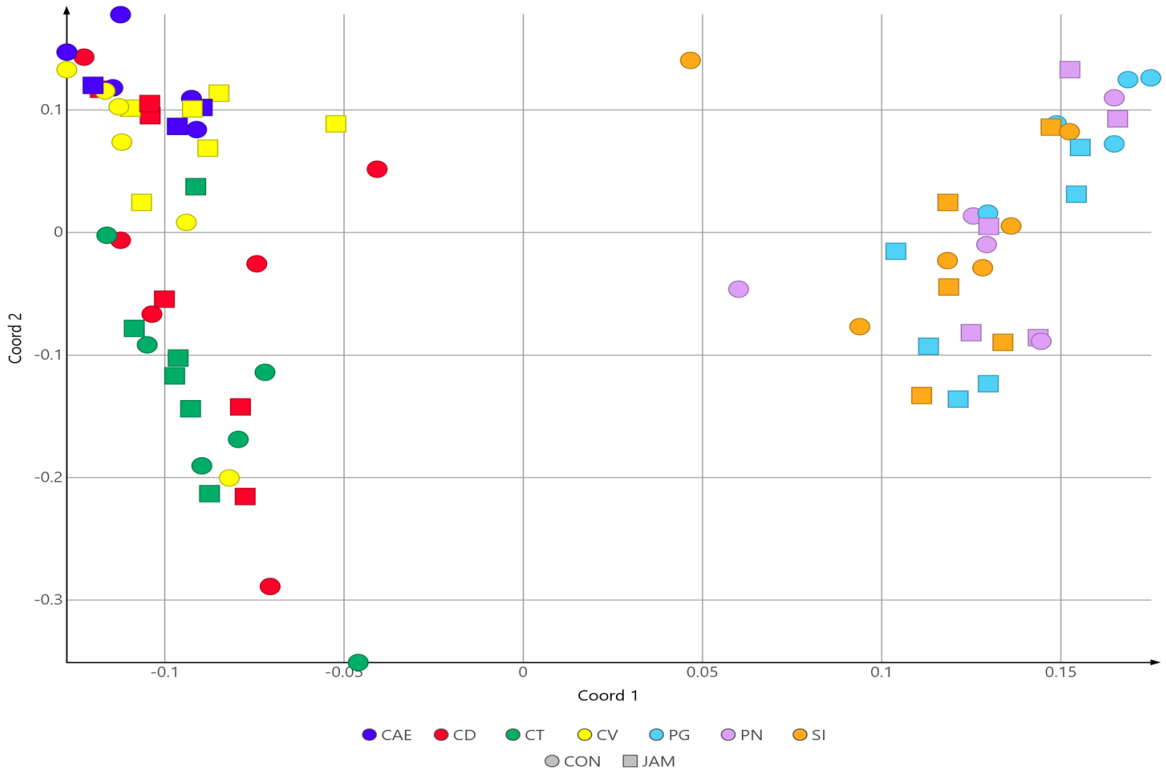


Fig. 2: Principal component analysis (PCoA) of the microbial composition in the digestive tract.

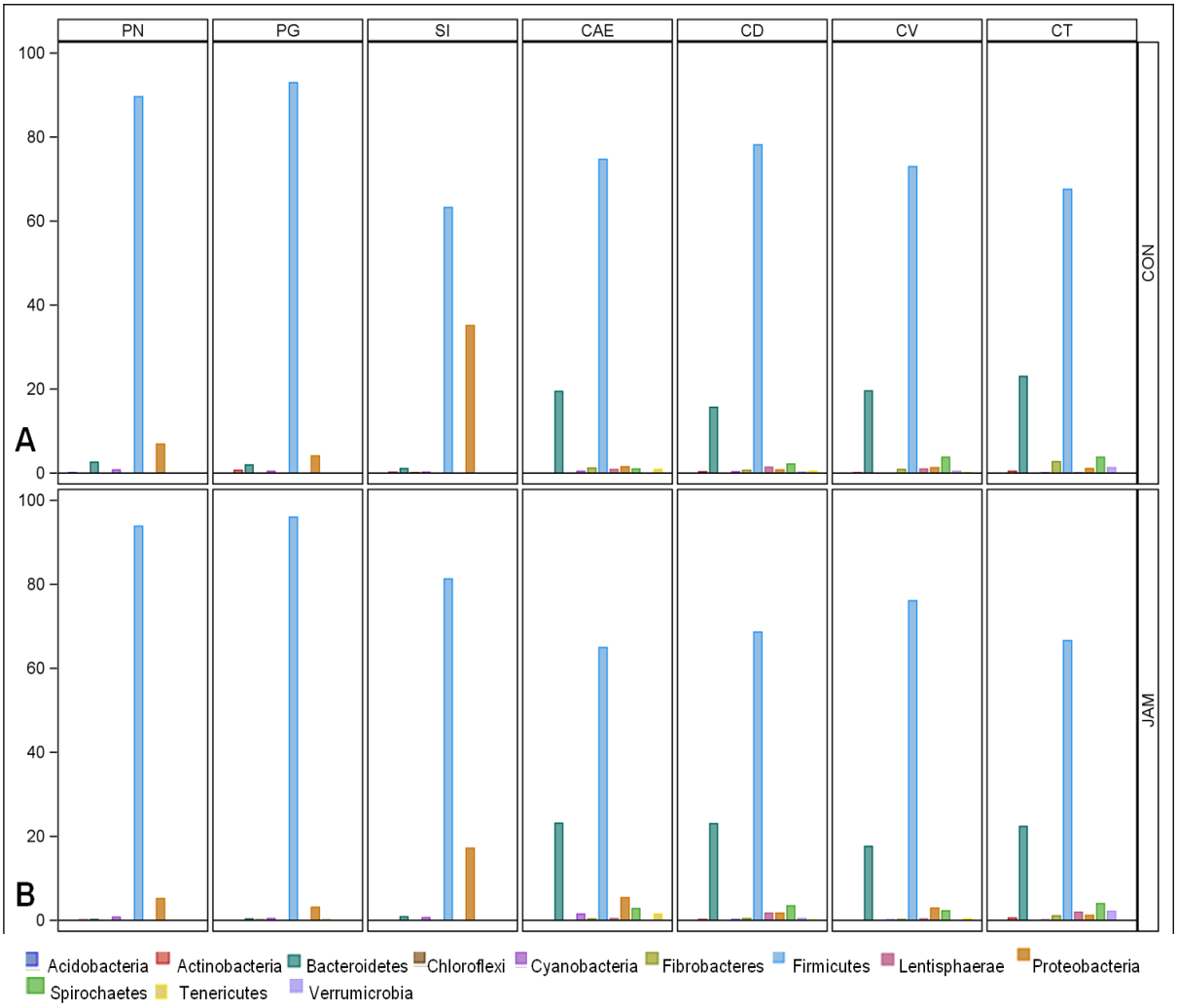
Unweighted UniFrac distance measures were used for the PCoA. The following parts of the GIT were examined: CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG (*pars glandularis*) PN (*pars nonglandularis*) and SI (small intestine) in the CON group (placebo group fed with maize cob meal without grains) and in the JAM group (Jerusalem artichoke meal).

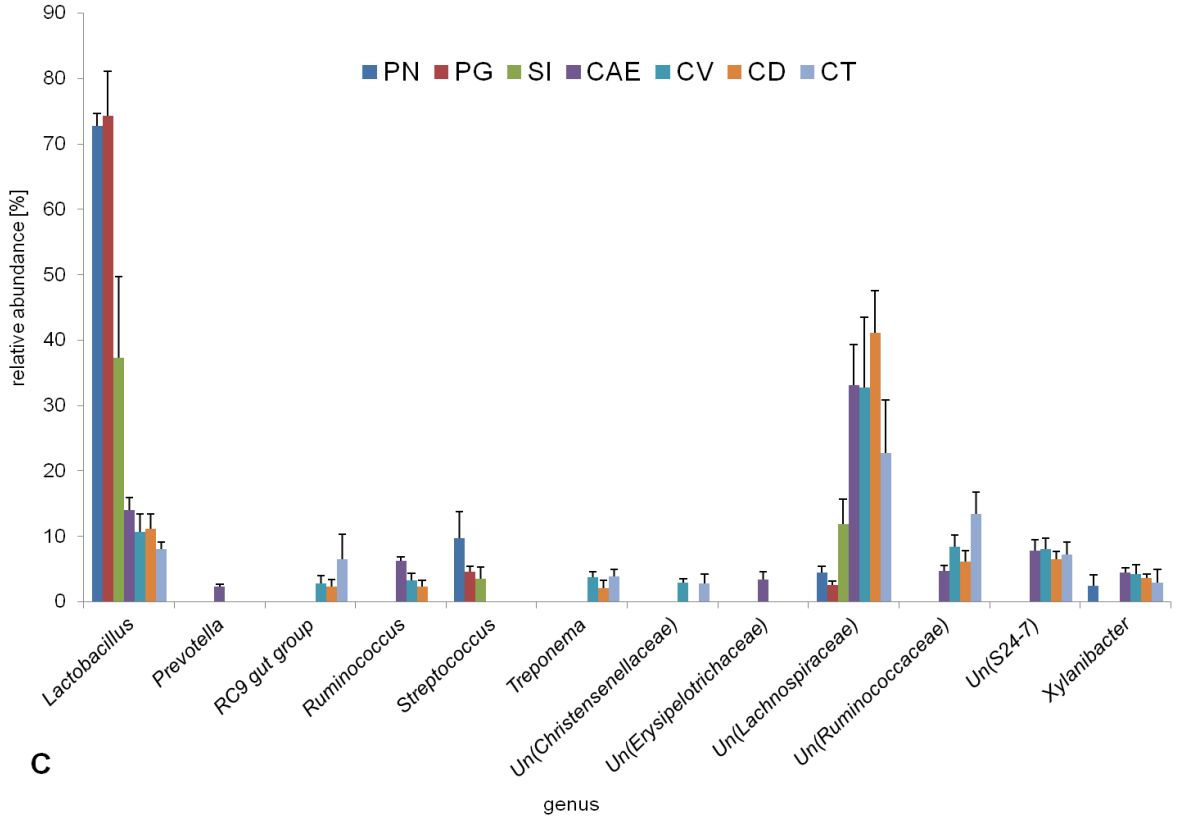
Table 5: Pairwise ANOSIM of Bray-Curtis similarity indices between the different regions of the GIT.

	PG	CV	CD	CT	PN	SI	CAE
PG	-	1.0000	0.1764	**0.0021**	**0.0021**	**0.0021**	**0.0021**
CV	0.0805	-	0.1260	**0.0021**	**0.0021**	**0.0021**	**0.0021**
CD	**0.0084**	**0.0060**	-	**0.0021**	**0.0021**	**0.0021**	**0.0021**
CT	**0.0001**	**0.0001**	**0.0001**	-	1.0000	1.0000	**0.0336**
PN	**0.0001**	**0.0001**	**0.0001**	0.2709	-	1.0000	0.4956
SI	**0.0001**	**0.0001**	**0.0001**	0.6151	0.9111	-	1.0000
CAE	**0.0001**	**0.0001**	**0.0001**	**0.0016**	**0.0236**	0.1190	-

The results indicate the one-way ANOSIM testing of ranked Bray-Curtis similarity indices between the different regions of the GIT: CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG (*pars glandularis*) PN (*pars nonglandularis*) and SI (small intestine). The p values are shown in the upper left and the Bonferroni corrected p values of the pairwise comparisons are shown in the upper right. Differences written in bold were significant.

In the foregut (including the PN, PG and SI), the supplemental feeding of JAM resulted in a generally higher relative abundance of the phylum *Firmicutes* (Figs 3A and 4B, no significant difference) and a lower abundance of *Bacteroidetes* (PG: $P < 0.05$) and *Proteobacteria* (no significant difference) compared to supplemental feeding of the placebo (CON). In the large intestine, the addition of JAM increased the relative abundance of *Bacteroidetes* (no significant difference), *Proteobacteria* (no significant difference) and *Spirochaetes* (no significant difference) in the CAE, as well as in the CD, but it reduced the abundance of *Firmicutes* (no significant difference) in both GIT regions. Furthermore, the relative abundance of the phylum *Proteobacteria* increased in the CV of the JAM group, but almost no variation was observed in the CT in comparison to the CON group (no significant difference).

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20
30
40
50
60
70
80
90
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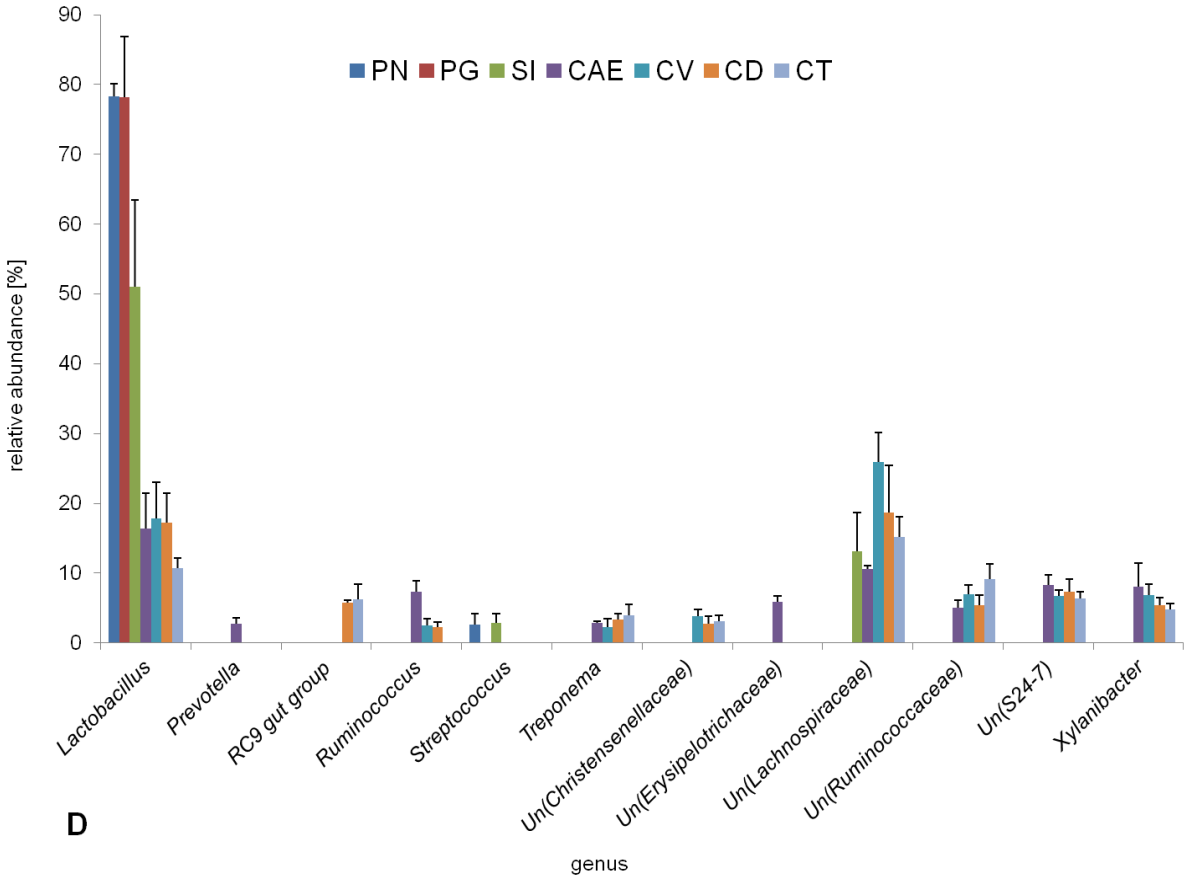
Ruminococcus
Streptococcus
Treponema
Un(Christensenellaceae)
Un(Erysipelotrichaceae)
Un(Lachnospiraceae)
Un(Ruminococcaceae)
Un(S24-7)
Xylanibacter
genus

C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relative abundance [%]
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30
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70
80
90
Lactobacillus
Prevotella
RC9 gut group
Ruminococcus
Streptococcus
Treponema
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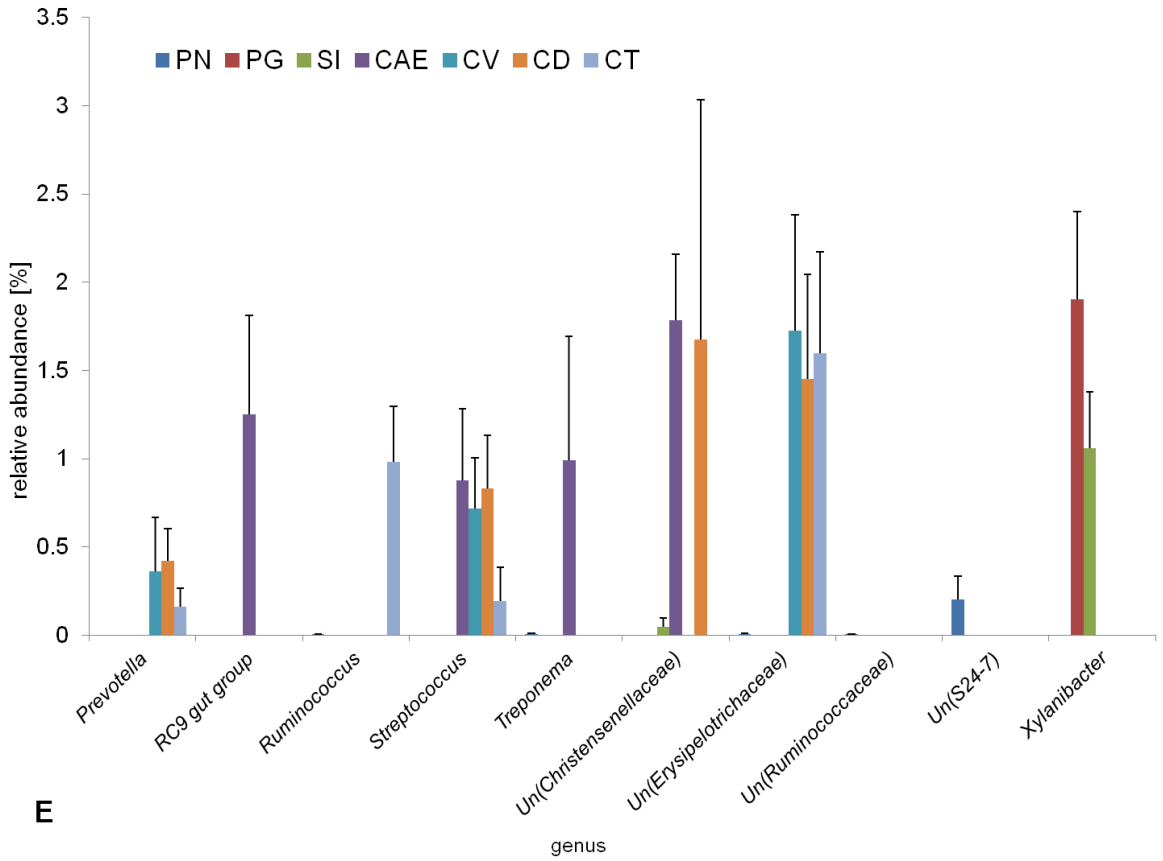
Un(Ruminococcaceae)
Un(S24-7)
Xylanibacter
genus

D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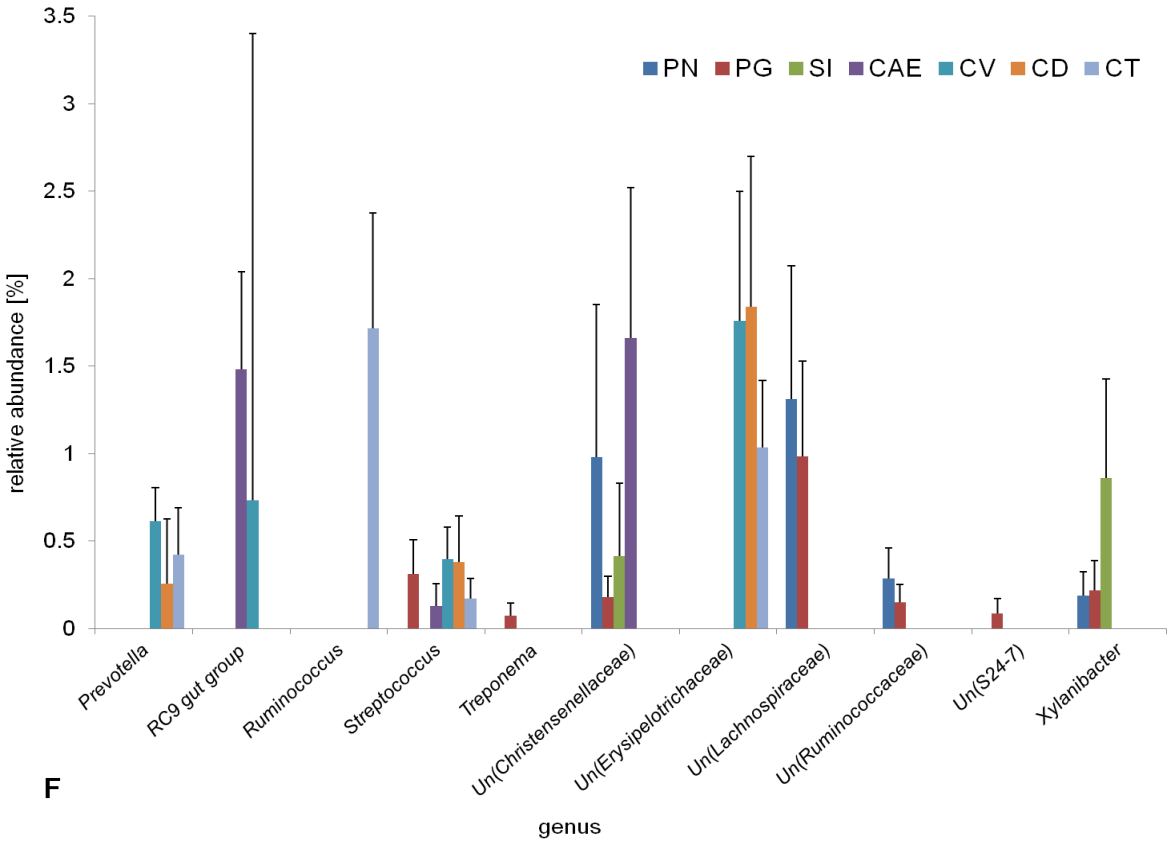
2.5
2
1.5
1
0.5
0
Prevotella
RC9 gut group
Ruminococcus
Streptococcus
Treponema
Un(Christensenellaceae)
Un(Erysipelotrichaceae)
Un(Ruminococcaceae)
Un(S24-7)
Xylanibacter
genus

E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relative abundance [%]
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3
2.5
2
1.5
1
0.5
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Prevotella
RC9 gut group
Ruminococcus
Streptococcus
Treponema
Un(Christensenellaceae)
Un(Erysipelotrichaceae)
Un(Lachnospiraceae)
Un(Ruminococcaceae)
Un(S24-7)
Xylanibacter
genus

F

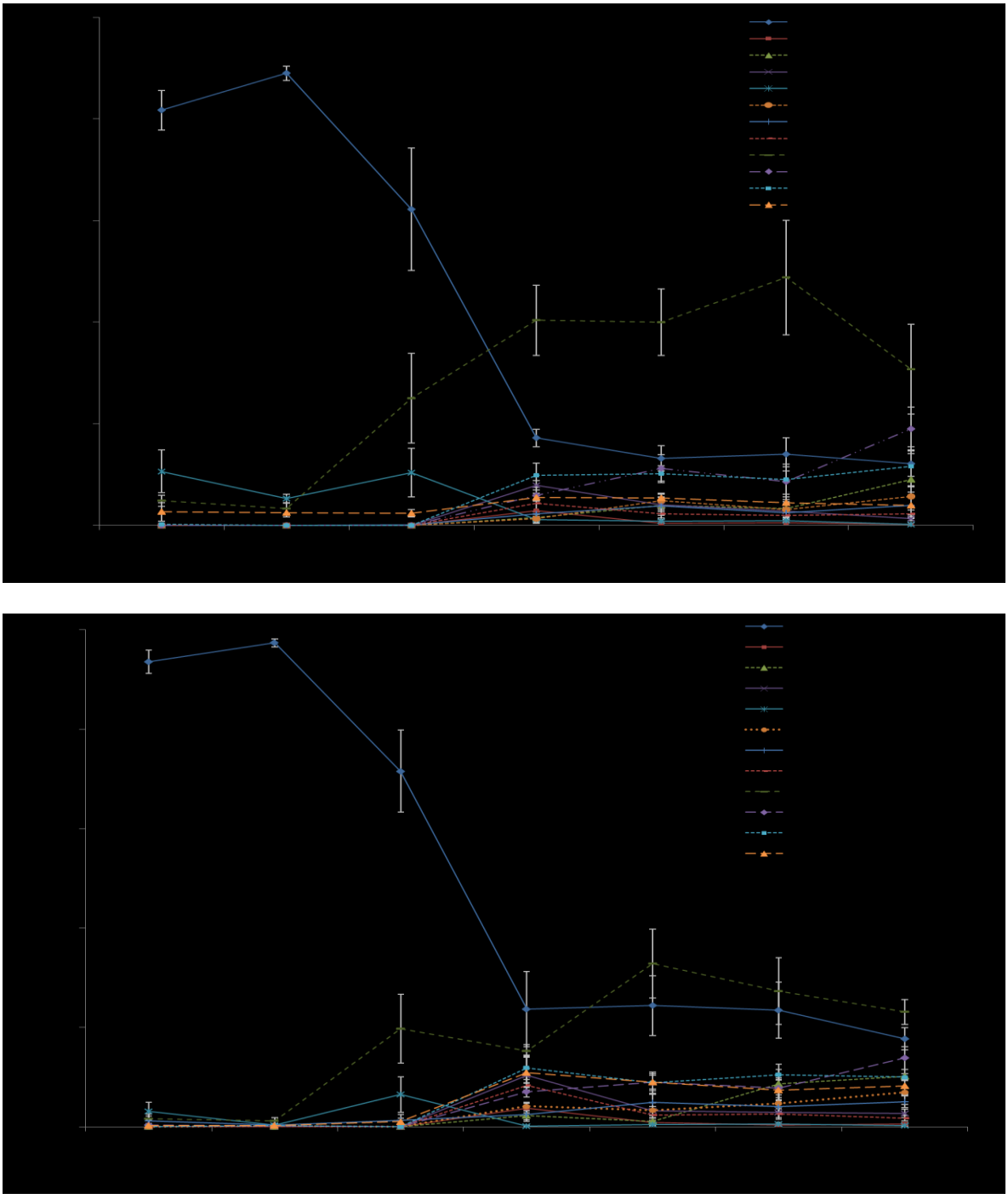

Fig. 3: Mean relative abundance (% ± SE) of the different phyla (A –B), the most abundant genera (mean relative abundance ≥ 2 %, C-D), rare genera (mean relative abundance < 2 %, E-F) and the distribution of the 12 most abundant genera (G-H) along the equine gastrointestinal tract.

The following parts of the digestive tract were examined: CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG (*pars glandularis*), PN (*pars nonglandularis*) and SI (small intestine). Figs 3A, 3C, 3E and 3G represent the CON group (placebo). Figs 3B, 3D, 3F and 3H illustrate the JAM group (Jerusalem artichoke meal). Both feeding groups included Un (an unknown member of a specific family).

The sequencing data revealed the presence of 100 different taxa at the genus level in the whole GIT (S5 Table). For statistical analysis, genera with a frequency of ≥ 2 % for at least one treatment (CON or JAM) and one intestinal part were included. Hence, 12 different genera remained for statistical comparison (Figs 3C-H). The most abundant genus in the stomach (both sections) and small intestine was *Lactobacillus* (no significant difference between feeding groups, Figs 3C-D). The second most abundant genus was *Streptococcus* in the CON group (PN and PG, CON vs. JAM: $P < 0.05$) but only partly in the JAM group (here: PN). In the SI, an unclassified member of the family *Lachnospiraceae* (*Un (Lachnospiraceae)*) was the second most abundant genus in both groups. In the PN, the unclassified member of the family *Lachnospiraceae* (*Un (Lachnospiraceae)*) was higher in the CON group compared to the JAM group ($P < 0.05$). *Lactobacillus* was abundant in all parts of the large intestine but not the dominant genus. The most abundant genus in the large intestine was represented by the same uncultured species from the family *Lachnospiraceae* (*Un (Lachnospiraceae)*), as in the foregut, with higher abundance in the CON group compared to the JAM group (significant difference only in the CAE, $P < 0.05$). Furthermore, the genus *Xylanibacter* was found at higher relative abundance in the JAM group than the CON group (only on the PG, $P < 0.05$).

Rare bacterial genera (mean relative abundance ≤ 2 %) were primarily present in the large intestine of the CON group (Fig 3E), whereas in the JAM group, these genera were also present in the foregut (Fig 3F). The hindgut in the CON and JAM groups was dominated by an unknown member of the family *Christensenellaceae* and *Erysipelotrichaceae*. The foregut of the JAM group was additionally dominated by an unknown member of the family *Lachnospiraceae*.

Figs 3G-H show the distribution of the most abundant genera along the equine gastrointestinal tract. The two feeding groups differed in the mean relative abundance of the genus *Lactobacillus*, which was higher in the JAM group, especially in the hindgut. Moreover, the mean relative abundance of an unknown member from the family *Lachnospiraceae* increased to a higher percentage in the CON group compared to the JAM group.

4. Discussion

The horses ingested the provided feed very well and showed no visible clinical signs of gastrointestinal disturbances or other diseases. Therefore, the calculated and ingested amounts of the prebiotic active compounds were likely within a healthy range. Surprisingly, one young mare of the CON group showed mucosal lesions alongside the *margo plicatus* (*pars nonglandularis*) in the stomach post mortem. According to the classification of the ECEIM (SYKES et al., 2015), the ulcers are primarily classified in the EGUS grade 3 with some individual lesions grade 2. Because the horses used in this study differed in a number of characteristics, e.g., age, body weight, feeding and housing conditions prior to the study, the young mare might have developed the mucosal lesions before starting the experiment.

The microbial composition in the equine GIT is prone to disturbances, primarily as a result of dietary changes. The horses in the present study had a starch intake of 1.2 g/kg bwt x d^{-1} *via* the feed concentrate with a hay to concentrate ratio of 87:13 (as fed basis), which was therefore clearly a forage-based diet. The starch supply was only slightly above what is recommended to be safe (LUTHERSSON et al., 2009; GFE, 2014; $\leq$ 1 g starch/kg bwt x d^{-1}) and should not have caused an overwhelming microbial fermentation and production of organic acids in the stomach.

A total of 0.2 g/kg bwt x d^{-1} of FOS + inulin for horses is recommended as an effective prebiotic dose in horses (JULLIAND and ZEYNER, 2013), meaning to achieve health-promoting effects *via* the GIT. Analyzing the prebiotic Jerusalem artichoke meal after completion of the current study revealed, however, a lower amount of the prebiotic active compound than declared (46.6 % analyzed *vs*. 62.7 % declared). Therefore, the horses ingested only 0.15 g/bwt x d^{-1} FOS + inulin instead of the intended 0.2 g/kg bwt x d^{-1}. Furthermore, the effect of a prebiotic on the GIT also depends on its chemical composition, especially the DP of oligo- and polymeric carbohydrates (VAN DE WIELE et al., 2007). The Jerusalem artichoke meal used in the study showed a lower percentage of higher DP molecules (23.3 % for chains from 16 - 20 monomers and 21.1 % for chains from 11 - 15 monomers) than described in the literature (inulin-type fructans: DP 2 – 60; ROBERFROID et al., 2010). As a consequence, the amount of the prebiotic active compound reaching the large intestine was likely lower than intended, and therefore, the effect on the microbial community might have been reduced. Recent literature confirmed the assumption,

that the natural prebiotic might in part already be degraded in the stomach (BACHMANN et al., 2019).

GLATTER et al. (2016), who used the same animal material as in the current study, reported a higher impact of Jerusalem artichoke meal on microbial fermentation in the foregut, especially the stomach, in comparison to the large intestine. Mainly in the digesta of the nonglandular part of the stomach, the concentration of SCFAs (primarily *n*-butyric acid) was twice as high after feeding of Jerusalem artichoke meal *vs.* control feeding. This finding is in accordance with other studies that also described the fermentation of inulin and/or grass fructans starting in the lower gut (COENEN et al., 2006; BACHMANN et al., 2019). The degradation might possibly started by acid hydrolysis in he stomach (INCE et al., 2014; STRAUCH et al., 2017). Furthermore, the WSC content in the large intestine was zero in the current study, indicating a largely complete degradation in the foregut. The high amount of short molecules (DP $\leq$ 10), especially in the stomach and the immediate shortening of carbohydrate chains in this organ supports the assumption that the stomach is a fructan-degrading organ most likely by the autochthonous microbiota (GLATTER et al., 2016). This hypothesis is supported by the results of the current study because the feeding of the Jerusalem artichoke meal resulted in an increased relative abundance of the specific microbial species. The phylum *Firmicutes,* and especially the genus *Lactobacillus* spp. was higher primarily in the stomach in the JAM group compared to the CON group. In accordance with the literature data, *Lactobacillus* was identified as one of the dominant genera in the horses´ stomachs (YUKI et al., 2000; AL JASSIM et al., 2005; AL JASSIM and ANDREWS, 2009; SADET-BOURGETEAU and JULLIAND, 2010; PERKINS et al., 2012; COSTA et al., 2015). In addition, we observed a decrease in the relative abundance of *Streptococcus* spp. after feeding JAM, which is also one of the dominant genera in the equine stomach (SADET-BOURGETEAU and JULLIAND, 2010; PERKINS et al., 2012; COSTA et al., 2015). PERKINS et al. (2012) showed that both genera form a thin layer at the gastric mucosa. Consequently, a shift in the microbial composition towards one genus might be crucial for stomach health. In vitro studies confirm the assumption that higher microbial activity with an increasing amount of fermentation products (SCFAs, *n*-butyric acid in particular) in the stomach might have harmful effects on the gastric mucosa (CEHAK et al., 2019).

In accordance to previously reported results (COSTA et al., 2015), our results showed that the most abundant phylum in the small intestinal digesta was *Firmicutes* followed by *Proteobacteria*. This was irrespective of the feeding group. In the current study, the digesta of the three different parts of the equine small intestine (duodenum, jejunum and ileum) was mixed and further used as an aggregate sample. Therefore, the results from the small intestine are limited to the mixed digesta whereas other studies have investigated the specific parts separately (COSTA et al., 2015). Previous studies have indicated that the concentration of bacteria, particularly proteolytic bacteria (MACKIE and WILKINS, 1988), increases along the foregut in the cranial to caudal direction. This might explain why our results were more likely comparable to the duodenum instead of the ileum.

The feeding of prebiotic active compounds aims to stimulate the metabolism of the autochthonous microbiota mainly in the large intestine (ROBERFROID et al., 2010). In accordance with other studies, we observed that the dominant phylum in the equine large intestine was *Firmicutes* followed by *Bacteroidetes* (SADET-BOURGETEAU and JULLIAND, 2010; DOUGAL et al., 2013). Feeding JAM reduced the relative abundance of an unclassified genus from the family *Lachnospiraceae* in all parts of the large intestine compared to feeding the placebo. Furthermore, several members of the family *Lachnospiraceae* mainly produce butyrate as a fermentation product (DOUGAL et al., 2014), which has, other as in the stomach (NADEAU et al., 2000; NADEAU et al., 2003; CEHAK et al., 2019), several health-promoting effects on the intestine, e.g., as a key energy source for the colonocytes, thus stimulating epithelial cell proliferation and improving barrier function (PLOEGER et al., 2012). A reduction of this family might has negative consequences for the health of the hindgut. Glatter et al. (2016) reported an increased concentration of *n*-butyric acid exclusively in the CV in response to feeding JAM indicating that the prebiotic effect was less marked than expected, with a lower intake of prebiotic substances than intended.

Except for the CAE, this was also the case for an unclassified genus from the family *Ruminococcaceae*. In contrast, the relative abundance of *Ruminococcus* spp. increased in the CAE and CT with the supplementation of JAM to the diet. DOUGAL et al. (2013) described a high abundance of the family *Ruminococcaceae* (in descending order) in the feces, CV and CAE. In the current study, no effect was observed on the relative abundance of fibrolytic *Ruminococcaceae* in the CV.

Lachnospiraceae and *Ruminococcaceae* have a greater relative abundance (in the feces) in healthy horses compared to colic patients (COSTA et al., 2012b). Therefore, a reduction of members from these families might have negative impacts concerning the health of the hindgut.

The sequencing data revealed a high number of genera, which were distributed in the GIT at low relative abundance. PROUDMAN et al. (2015) indicated that these were likely plant- or soil-attached bacteria and therefore belonged to the transient microflora of the GIT. Whether or not and to what extent these bacteria could contribute to metabolism in the digestive tract is currently not known. Furthermore, the recent study used DNA to determine the microbial composition in the different parts of the GIT but this approach is not able to make conclusions about the metabolic activity. The results are limited and do not distinguish between alive or dead microbial species in the GIT which has to be taken into consideration.

Moreover, an increase in bacterial diversity following prebiotic supply, as reported in the recent study, might lead to a more stable microbial community that is less sensitive to biotic/abiotic stressors. The calculated diversity indices (Simpson and Shannon-Wiener) varied between the feeding groups. In all parts of the gastrointestinal tract, the diversity was elevated with the supplementation of JAM to the diet. Furthermore, the species richness was higher in the JAM group than the CON group, which indicated that the different GIT sections might have reduced susceptibility to pathogens and increased microbial community stability (LOZUPONE et al., 2012). Considering the beta diversity, the different parts of the GIT are self-containing habitats but are not unaffected by each other because of their physiological and anatomical functions. Therefore, the calculated low levels of similarity between the different parts are not unexpected. Moreover, the feeding of JAM resulted in a higher beta diversity concerning in the PN than CAE and CT. This might lead to the assumption that the introduction of a prebiotic into the equine digestive tract (more precisely the microbial community) resulted in a more diverse microbiota at the beginning of the GIT (here: PN) compared to the hindgut. In general, higher diversity in the microbial communities is beneficial for the stability of the ecological system (LOZUPONE et al., 2012; HANSEN et al., 2015) however, it has been shown that microbial diversity declines with age (DOUGAL et al., 2014) but this was not explicit investigated in this study.

Future studies should include detailed chemical analyses of the content and DP distribution of prebiotic active substances in the prebiotic supplement in question, which is particularly important when no purified FOS/inulin preparations are used. To prevent the breakdown of these substances in the foregut, galenic treatment is required so that the release of the prebiotic active substances occurs first in the large intestine. Because of the expected higher prebiotic load of the large intestine, if such stomach protected prebiotics are used, both the effective and safe dose need a reappraisal based on experimental results.

Finally, future studies should include the application of prebiotics under different biotic and/or abiotic stressors as well as different breeds and ages of horses to better describe the impact of the prebiotics under different physiological conditions. Further considerations might include investigating the different mucosal and luminal microbiota compositions, which might have diverse metabolic functions: however, collecting such samples is invasive and often limited due to ethical reasons. Nevertheless, such studies would be of great advantage, as the results obtained from research projects that use feces provide very little information on the microbiota composition and function in the foregut.

5. Conclusion

Despite the large intestine being the declared target for prebiotic interventions, the results of this study show a clear effect in the foregut. The supplementation of Jerusalem artichoke meal containing prebiotic fructooligosaccharides and inulin increased the relative abundance of the dominant genus *Lactobacillus* and decreased the relative abundance of *Streptococcus* to a marked extent in the stomach. This alteration might cause a harmful impact on the stomach by increased bacterial metabolism (SCFA production) and possibly a decreased pH value. Recent literature data indicate, that stomach mucosal lesions might also occur without a markedly pH decrease. Feeding Jerusalem artichoke meal nevertheless increases the bacterial diversity in all parts of the digestive tract, which might be beneficial for the stability of the gastrointestinal microbial community.

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Conflicts of interest

There are no conflicts of interest.

Author contributions

Conceptualization: MG, AZ, MBa and GB. Animal experiments: MG, MBo and MBa. Chemical analysis: MG, KB, BB, HS, AZ and JMG. Statistical data analysis: MG, KB, BB and MW-D. Supervision: AZ. Wrote the article (original draft): MG, AZ, KB, MBo, GB and HS; review/editing: MG, AZ and HS.

References

Al Jassim R. A. M., Scott P. T., Trebbin A.L., Trott D., Pollitt C. C. (2005): The genetic diversity of lactic acid producing bacteria in the equine gastrointestinal tract. *FEMS Microbiology Letters* 248: 75–81.

Al Jassim R. A. M., Andrews F.M. (2009): The bacterial community of the horse gastrointestinal tract and its relation to fermentative acidosis, laminitis, colic, and stomach ulcers. *Veterinary Clinics of North America: Equine practice* 25: 199–215.

Bachmann M., Glatter M., Bochnia M., Greef J. M., Breves G., Zeyner A. (2019): Estimating compartmental and total tract apparent digestibility in horses using internal and external markers. *Livestock Science* 223: 16-23.

Biddle A., Stewart L., Blanchard J., Leschine S. (2013): Untangling the Genetic Basis of Fibrolytic Specialization by Lachnospiraceae and Ruminococcaceae in Diverse Gut Communities. *Diversity* 5: 627–640.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N.; Peña A. G., Goodrich J.K., Gordon J. I., Huttley G. A., Kelley S.T., Knights D., Koenig J. E., Ley R. E., Lozupone C. A., McDonald D., Muegge B. D., Pirrung M., Reeder J., Sevinsky J. L., Turnbaugh P. J., Walters W. A., Widmann J., Yatsunenko T., Zaneveld J., Knight R. (2010): QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7: 335–336.

Cehak A., Marholt L., Kraegeloh T., Zuraw A., Kershaw O., Brehm R., Breves G. (2019): Does prebiotic feeding affect equine gastric health? A Study on the effects of prebiotic-induced gastric butyric acid production on mucosal integrity of the equine stomach. *Research in Veterinary Science* 124: 303-309.

Coenen M., Moeßeler A., Vervuert I. (2006): Fermentative gases in breath indictae that inulin and starch start to be degrated by microbial fermentation in the stomach and small intestine of the horse in contrast to pectin and cellulose. *The Journal of Nutritition* 136: 2108S-2110S.

Coenen M., Kienzle E., Vervuert I., Zeyner A. (2011): Recent German developments in the formulation of energy and nutrient requirements in horses and the resulting feeding recommendations. *Journal of Equine Veterinary Science* 31: 219–229.

Costa M.C., Weese J.S. (2012a): The equine intestinal microbiome. *Animal Health Research Reviews* 13: 121–128.

Costa M. C., Arroyo L. G., Allen-Vercoe E., Staempfli H. R., Kim P. T., Sturgeon A., Weese J. S. (2012b): Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. *PLoS ONE* 7 (7): e41484.

Costa M. C., Silva G., Ramos R. V., Staempfli H. R., Arroyo L. G., Kim P., Weese J. S. (2015): Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. *The Veterinary Journal* 205 (1): 74–80.

Destrez A., Grimm P., Cézilly F., Julliand V. (2015): Changes of the hindgut microbiota due to high-starch diet can be associated with behavioral stress response in horses. *Physiology & Behavior* 149: 159–164.

Dicks L., Botha M., Dicks E., Botes M. (2014): The equine gastro-intestinal tract: An overview of the microbiota, disease and treatment. *Livestock Science* 160: 69–81.

Dougal K., De la Fuente G., Harris P. A., Girdwood S. E., Pinloche E., Newbold C. J ., Schuch R. (2013): Identification of a Core Bacterial Community within the Large Intestine of the Horse. *PLoS ONE* 8: e77660.

Dougal K., De la Fuente G., Harris P. A., Girdwood S. E., Pinloche E., Geor R. J., Nielsen B. D., Schott H. C., Elzinga S., Newbold C. J., Ibekwe A. M. (2014): Characterisation of the Faecal Bacterial Community in Adult and Elderly Horses Fed a High Fibre, High Oil or High Starch Diet Using 454 Pyrosequencing. *PLoS ONE* 9: e87424.

Gesellschaft für Ernährungsphysiologie. Energie und Nährstoffbedarf landwirtschaftlicher Nutztiere Vol. 11: Empfehlungen zur Energie- und Nährstoffversorgung von Pferden. Frankfurt am Main: DLG-Verlag; 2014. 192 p.

Glatter M., Wiedner K., Hirche F., Mielenz N., Hillegeist D., Bochnia M., Cehak A., Bachmann M., Greef J. M., Glaser B., Wolf P., Breves G., Zeyner A. (2016): Fermentation characteristics along the gastrointestinal tract after feeding of Jerusalem artichoke meal to adult healthy warmblood horses. *Journal of Animal Nutrition* 1: 1-11.

Haas B. J., Gevers D., Earl A. M., Feldgarden M., Ward D. V., Giannoukos G., Ciulla D., Tabbaa D., Highlander S. K., Sodergren E., Methe B., De Santis T. Z., Petrosino J. F., Knight R., Birren B. W. (2011): Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research* 21: 494–504.

Hammer Ø., Harper, D.A.T., Ryan, P.D. (2001): PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9pp

Hansen N. C. K., Avershina E., Mydland L. T., Naesset J. A., Austbø D., Moen B., Mage I., Rudi K. (2015): High nutrient availability reduces the diversity and stability of the equine caecal microbiota. *Microbial Ecology in Health and Disease* 26: 1-8.

Ince J. C., Longland A. C., Moore-Colyer M. J. S., Harris P. A. (2014): In vitro degradation of grass fructan by equid gastrointestinal digesta. *Grass and Forage Science* 69: 514–523. doi: 10.1111/gfs.12061

Julliand V., Zeyner A. (2013): Dietary strategies for optimizing gastro intestinal health: an update on pre- and probiotics. In: Horse Health Nutrition Vol. 6, *Proceedings of the European Equine Health & Nutrition Congress:* Ghent; p. 75–84.

Julliand V., Grimm P. (2016): HORSE SPECIES SYMPOSIUM: The microbiome of the horse: History and current knowledge. *Journal of Animal Science*: 2262–2274.

Julliand V., Grimm P. (2017): The Impact of diet on the hindgut microbiome. *Journal of Equine Veterinary Science* 52: 23–28.

Kienzle E., Zeyner A. (2010): The development of a metabolizable energy system for horses. *Journal of Animal Physiology and Animal Nutrition* 94: e231-e240.

Longland A. C., Byrd B. M. (2006): Pasture nonstructural carbohydrates and equine laminitis. *The Journal of Nutrition* 136: 2099S–2102S.

Lozupone C. A., Knight R. (2005): UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology* 71: 8228-8235.

Lozupone C. A., Stombaugh J. I., Gordon J. I., Jansson J. K., Knight R. (2012): Diversity, stability and resilience of the human gut microbiota. *Nature* 489: 220–230.

Luthersson N., Nielsen K. H., Harris P. A., Parkin T. D. H. (2009): Risk factors associated with equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. *Equine Veterinary Journal* 41: 625–630.

Mackie R. I., Wilkins C. A. (1988): Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. *Applied and Environmental Microbiology* 54: 2155–2160.

Milinovich G. J., Trott D. J., Burrell P. C., Van Eps A. W., Thoefner M. B., Blackall L. L., Al Jassim R. A. M., Morton J. M., Pollit C. C. (2006): Changes in equine hindgut bacterial populations during oligofructose-induced laminitis. *Environmental Microbiology* 8: 885–898.

Milinovich G. J., Klieve A. V., Pollitt C. C., Trott D. J. (2010): Microbial events in the hindgut during carbohydrate-induced equine laminitis. *Veterinary Clinics of North America: Equine Practice* 26: 79–94.

Nadeau J. A., Andrews F. M., Mathew A. G., Argenzio R. A., Blackford J. T., Sohtell M., Saxton A. M. (2000): Evaluation of diet as cause of gastric ulcers in horses. *American Journal of Veterinary Research* 61: 784–790.

Nadeau J. A., Andrews F. M., Patton C. S., Argenzio R. A., Mathew A. G., Saxton A. M. (2003): Effects of hydrochloric, acetic, butyric, and propionic acids on pathogenesis of ulcers in the nonglandular portion of the stomach of horses. *American Journal of Veterinary Research* 64: 404–412.

Naumann K., Bassler R. (1976): Die chemische Untersuchung von Futtermitteln. In: Methodenbuch Vol. 3. Darmstadt: VDLUFA-Verlag; 1976.

Pavis N, Chatterton NJ, Harrison PA, Baumgartner S, Praznik W, Boucard J et al. (2001) Structure of fructans in roots and leaf tissues of Lolium perenne. New Phytologist 150: 83–95.

Perkins G. A., Den Bakker H. C., Burton A. J., Erb H. N., McDonough S. P., McDonough P. L., Parker J., Rosenthal R. L., Wiedmann M., Dowd S. E., Simpson K. W. (2012): Equine stomachs harbour an abundant and diverse mucosal microbiota. *Applied and Environmental Microbiology* 78: 2522–2532.

Ploeger S., Stumpff F., Penner G. B., Schulzke J. D., Gäbel G., Martens H., Shen Z., Guenzel D., Aschenbach J. R. (2012): Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Annals of the New York Academy of Sciences* 1258: 52–59.

Proudman C. J., Hunter J. O., Darby A. C., Escalona E. E., Batty C., Turner C. (2015): Characterisation of the faecal metabolome and microbiome of Thoroughbred racehorses. *Equine Veterinary Journal* 47: 580–586.

Pruesse E., Quast C., Knittel K., Fuchs B. M., Ludwig W., Peplies J., Glockner F. O. (2007): SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35: 7188–7196.

Respondek F., Goachet A. G., Julliand V. (2008): Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. *Journal of Animal Science* 86: 316–323.

Roberfroid M., Gibson G. R., Hoyles L., McCartney A. L., Rastall R., Rowland I., Wolvers D., Watzl B., Szajewska H., Stahl B., Guarner F., Respondek F., Whelan K., Coxam V., Davicco M.-J., Léotoing L., Wittrant Y., Delzenne N. M., Cani P. D., Neyrinck A. M., Meheust A. (2010): Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition* 104: S1-S62.

Sadet-Bourgeteau S., Julliand V. (2010): Equine microbial gastro-intestinal health. In: The impact of nutrition on health and welfare of horses. EAAP Publications; p. 161–182.

Santos A. S., Rodrigues M. A. M., Bessa R. J. B., Ferreira L. M., Martin-Rosset W. (2011): Understanding the equine cecum-colon ecosystem: current knowledge and future perspectives. *Animal* 5: 48–56.

Schoster A., Arroyo L. G., Staempfli H. R., Weese J. S. (2013): Comparison of microbial populations in the small intestine, large intestine and feces of healthy horses using terminal restriction fragment length polymorphism. *BMC Research Notes* 6: 91.

Schoster A., Mosing M., Jalali M., Staempfli H. R., Weese J. S. (2016): Effects of transport, fasting and anaesthesia on the faecal microbiota of healthy adult horses. *Equine Veterinary Journal* 48: 595–602.

Strauch S., Wichert B., Greef J. M., Hillegeist D., Zeyner A., Liesegang A. (2017): Evaluation of an in vitro system to simulate equine foregut digestion and the influence of acidity on protein and fructan degradation in the horse's stomach. *Journal of Animal Physiology and Animal Nutrition* 101 Suppl 1: 51–58.

Sykes B. W., Hewetson M., Hepburn R. J., Luthersson N., Tamzali Y. (20015): European College of Equine Internal Medicine consensus statement- Equine Gastric Ulcer Syndrome in adult horses. *Journal of Veterinary Internal Medicine* 29: 1288-1299.

Tian L., Scholte J., Borewicz K., Van den Bogert B., Smidt H., Scheurink A. J. W., Gruppen H., Schols H. A. (2016): Effects of pectin supplementation on the fermentation patterns of different structural carbohydrates in rats. *Molecular Nutrition & Food Research* 60: 2256–2266.

Van de Wiele T., Boon N., Possemiers S., Jacobs H., Verstraete W. (2007): Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. *Journal of Applied Microbiology* 102: 452–460.

Van Eps A. W., Pollit C. C. (2006): Equine laminitis induced with oligofructose. *Equine Veterinary Journal* 38: 203–208.

Vosmer J., Liesegang A., Wanner M., Zeyner A., Suter D., Hoelzle L., Wichert B. (2012): Fermentation of six different forages in the semi-continuous fermentation technique Caesitec. *Journal of Animal Physiology and Animal Nutrition* 96: 860–869.

Yuki N., Shimazaki T., Kushiro A., Watanabe K., Uchida K., Yuyama T., Morotomi M. (2000): Colonization of the stratified squamous epithelium of the nonsecreting area of horse stomach by Lactobacilli. *Applied and Environmental Microbiology* 66: 5030–5034.

Zeyner A., Gefrom A., Hillegeist D., Sommer M., Greef J. M. (2015): Contribution to the method of sugar analysis in legume grains for ensiling - A pilot study. *International Journal of Scientific Research in Science and Technology* 1: 74-80.

4.3 Paper III

Glycaemic and insulinaemic responses of adult healthy warm-blooded mares following feeding with Jerusalem artichoke meal

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Summary

This study aimed to investigate the impact of the supplementation of a prebiotic compound (Jerusalem artichoke meal) on the glycemic and insulinemic response in healthy, non-obese warm-blooded horses. Six adult mares (mean body weight [bwt] 529 ± 38.7 kg; body condition score: 5.1 ± 0.49/9) were used. In two equal meals per day, the horses received crushed oat grains (1 g starch/kg bwt x d^{-1}) and meadow hay (2 kg/100 kg bwt x d^{-1}) which together were likely to meet the energy recommendation for light work (GFE, 2014). Additionally, they received either 0.15 g fructo-oligosaccharides and inulin (FOS+INU)/kg bwt x d^{-1} *via* commercial Jerusalem artichoke meal (JAM) or maize cob meal without grains as control (CON) in 2 x 3-week periods according to a crossover design. Blood was collected on d21 of the feeding period at different ante- and postprandial (PP) time points (-60, 0, 30, 60, 90, 120, 180, 240 and 300 min), and the plasma glucose and serum insulin levels were determined. Feeding JAM *vs.* CON did not change the PP peak of glucose or insulin (glucose: 6.3 ± 0.40 *vs* 7.0 ± 0.87 mmol/L; insulin: 0.508 ± 0.087 *vs* 0.476 ± 0.082 nmol/L) nor did it cause different AUCs until 120 and 300 min PP for the glucose and insulin, respectively, (AUC_{120}, glucose: 997 ± 41.6 *vs* 1,015 ± 41.63 mmol/L x min^{-1}, insulin: 49 ± 6.3 *vs* 42 ± 6.3 nmol/L x min^{-1}; AUC_{300}, glucose: 1,943 ± 142.3 *vs* 2,115 ± 142.3 mmol/L x min^{-1}, insulin: 94 ± 14.8 *vs* 106 ± 14.8 nmol/L x min^{-1}; $P > 0.05$). Following JAM *vs.* CON feeding, glucose and insulin levels declined more rapidly until 240 min PP and tended to be lower ($P = 0.053$ and $P = 0.056$, respectively) at this time point. This result might be promising and should further be studied in more detail.

Keywords: Prebiotic, equine nutrition, insulin, glucose

1. Introduction

Common prebiotics used in horse nutrition are often fructans, which consist of several fructose units and one terminal glucose unit (ROBERFROID, 2007). Depending on the chemical composition, fructans are classified as inulin-type fructans (ITFs, common in *Compositae*) or phlein-type fructans (common in *Poales*; GLATTER et al., 2016a). Depending on the dose, both types of fructans can have harmful or beneficial effects on a horse´s health. With very high intake, particularly by not or only insufficiently adapted horses, phlein-type fructans (LONGLAND and

BYRD, 2006) as well as inulin-type fructans (VAN EPS and POLLIT, 2006, 2009) can trigger laminitis. Laminitis is often associated with endocrine insulin dysregulation, which leads secondarily to permanent stimulation and subsequent downregulation of the insulin receptors (DE LAAT et al., 2016). The development of insulin resistance (IR) related to hyperinsulinemia is often linked to clinical signs of laminitis (KRONFELD et al., 2005; ASPLIN et al., 2007; BAILEY et al., 2007; DE LAAT et al., 2010). Ponies predisposed to laminitis showed a 5.5-fold higher serum insulin concentration after feeding of 3 g inulin/kg bwt x d^{-1} (over 48 h) in comparison to control ponies (BAILEY et al., 2007). The respective differences in serum insulin between laminitis prone and normal ponies were however of minor importance following only 1 g inulin/kg bwt x d^{-1} over the course of 3 days (BORER et al., 2012). Dietary supplementation with 45 g of short chain fructo-oligosaccharides (scFOS) during 6 weeks significantly increased insulin sensitivity and reduced acute insulin response to glucose in comparison with maltodextrin in obese Arabian geldings (body condition score, [BCS] 8/9) without affecting body weight and BCS, but did not alter glucose effectiveness (RESPONDEK et al., 2011). In this study, resting serum insulin concentration was reduced by scFOS but not maltodextrin supplementation. The aim of the present study was to investigate the effect of a natural prebiotic (Jerusalem artichoke, *Helianthus tuberosus*) on the glycemic and insulinemic responses in adult healthy, non-obese, warm-blooded mares with no known history of laminitis. We hypothesized that even clinically normal, non-obese horses might benefit from daily prebiotic doses of FOS and inulin from Jerusalem artichoke meal by improved insulin sensitivity and thus postprandial (PP) glucose clearance following a hay-concentrate meal.

2. Materials and methods

Animals

Six healthy, warm-blooded mares (age: 6 – 13 years) with a mean body weight (bwt) of 529 ± 38.7 kg and a BCS of 5.1 ± 0.49/9 (KIENZLE and SCHRAMME, 2004) were individually housed in box stalls, bedded on wood shavings and had *ad libitum* access to tap water and a salt block (consisting of sodium chloride only). The horses were longed for 30 minutes/day for 5 days/week. The experimental procedure was approved by the Animal Welfare Commissioner of the Martin Luther University Halle-

Wittenberg and the state administration department in Halle/Saale in accordance with the German Animal Welfare Law (reference number: 42502-3-733 MLU).

Diets

During a 2 x 3-week trial period, the horses received two quantitatively equal meals per day consisting of crushed oat grains (1 g starch/kg bwt x d^{-1}; Table 1, 2) and meadow hay (2 kg/100 kg bwt x d^{-1}; Table 1, 2) to meet the energy requirements for light work (GFE, 2014). It was further intended that they additionally receive either 0.2 g fructooligosaccharides and inulin (FOS+INU)/kg bwt x d^{-1} *via* commercial JAM (Tables 1 and 2) or an equal amount of maize cob meal without grains as the control (CON; Tables 1 and 2) according to a crossover design. The crossover experiment was a simple design with two treatments (JAM, CON) applied over a time period of 21 days each and accordingly two feeding sequences. During the adaptation period, the animals were randomly allocated to one of the both feeding sequences: JAM-CON or CON-JAM. Because of the dry consistency of the supplemental feed, water was added prior to feeding at 15 % of the total concentrate meal (w/w). The hay was changed at the end of the second trial (6 days before the second blood collection) due to the reluctance of the horses to eat the initially used hay.

Feed sampling and control of water intake

All feedstuffs were sampled as aggregate samples during the entire study period and stored at room temperature until immediate analysis. The water intake per horse and day was routinely monitored at the same time of day (7:00 PM) by a water clock (Maddalena, CD SD Plus, Povoletto, Italy; accuracy: ± 5%) permanently attached to the drinking system.

Table 1: Analyzed contents of dry matter, proximate nutrients and calculated contents of pre-caecal digestible crude protein and metabolizable energy in the individual feedstuffs used in the study

Item	Hay 1	Hay 2	Oat grains	JAM[1]	CON[2]
DM [g/kg, as fed]	925	916	912	939	927
CA [g/kg DM]	77	69	24	136	26
CP [g/kg DM]	68	98	108	63	36
AEE [g/kg DM]	9	13	46	6	8
CF [g/kg DM]	358	326	151	14	343
NDF [g/kg DM]	652	637	305	3	799
ADF [g/kg DM]	389	369	203	11	415
ADL [g/kg DM]	46	40	33	4	39
pcdCP[3] [g/kg DM]	29	49	76	56	13
ME[4] [MJ/kg DM]	5.3	6.0	11.6	11.8	6.9

DM = dry matter; CA = crude ash; CP = crude protein; AEE = acid ether extract; CF = crude fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; pcdCP = pre-caecal digestible crude protein ; ME = metabolizable energy
[1]JAM = Jerusalem artichoke meal
[2]CON = placebo (maize cob meal without grains)
[3]calculated according to KIENZLE and ZEYNER (2010) and GFE (2014)
[4]calculated according to GFE (2014) and ZEYNER et al. (2015a)

Table 2: Analyzed content of non-structural carbohydrates in the individual feedstuffs used in the study (in g/kg DM)

Feedstuff	glucose	fructose	sucrose	fructan	starch
Hay 1	16	40	3	51	0
Hay 2	22	40	2	18	0
Oat grains	9	0	0	0	373
JAM[1]	9	62	99	484	0
CON[2]	9	10	16	14	0

[1]JAM = Jerusalem artichoke meal
[2]CON = placebo (maize cob without grains)

Blood collection

Blood was collected at the end of each trial period (d21) from the *Vena jugularis externa* by a veterinarian at defined time points ante- and postprandial (PP). In detail, fasting blood samples were taken after 12 h without any access to feed, which was tantamount, in this case, to 60 min prior to the concentrate feeding (min – 60). Immediately after blood collection, the horses received 1 kg of meadow hay according to the recommendation to provide roughage prior to the concentrates (ZEYNER et al., 2004). After 1 h, blood was collected again to determine the basal glucose and insulin concentrations (min 0). After this second blood collection, the horses received the morning concentrate meal, including the supplement (JAM or CON). Before they got their concentrate meal, residual hay, when it occurred, was collected and weighed. Thereafter, blood was sampled at seven defined time points (30, 60, 90, 120, 180, 240 and 300 min PP). Blood was collected in tubes containing clot activators to separate the serum (for insulin) as well as in tubes with EDTA and fluoride to separate the plasma (for glucose). The tubes for serum separation were allowed to clot at room temperature for 30 min, and the collection tubes for plasma separation were kept in a refrigerator (4°C) until centrifugation. The samples were subsequently centrifuged at 2,054 *g* for 10 min. The plasma and serum were separated in new Eppendorf tubes and stored at -20°C until analysis.

Analytical methods

The dietary components were analyzed according to the Association of German Agricultural Research Laboratories (Verband Deutscher Landwirtschaftlicher Untersuchungs – und Forschungsanstalten; VDLUFA; NAUMANN and BASSLER, 1976). The content of the non-structural carbohydrates (starch, fructans, mono- and dimeric sugars) was investigated as described by ZEYNER et al. (2015a, b). The plasma glucose was determined by use of the Hitachi 912 automated analyser (Roche Diagnostics GmbH, Mannheim, Germany; ZEYNER et al. 2002), and the serum insulin was determined *via* an immunoradiometric assay (Insulin-Coa-Acount-RIA-Kit, BioSource Europe S.A., Belgium; GOTTSCHALK et at. 2011).

Statistical analyses

The fasting and basal plasma glucose and serum insulin concentrations as well as the maximum concentrations of both blood parameters, were calculated. Fasting and baseline concentrations are understood as concentrations at time points -60 min and immediately (0 min) prior to the concentrate meal. Furthermore, the time to reach the PP plasma and serum peak ('time to peak'), respectively, was computed for glucose and insulin separately. The area under the curve (AUC) was determined for glucose (in mmol/L x min^{-1}) and insulin (in nmol/L x min^{-1}) by using non-overlapping rectangles and triangles (trapezoidal method). The AUC was calculated for both the immediate phase 0 – 120 min PP (AUC_{120}) and further from 0 – 300 min PP (AUC_{300}). Furthermore, the quotients of AUC_{120} (glucose/insulin) and AUC_{300} (glucose/insulin) as well as the insulin/glucose and glucose/insulin quotient were calculated. Statistical analysis was performed using the MIXED procedure of SAS (version 9.4, SAS Inst. Inc., Cary, NC). The insulin, and glucose concentration as functions of the nine time points (TPO; -60, 0, 30, 60, 90, 120, 180, 240 and 300 min PP) were analyzed using models with fixed effects of treatment (TRM), period (PER), TPO, and of the interactions TRM × PER and TRM × TPO. The calculated AUC_{120} and AUC_{300} (for glucose and insulin separately) and also the quotients AUC_{120} (glucose/insulin), AUC_{300} (glucose/insulin), insulin/glucose (fasting and baseline) and glucose/insulin (fasting and baseline) were analysed using models with fixed effects of TRM, PER and of the interaction TRM × PER. The repeated measures per animal over the periods were taken into account by random animal effects. Additionally, to determine the correlation structure of the repeated measures per animal and the period over the time points, different variance-covariance structures with inhomogeneous residual variances (autoregressive: type=arh (1), compound-symmetry: type=csh and Toeplitz: type=toeph) were tested. Finally, the correlation structure with the lowest Akaike's information criterion (AIC) value was selected. For the detection of extreme outliers at the time points, the 3.0 × interquartile range was used. The Shapiro-Wilk test was used to check the normality of the studentized residuals. The differences of means were tested using the multiple *t*-test implemented in MIXED. The Kenward-Roger method was used for approximation to the degrees of freedom. Statistical significance was accepted at $P < 0.05$. The least square means (LSM) of all traits (± SE) are reported.

3. Results

General observations

During the study, no horse showed any sign of gastrointestinal disorders or was otherwise clinically conspicuous. Furthermore, the horses lost weight (mean: 13 kg; range: 5 – 30 kg) during the study because of their reluctance to eat the hay provided initially. After changing the batch of hay, all horses gained weight (6 – 16 kg per horses), except of one mare, which lost 2 kg of body weight (Fig. 1). Moreover, the ambient temperature was higher during the first blood sampling day in comparison to the second testing day with fairly consistent relative humidity throughout the study (apart from a conspicuous decline in week 5; Fig. 2). The daily water intake of the horses tended to vary conversely with the ambient temperature (Fig. 1 and Fig. 2). There were no differences in the daily water intake between the feeding groups (Lsmeans ± SE; JAM: 27.9 ± 1.3 L/d *vs*. CON: 27.2 ± 1.3 L/d; P = 0.195). On the blood sampling days, the horses consumed only half of the presented meadow hay during the given hour prior the concentrate meal (means ± SE; JAM: 540 ± 82 g; CON: 501 ± 40 g). The concentrate meal and the supplemental feeding (JAM or CON) were completely consumed.

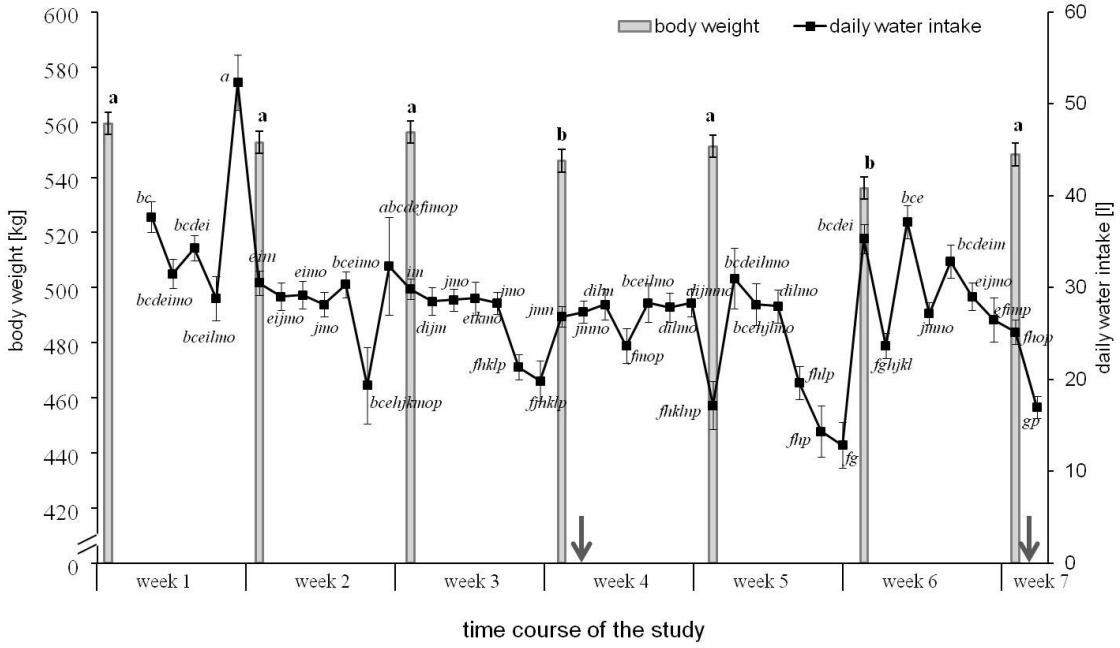

Fig. 1**:** Least square means (LSM ± SE) of the daily water intake and the body weight over the course of the study; arrows indicate days of blood collection; [a,b] indicates significant differences between means (bold letters: body weight; italics: daily water intake)

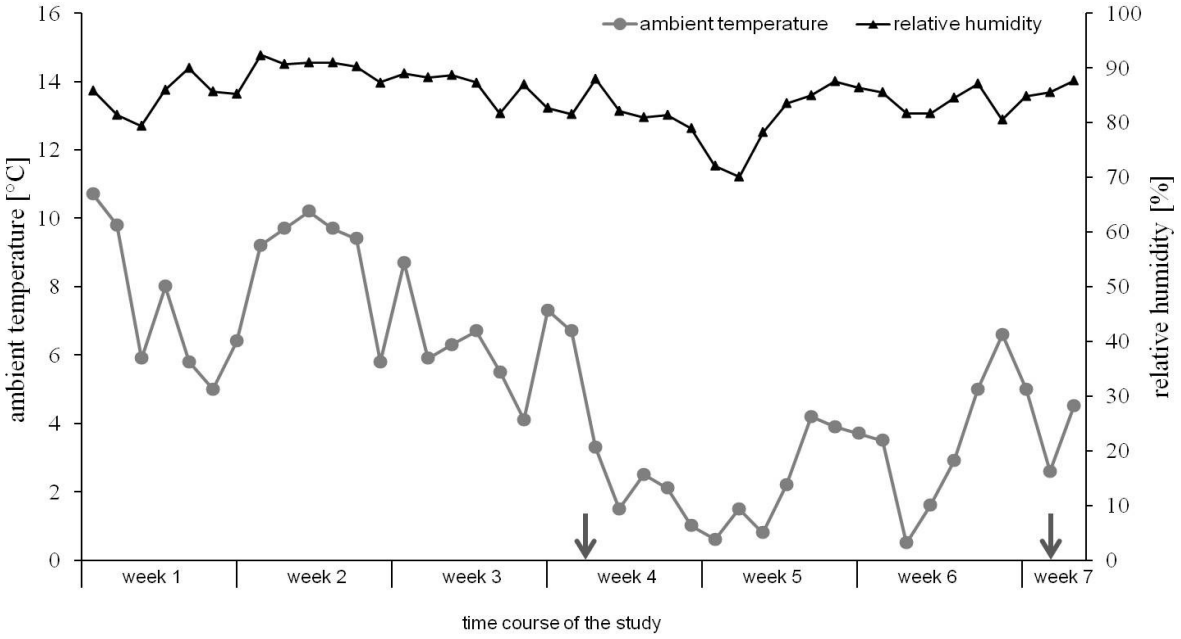

Fig. 2: Daily ambient temperature and relative humidity over the course of the study (arrows indicate days of blood collection)

Chemical composition of the feed

The analysed contents of the proximate nutrients, non-structural carbohydrates and from this calculated concentrations of pre-caecal digestible crude protein (pcdCP) and metabolizable energy (ME) in all feedstuffs used in this study are given in Tables 1 and 2. Note that the second batch of hay had a different chemical composition and energy content with particularly lower and higher contents of crude fiber and metabolizable energy, respectively, compared to the first batch (Table 1). Of particular importance in the context discussed here is the result that the commercial Jerusalem artichoke meal revealed a much lower content of the analysed prebiotic active ingredients than was declared and thus expected (46.6 % as analyzed *vs* 62.7 % as declared). This resulted in a total lower intake of prebiotic active substances namely FOS+INU (0.15 instead of 0.2 g/kg bwt x d^{-1}) as initially intended.

Blood Parameters

Fasting as well as baseline plasma glucose concentrations were similar for the CON and the supplemental group (JAM, Table 3; $P > 0.05$). Neither the peak for the plasma glucose concentration nor the time to reach the peak differed significantly

between the two groups (Table 3; Fig: 3; $P > 0.05$). A significant increase in the plasma glucose concentration compared to the baseline was measured 30 min after the horses received the concentrate meal in both groups (Fig. 3; $P < 0.05$). One mare supplemented with CON showed the highest plasma glucose and also the highest serum insulin concentration (glucose: 11.4 mmol/L; insulin: 0.846 nmol/L). The supplementation of JAM did not influence the fasting and baseline serum insulin concentration, the peak insulin concentration or the time to reach the peak in comparison with CON (Table 3; $P > 0.05$). The serum insulin concentration was significantly elevated 30 min after feeding oat grains plus JAM or CON (Fig. 4; $P < 0.05$). The AUC for glucose and insulin until 120 min or 300 min PP was not affected by JAM (Table 3; $P > 0.05$).

Table 3**:** Least square means (LSM ± SE) of fasting and baseline plasma glucose and serum insulin concentrations, peak glucose and insulin concentrations, time to reach the peak and area under the curve (AUC) for the immediate phase (0 – 120 min; AUC_{120}) and for the total curve of the shape (0 – 300 min. AUC_{300})

item	CON	JAM	*P* value
glucose			
fasting blood level [mmol/L]	4.7 ± 0.42	4.8 ± 0.42	0.955
baseline concentration [mmol/L]	5.0 ± 0.42	5.1 ± 0.42	0.905
peak [mmol/L]	7.0 ± 0.87	6.3 ± 0.40	0.560
time to peak [min]	90 ± 22.4	105 ± 15.0	0.639
AUC_{120} [mmol/L x min^{-1}]	1,015 ± 41.6	997 ± 41.6	0.783
AUC_{300} [mmol/L x min^{-1}]	2,115 ± 142.3	1,943 ± 142.3	0.464
insulin			
fasting blood level [nmol/L]	0.048 ± 0.055	0.051 ± 0.055	0.976
baseline concentration [nmol/L]	0.081 ± 0.055	0.125 ± 0.055	0.575
peak [nmol/L]	0.476 ± 0.082	0.508 ± 0.087	0.836
time to peak [min]	125 ± 25.0	110 ± 15.8	0.288
AUC_{120} [nmol/L x min^{-1}]	42 ± 6.3	49 ± 6.3	0.545
AUC_{300} [nmol/L x min^{-1}]	106 ± 14.8	94 ± 14.8	0.673
quotients			
AUC_{120} glucose/insulin	24 ± 2.7	23 ± 2.7	0.714
AUC_{300} glucose/insulin	21 ± 2.2	22 ± 2.2	0.898
fasting insulin/glucose	0.010 ± 0.001	0.011 ± 0.001	0.639
baseline insulin/glucose	0.016 ± 0.005	0.024 ± 0.005	0.327
fasting glucose/insulin (FGIR)	10.6 ± 0.30	10.4 ± 0.76	0.852
baseline glucose/insulin	7.3 ± 1.0	6.2 ± 1.2	0.517

CON = placebo (maize cob without grains); JAM = Jerusalem artichoke meal; FGIR = fasting glucose to insulin ratio

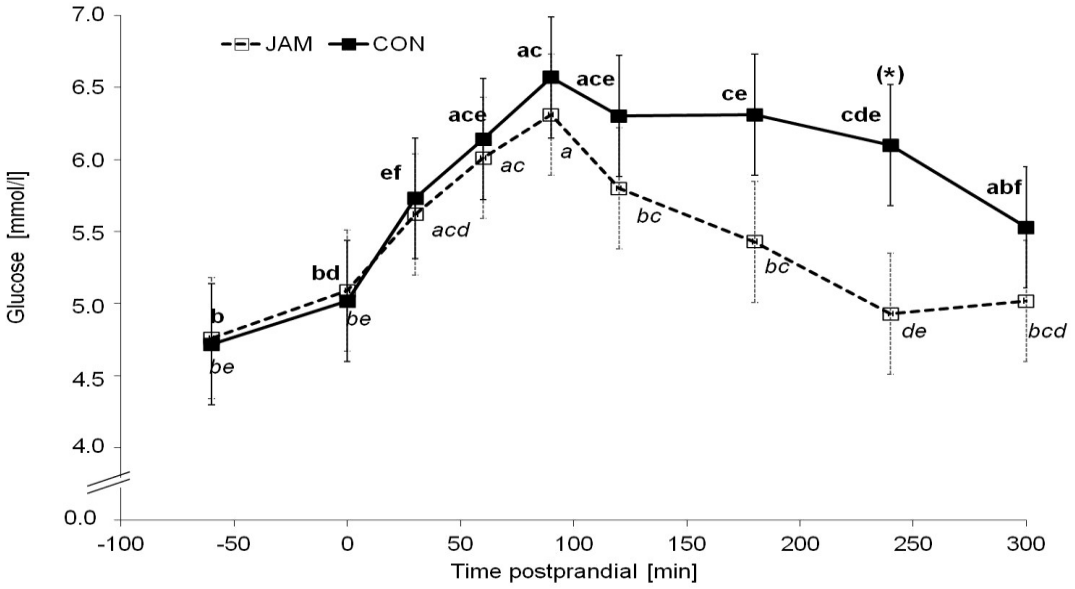

Fig. 3: Least square means (LSM) ± 95 % confidence interval for the postprandial (min) plasma glucose concentration (mmol/L) after feeding with Jerusalem artichoke meal (JAM) or placebo (CON); [a,b] indicates significant ($P < 0.05$) differences within the treatment group (CON: bold letters; JAM: italics) dependent on the time; [(*)] indicates tendential differences between the treatment groups at a specific time point ($P = 0.053$)

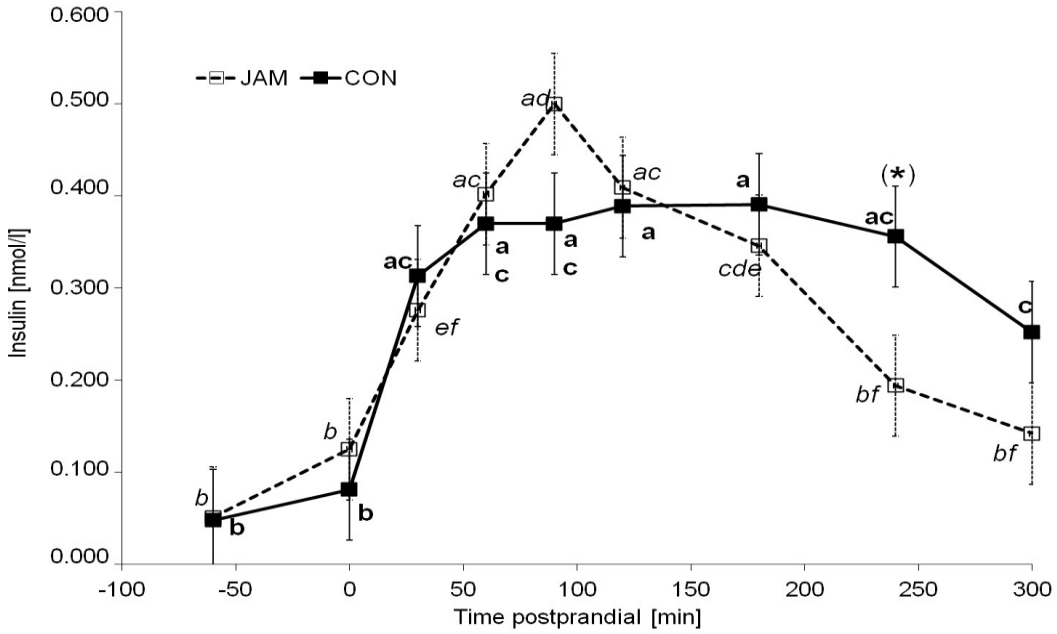

Fig. 4: Least square means (LSM) ± 95 % confidence interval for the postprandial (min) serum insulin concentration (nmol/L) after feeding with Jerusalem artichoke meal (JAM) or placebo (CON); [a,b] indicates significant ($P < 0.05$) differences within the treatment group (CON: bold letters; JAM: italics) dependent on the time; [(*)] indicates tendential difference between the treatment groups at a particular postprandial time point ($P = 0.056$)

4. Discussion

The recommended prebiotic dosage of FOS+INU for horses is 0.2 g/kg bwt x d^{-1} (equivalent to 0.1 kg/d for a 500 kg horse; JULLIAND and ZEYNER, 2013), which was the dosage that was intended to be applied in this study. Because the analysis revealed that the content of FOS+INU in the commercial JAM used in this study was 46.6 % (instead of declared 62.7 %), the actual intake amounted to only 0.15 g/kg bwt x d^{-1}. This is substantially lower than the level supplied by BAILEY et al. (2007), where a dose of 3 g inulin/kg bwt caused a 5.5-fold increase of the PP insulinaemic response and lower than the amount supplied by BORER et al. (2012), where 1 g inulin/kg bwt induced a doubled PP insulinaemic response. Contrary to the current study, however, ponies were used in both of the cited studies, where BAILEY et al. (2007) and BORER et al. (2012) addressed individuals prone to laminitis, and only BORER et al. (2012) studied normal ponies, too.

The prebiotic potentiality regarding the hindgut might have been much weaker than expected because fewer effective prebiotic substrates might have been able to reach the hindgut. In horse nutrition, the dosage of feeding prebiotic compounds is marginally variable because high amounts of fructans (inulin- or phlein-type) have the potential to trigger severe health disorders such as laminitis (LONGLAND and BYRD, 2006; VAN EPS and POLLIT, 2006, 2009). Investigations of fermentation products in different segments of the digestive tract of adult healthy, warm-blooded horses performed with the same batch and quantity of JAM as in the current study indicated the fermentative activity of the microbes in the terminal tract was stimulated to a much lower degree than expected (GLATTER et al., 2016b). Several studies, however, indicate that the effect of a prebiotic ingredient depends on various factors, such as its composition, degree of polymerization (DP) or dosage (SAMANTA et al., 2013). Prebiotics with a higher DP exhibited more pronounced effects on the terminal tract than shorter ones or a mixture of different chain lengths (VAN DE WIELE et al., 2007; AZORÍN-ORTUÑO et al., 2009). The commercial JAM used in this study had a DP predominantly (55 %) ranging from 11 to 25 units, which is not as large as the DP indicated for inulin-type fructans (DP 2-60; ROBERFROID et al., 2010). According to COENEN et al. (2006), INCE et al. (2014) and STRAUCH et al. (2016), fructans are prone to be already decomposed in the lower gut, including the stomach where the DP might be of particular importance (STRAUCH et al., 2016). GLATTER et al. (2016b) actually measured an increased content of organic acids in the stomach of

horses fed inulin-type fructans according to a dietary approach identical to that of the current study. For further studies, a detailed analysis of the prebiotic compound, which is intended to be used, is recommended to determine its actual contents and DP prior to the experiment and thus to allow a more purposeful application of the prebiotic and valuation of the results of its application.

Further imponderables resulted from the exchange of hay batches performed just a few days before the end of the study. To assess the likely short-term effects on the glycaemic and possibly insulinaemic response, the contents of mono- and dimeric sugars (SU) in the hay batches and the quantities the horses consumed immediately before blood samples were taken have been used to calculate the SU intakes (Tables 1 and 2; 0.5 kg of hay; mean body weight 529 kg). The difference, however, was 0.003 g SU/kg body weight between the two batches of hay; thus, the impact on the measured blood parameters might be negligible.

Neither fasting and basal glucose nor insulin concentrations (Table 3) differed significantly between the treatment groups and were further i) within normal ranges (KOELLER et al., 2014) and in particular ii) in the range of similar feeding quantities of easily available energy from starch following a 12 hr overnight fasting period (VERVUERT et al., 2009b). In the current study, the feeding of JAM vs. CON resulted in a particularly fast and pronounced peak of serum insulin followed by a more rapid decline in both plasma glucose and serum insulin PP (Figs. 3 and 4). The return to baseline of plasma glucose until 300 min PP was nearly complete in JAM but not CON. Jerusalem artichoke meal consists of several fructose units and one terminal glucose unit, which are linearly connected to each other at the β-2, 1-linkage, which cannot be hydrolysed by mammalian enzymes (ROBERFROID, 2007). Therefore, fructose is the dominant available monosaccharide after the microbial breakdown of the prebiotic. In comparison to glucose, fructose causes minor changes in the glycaemic and insulinaemic responses PP (BORER et al., 2012). Previously, PP exhalation of methane and hydrogen in equines indicated that inulin started to be fermented already in the foregut (COENEN et al., 2006). *In vitro* studies with grass fructans suggested that under specific conditions, acid hydrolysis in the stomach leads to the decomposition of grass fructans (INCE et al., 2014; STRAUCH et al., 2016). When fructose is released and not immediately be fermented by gut microbes it might be absorbed in the equine digestive tract by specific transporters (Glut 5), which are highly abundant in the small intestine (FERNANDEZ CASTAÑO MEREDIZ

et al., 2004; TAYLOR et al., 2012), and is transported through the bloodstream into the liver. In the liver, it is metabolized to fructose-1-phosphate, which can be further used to generate glucose, glycogen or triglycerides (JOHNSON et al., 2013). Nevertheless, it is not clear, whether, and to what extent, this monomeric sugar might be absorbed pre-caecally and induce an insulin reaction, but the changes are seemingly less pronounced in comparison to glucose (BORER et al., 2012).

The higher PP concentration of insulin after the feeding of JAM in comparison to CON is critical to note. Permanent or frequently elevated circulating insulin concentrations, manifested as hyperinsulinemia, might be a trigger for metabolic disorders such as laminitis (TREIBER et al., 2006; GEOR, 2008) at least in prone individuals. Furthermore, high amounts of circulating insulin, for example after feeding high-glycaemic meals (TREIBER et al., 2005a), decrease the responsiveness of insulin-sensitive cells and lead to an insulin resistance accompanied by a reduced insulin sensitivity (KRONFELD et al., 2005). Nevertheless, the tendentially more pronounced decrease of the both plasma glucose and serum insulin concentration at 240 min PP (P = 0.053 and 0.056, respectively; Fig. 4) might be a positive impact of the long-term prebiotic supply. The basal serum insulin concentration (meaning that after ingestion of around 0.5 kg of meadow hay) was recovered approximately 300 min PP (0 min: 0.125 nmol/L vs. 300 min: 0.142 nmol/L) with the supplementation of JAM in the diet but not after feeding of the placebo. Recent studies (BAMFORD et al., 2016a, b) indicate a high PP insulin concentration after ingestion of a once-daily high-glycaemic meal might not cause a down-regulation of the insulin receptor and therefore lead to a decreased insulin sensitivity. Moreover, a chronic stimulation of the pancreas by more than one high-glycaemic meal is required to modify the insulin receptor sensitivity.

Prebiotics are supposed to contribute to glucose homeostasis via increasing insulin secretion or enhancing insulin sensitivity (ROBERFROID et al., 2010), depending on the initial condition (e. g., obese, diabetic). Feeding obese horses (BCS 8 ± 1/9) with 45 g scFOS/d (over 6 weeks) led to an improved insulin sensitivity (RESPONDEK et al., 2011). In the present study, the mares had a normal body condition score being around 5/9. The trend for a more pronounced decline of plasma glucose and serum insulin following the PP peak revealed in our study indicate that an improvement of the glucose homeostasis after feeding the prebiotic compound is realistically possible also in non-obese horses. However, the impact of the very high glucose (peak: 11.4

mmol/L) and insulin concentration (peak: 0.846 nmol/L) in one of the mares included in the current study after being fed the control diet has to be taken into consideration. Thus, the results should be treated carefully because of the small number of animals and the divergent glycaemic and insulinaemic responses, especially of one clinically normal mare.

An improvement of insulin sensitivity might be achieved by an increased release of incretins, such as glucagon-like peptide 1 (GLP-1) and glucose-dependent nsulinotrophic polypeptide (GIP), which are involved in glucose disposal through stimulation of the pancreatic β- cells associated with an increasing insulin secretion (CANI et al., 2004, 2007; KAZAKOS, 2011). In horses, the relative contribution of incretins to insulin production is unclear (DE LAAT et al., 2016). Only a few studies investigated the PP response of GLP-1 to different diets (BAMFORD et al., 2015; CHAMEROY et al., 2016), but, to the author's knowledge, there is no information about the impact of prebiotics on incretin secretion in horses. In rats, the supplementation of prebiotic compounds leads to an increased amount of endocrine L-cells in the colon (CANI et al., 2007), which stimulates the expression of the proglucagon gene in these cells and, therefore, enhances the secretion of different peptides, such as the glucagon-like peptides (GLP-1).

Moreover, prebiotics are supposed to enhance microbial fermentation predominantly in the hindgut and therefore lead to an increased amount of fermentation products, such as short chain fatty acids (SCFA) or lactate (ROBERFROID et al., 2010). SCFA (mainly acetate, propionate and *n*-butyrate) are metabolized, that is, locally in the gut epithelium, in the liver or in peripheral tissues (fat and muscle cells), and have indirect effects on glucose homeostasis and cholesterol synthesis (BERGMANN, 1990). Propionate is metabolized in the liver via oxaloacetate to glucose and thus increases the blood glucose concentration. By stimulating the hepatic gluconeogenesis from propionate, the supplemental feeding of prebiotics with coincident increasing production of SCFA (especially propionate) leads to an elevated secretion of insulin. The formation of glucose from propionate in the liver is proven for ruminants (HUNTINGTON, 1990), but only assumed for horses. Feeding the prebiotic over a longer period of time might result in a permanent slightly elevated glucose concentration PP in the blood and an equally increased insulin secretion. Moreover, the insulin sensitivity is linked to the size of adipocytes (ROBERTSON, 2007). SCFA have the potential to affect the differentiation as well as the size of the

adipocyte cells, and vice versa, and therefore might improve insulin sensitivity. If the adipocytes reach a critical size, their physiological function could be dysregulated, resulting in a higher production of adipokines and inflammatory cytokines, an increased recruitment of monocytes and the release of medium chain fatty acids as well as predominantly long chain fatty acids (GEOR, 2008). As a result, the insulin signaling pathways in multiple tissues might be disrupted and lead to the development or enhancement of insulin resistance. Therefore, the stimulatory effect of fatty acids on adipocytes has limitations.

Furthermore, the calculated quotients (glucose/insulin and insulin/glucose, Table 3; FIRSHMAN and VALBERG, 2007) from the fasting and baseline levels are recommended to be in principle suitable to indicate an altered glucose homeostasis. Fasting glucose to insulin ration (FGIR) and insulin to glucose quotient showed no modification after feeding JAM. FGIR levels below 4.5 indicate insulin resistance (DE LAAT et al., 2016) but the calculated values are in the normal range of about 10 (FGIR, CON: 10.6 ± 0.30 vs. JAM: 10.4 ± 0.76; Table 3). Moreover, the baseline insulin/glucose quotient might be suitable to indicate an increased pancreatic release of insulin after feeding JAM to the horses (FIRSHMAN and VALBERG, 2007). Comparing both calculated baseline quotients (glucose to insulin and insulin to glucose) in the current study they are far from any statistically significant difference.

Nevertheless, the data permit no interference on the insulin sensitivity in the peripheral tissues, because this was not determined by specific tests during the study (e.g., oral glucose tolerance test, frequently sampled intravenous glucose tolerance test [FSIGT] or euglycaemic-hyperglycaemic clamp; FIRSHMAN and VALBERG, 2007). This aspect has to be taken into consideration in following investigations.

5. Conclusion

The feeding of 0.15 g fructooligosaccharides and inulin per kg of bwt and day to adult healthy, non-obese mares does not significantly alter fasting, basal and postprandial glycaemic and insulinaemic responses. However, a tendency for a more rapid and complete decrease of both plasma glucose and serum insulin following the postprandial peak might indicate an improved glucose clearance from the bloodstream following starchy meals, which in turn may represent an unexpected but positive effect of prebiotic supplementation even in non-obese equines being not insulin resistant.

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References

Asplin, K. E.; Sillence, M. N.; Pollitt, C. C.; McGowan, C. M. (2007): Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *The Veterinary Journal* 174 (3): 530–535.

Azorín-Ortuño, M.; Urbán, C.; Cerón, J. J.; Tecles, F.; Allende, A.; Tomás-Barberán, F. A.; Espín, J. C. (2009): Effect of low inulin doses with different polymerisation degree on lipid metabolism, mineral absorption, and intestinal microbiota in rats with fat-supplemented diet. *Food Chemistry* 113 (4): 1058–1065.

Bailey, S. R.; Menzies-Gow, N.; Harris, P. A.; Habershon-Butcher, J. L.; Crawford, C.; Berhane, Y.; Boston, R. C.; Elliot, J. (2007): Effect of dietary fructans and dexamethasone administration on the insulin response of ponies predisposed to laminitis. *Journal of the American Veterinary Medical Association* 231 (9): 1365–1373.

Bamford, N. J.; Baskerville, C. L.; Harris, P. A.; Bailey, S. R. (2015): Postprandial glucose, insulin, and glucagon-like peptide-1 responses of different equine breeds adapted to meals containing micronized maize. *Journal of Animal Science* 93 (7): 3377–3383.

Bamford, N. J.; Potter, S. J.; Harris, P. A.; Bailey, S. R., (2016a): Effect of increased adiposity on insulin sensitivity and adipokine concentrations in horses and ponies fed a high fat diet, with or without a once daily high glycaemic meal. *Equine Veterinary Journal* 48 (3): 368–373.

Bamford, N. J.; Potter, S. J.; Baskerville, C. L.; Harris, P. A.; Bailey, S. R. (2016b): Effect of increased adiposity on insulin sensitivity and adipokine concentrations in different equine breeds adapted to cereal-rich or fat-rich meals. *Veterinary Journal* 214: 14–20.

Bergmann, E. N. (1990): Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70 (2): 567–590.

Borer, K. E.; Bailey, S. R.; Menzies-Gow, N. J.; Harris, P. A.; Elliott, J. (2012): Effect of feeding glucose, fructose, and inulin on blood glucose and insulin concentrations in normal ponies and those predisposed to laminitis. *Journal of Animal Science* 90 (9): 3003–3011.

Cani, P. D.; Dewever, C.; Delzenne, N. M. (2004): Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *The British Journal of Nutrition* 92 (3): 521-526.

Cani, P. D.; Hoste, S.; Guiot, Y.; Delzenne, N. M. (2007): Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. *The British Journal of Nutrition* 98 (1): 32–37.

Chameroy, K. A.; Frank, N.; Elliott, S. B.; Boston, R. C. (2016): Comparison of plasma active glucagon-like peptide 1 concentrations in normal horses and those with equine metabolic syndrome and in horses placed on a high-grain diet. *Journal of Equine Veterinary Science* 40: 16–25.

Coenen, M.; Mößeler, A.; Vervuert, I. (2006): Fermentative gases in breath indicate the inulin and starch start to be degrated by microbial fermentation in the stomach and small intestine of the horse in contrast to pectin and cellulose. *The Journal of Nutritition* 136: 2108S–2110S.

De Laat, M. A.; McGowan, C. M.; Sillence, M. N.; Pollitt, C. C. (2010): Equine laminitis: induced by 48 h hyperinsulinaemia in Standardbred horses. *Equine Veterinary Journal* 42 (2): 129–135.

De Laat, M. A.; McGree, J. M.; Sillence, M. N. (2016): Equine hyperinsulinaemia: investigation of the enteroinsular axis during insulin dysregulation. *American Journal of Physiology, Endocrinology and Metabolism* 310 (1): E61-E72.

Fernandez-Castaño Merediz, E.; Dyer, J.; Salmon, K. S. H.; Shirazi-Beechey, S. P. (2004): Molecular characterization of fructose transport in equine small intestine. *Equine Veterinary Journal* 36 (6): 532–538.

Firshman, A. M.; Valberg, S. J. (2007): Factors affecting clinical assessment of insulin sensitivity in horses. *Equine Veterinary Journal* 39 (6): 567–575.

Geor, R. J. (2008): Metabolic Predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *Journal of Equine Veterinary Science* 28 (12): 753–759.

GfE (Gesellschaft für Ernährungsphysiologie; ed.) (2014): Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere Nr. 11, Empfehlungen zur Energie- und Nährstoffversorgung von Pferden, DLG-Verlag Frankfurt am Main.

Glatter, M.; Breves, G.; Zeyner, A. (2016a): Anwendung von Präbiotika in der Ernährung von Pferden. Tierärztliche Umschau 71: 65-71.

Glatter, M.; Wiedner, K.; Hirche, F.; Mielenz, N.; Hillegeist, D.; Bochnia, M.; Cehak, A.; Bachmann, M.; Greef, J. M.; Glaser, B.; Wolf, P.; Breves, G.; Zeyner, A. (2016b): Fermentation characteristics along the gastrointestinal tract after feeding of Jerusalem artichoke meal to adult healthy warmblood horses. Journal of Animal Nutrition 1: DOI: 10.21767/2572-5459.100016.

Gottschalk, J., Einspanier, A., Ungemach, F.R., Abraham, G. (2011): Influence of topical dexamethasone applications on insulin, glucose, thyroid hormone and cortisol levels in dogs. *Research in Veterinary Science* 90: 491-497.

Huntington, G. B. (1990): Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. *Reproduction Nutrition Development* 30: 35–47.

Ince, J. C.; Longland, A. C.; Moore-Colyer, M. J. S.; Harris, P. A. (2014): *In vitro* degradation of grass fructan by equid gastrointestinal digesta. *Grass Forage Science* 69 (3): 514–523.

Johnson, R. J.; Rivard, C.; Lanaspa, M. A.; Otabachian-Smith, S.; Ishimoto, T.; Cicerchi, C., Cheeke, P. R.; Macintosh, B.; Hess, T. (2013): Fructokinase, fructans, intestinal permeability, and metabolic syndrome: An equine connection? *Journal of Equine Veterinary Science* 33 (2): 120–126.

Julliand, V.; Zeyner, A. (2013): Dietary strategies for optimizing gastro intestinal health: an update on pre- and probiotics. In: *Horse Health Nutrition, Vol.* 6. European Equine Health & Nutrition Congress. Ghent, 75–84.

Kazakos, K. (2011): Incretin effect. GLP-1, GIP, DPP4. *Diabetes Research and Clinical Practice* 93: S32-S36.

Kienzle, E.; Schramme, S. (2004): Beurteilung des Ernährungszustandes mittels Body Condition Scores und Gewichtsschätzung beim adulten Warmblutpferd. *Pferdeheilkunde* 20 (6): 517–524.

Kienzle, E.; Zeyner, A. (2010): The development of a metabolizable energy system for horses. *Journal of Animal Physiology and Animal Nutrition* 94: e231-e240.

Koeller, G.; Gieseler, T.; Schusser, G. F. (2014): Hämatologische und blutchemische Referenzbereiche bei Pferden unterschiedlicher Rasse und Altersgruppen basierend auf neuesten labordiagnostischen Methoden. *Pferdeheilkunde* 30: 381-393.

Kronfeld, D. S.; Treiber, K. H.; Hess, T. M.; Boston, R. C. (2005): Insulin resistance in the horse: Definition, detection, and dietetics. *Journal of Animal Science* 83 (suppl.): E22-E31.

Longland, A. C.; Byrd, B. M. (2006): Pasture nonstructural carbohydrates and equine laminitis. *Journal of Nutrition* 136 (7): 2099S–2102S.

Naumann, K.; Bassler, R. (1976): Die chemische Untersuchung von Futtermitteln, Verband deutscher Landwirtschaftlicher Untersuchungs - und Forschungsanstalten, VDLUFA-Verlag.

Respondek, F.; Myers, K.; Smith, T. L.; Wagner, A.; Geor, R. J. (2011): Dietary supplementation with short-chain fructo-oligosaccharides improves insulin sensitivity in obese horses. *Journal of Animal Science* 89 (1): 77–83.

Roberfroid, M. (2007): Prebiotics: The concept revisited. *Journal of Nutrition* 137: 830S–837S.

Roberfroid, M.; Gibson, G. R.; Hoyles, L.; McCartney, A. L.; Rastall, R.; Rowland, I.; Wolvers, D.; Watzl, B.; Szajewska, H.; Stahl, B.; Guarner, F.; Respondek, F.; Whelan, K.; Coxam, V.; Davicco, M.-J.; Léotoing, L.; Wittrant, Y.; Delzenne, N. M.; Cani, P. D.; Neyrinck, A. M.; Meheust, A. (2010): Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition* 104 (S2): S1-S62.

Robertson, D. M. (2007): Metabolic cross talk between the colon and the periphery: implications for insulin sensitivity. *The Proceedings of the Nutrition Society* 66 (3): 351–361.

Samanta, A. K.; Jayapal, N.; Senani, S.; Kolte, A. P.; Sridhar, M. (2013): Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. *Sociedade Brasileira de Microbiologia* 44 (1): 1–14.

Strauch, S.; Wichert, B.; Greef, J. M.; Hillegeist, D.; Zeyner, A.; Liesegang, A. (2016): Evaluation of an *in vitro* system to simulate equine foregut digestion and the influence of acidity on protein and fructan digestion in the horse´s stomach. *Journal of Animal Physiology and Animal Nutrition* (in press)

Taylor, N. P.; Manjarin, R.; Woodward, A. D.; Holcombe, S. J.; Trottier, N. L. (2012): Transcript profiles of hexose transporters in the equine gastrointestinal tract. *Journal of Equine Veterinary Science* 32 (6): 346–351.

Treiber, K. H.; Boston, R. C.; Kronfeld, D. S.; Staniar, W. B.; Harris, P. A. (2005): Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycaemic meals. *Journal of Animal Science* 83: 2357–2364.

Treiber, K. H.; Kronfeld, D. S.; Geor, R. J. (2006): Insulin resistance in equids: Possible role in laminitis. *Journal of Nutrition* 136 (7): 2094S–2098S.

Van de Wiele, T.; Boon, N.; Possemiers, S.; Jacobs, H.; Verstraete, W. (2007): Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. *Journal of Applied Microbiology* 102 (2): 452–460.

Van Eps, A. W.; Pollit, C. C. (2006): Equine laminitis induced with oligofructose. *Equine Veterinary Journal* 38, 203–208.

Van Eps, A. W.; Pollit, C. C. (2009): Equine laminitis model: Lamellar histopathology seven days after induction with oligofructose. *Equine Veterinary Journal* 41: 735–740.

Vervuert, I.; Voigt, K.; Hollands, T.; Cuddeford, D.; Coenen, M. (2009): Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. *The Veterinary Journal* 182 (1), 67–72.

Zeyner, A.; Bessert, J.; Gropp, J.M. (2002): Effect of feeding exercised horses on high-starch or high-fat concentrates for 390 days. *Equine Veterinary Journal (Suppl.)* 34: 50-57.

Zeyner, A.; Geißler, C.; Dittrich, A. (2004): Effects of hay intake and feeding sequence on variables in faeces and faecal water (dry matter, pH value, organic acids, ammonia, buffering capacity) of horses. *Journal of Animal Physiology and Animal Nutrition* 88: 7–19.

Zeyner, A.; Kirchhof, S.; Susenbeth, A.; Südekum, K.-H.; Kienzle, E. (2015a): A new protein evaluation system for horse feed from literature data. *Journal of Nutritional Sciences* 4: 1-3. DOI: 10.1017/jns.2014.66

Zeyner, A.; Gefrom, A.; Hillegeist, D.; Sommer, M.; Greef, J.M. (2015b): Contribution to the method of sugar analysis in legume grains for ensiling – a pilot study. *International Journal of Scientific Research in Science and Technology* 1: 74-80.

4.4 Paper IV

Estimating compartmental and total tract digestibility in horses using internal and external markers

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Abstract

Partitioning of apparent digestibility is helpful to assess the nutritive value of feedstuffs and to identify possible risks for digestive disorders. In horses, information on partial digestibility is less available and addresses less on carbohydrate fractions. It is suggested that large parts of water-soluble carbohydrates (WSC) are degraded already in the stomach, which elevates the risk for mucosal ulceration. Our objective was to investigate apparent digestibility of dry matter (DM), proximate nutrients, and WSC along the digestive tract using internal and external markers. Twelve adult horses were adapted for 20 d to meadow hay (1.5 kg/100 kg body weight per day) and crushed oats (1.2 g starch/kg body weight per day), which were offered 2 times a day. Six horses on the treatment group also received 0.15 g of fructooligosaccharides + inulin (via Jerusalem artichoke meal) per kg body weight per day. The other 6 horses on the control group received corncob meal without grains. The tested markers were 4N acid insoluble ash (AIA), acid detergent lignin (ADL), *n*-heptacosane, *n*-nonacosane, *n*-hentriacontane, and *n*-tritriacontane. Additionally, Cr_2O_3 (approximately 2.8 g/bolus) and TiO_2 (approximately 2.5 g/bolus) were provided as a bolus 2 times a day. The horses were euthanized at d 21 approximately 1 h after the morning meal. Samples of digesta were collected from the stomach (*pars nonglandularis* and *pars glandularis*), cecum, ventral and dorsal colon ascendens, and colon transversum. Feces were sampled at 5 d ante mortem. Compartmental and total tract apparent digestibility was calculated for each horse. The stomach seemed to be considerably involved in nutrient degradation with particular great disappearance of simple sugars, starch, and fructans (apparent digestibility was up to 78, 74, and 56%). No WSC were measured in the cecum. In the hindgut, apparent digestibility increased from the cecum to the colon transversum (e.g., for DM: 0.49-0.79 with AIA, and 0.44-0.56 with *n*-tritriacontane; for crude protein: 0.51-0.82 with AIA, and 0.47-0.63 with *n*-tritriacontane; and for neutral detergent fiber: 0.43-0.76 with AIA, and 0.37-0.50 with *n*-tritriacontane; $P < 0.05$). Supplying of fructooligosaccharides and inulin had no effect on apparent digestibility. Differences among apparent digestibility estimates did mainly belong to the gut compartments ($P < 0.05$) and the markers ($P < 0.001$). For all nutrients, apparent digestibility estimates did differ less among the plant alkanes, but they had a vast variation among the horses when based on Cr_2O_3 or TiO_2. The extensive

disappearance of WSC, including starch, in the stomach should be considered for dietary composition and risk assessment concerning stomach ulceration. Parts of WSC possibly had not been degraded in the stomach, but rapidly flowed with the liquid phase into the intestine. The probable underestimation of apparent digestibility by ADL confirms the known instability of ADL during the digestive process. Acid insoluble ash probably overestimated apparent digestibility along the hindgut, especially for the fiber fractions. Thus, apparent digestibility seemed to be best estimated by cell wall-associated plant alkanes. The estimations made by Cr_2O_3 and TiO_2 were only accurate for the total tract, considering mealtime feeding of mixed diets.

Keywords: Apparent digestibility; Partitioning; Internal markers; External markers; Water-soluble carbohydrates

1. Introduction

Knowledge on compartmental apparent digestibility of nutrients is helpful to assess the nutritive value of feedstuffs or a whole diet, and to identify possible risks for digestive disorders. Investigations on the nutrient apparent digestibility in single compartments of the digestive tract of horses are very limited (HINTZ et al., 1971; MIYAJI et al., 2008; VARLOUD et al., 2004), and do hardly address more sophisticated carbohydrate fractions. It is generally assumed that cell wall carbohydrates are initially fermented in the hindgut. Recent studies on fermentation products of gut microbes (COENEN et al., 2006; GLATTER et al., 2016) and on *in vitro* digestibility (INCE et al., 2014; STRAUCH et al., 2017) have indicated that besides simple sugars and starch also fructans as further components of water-soluble carbohydrates (WSC) are largely degraded by the time reaching the end of the small intestine, although currently no specific endogenous digestive enzymes are known. To investigate this in a horse model *in vivo*, euthanized horses are required and the use of digestibility markers is the method of choice. Particularly with mealtime feeding, the passage of external markers like Cr_2O_3, TiO_2, lanthanides, or synthetic alkanes is subjected to large variations affected by administration frequency, composition of the diet, and the application form. Internal markers such as

acid insoluble ash (AIA), acid detergent lignin (ADL), and plant alkanes may be more suitable under these conditions (BACHMANN et al., 2016a, 2018b).

The objective of the current study was to investigate the apparent digestibility of dry matter (DM), proximate nutrients, and carbohydrate fractions along the entire digestive tract using euthanized horses, and several internal and external markers. We hypothesized that WSC, including fructans, are degraded to a large extent already in the stomach and the small intestine, and that internal markers of plant origin provide more accurate digestibility estimates than external markers.

2. Materials and methods

Animals, diets, and experimental design

The horses used in this experiment were kept and cared for by the University of Veterinary Medicine Hannover, Foundation. The experiment was performed after approval by the Animal Welfare Commissioner of the University of Veterinary Medicine Hannover, Foundation.

Twelve Warmblood horses (10 mares, 1 stallion, and 1 gelding) with 534 ± 64.5 kg body weight, and between 3 and 24 years, were used in this study. The horses were kept in single boxes on wood shavings and had free access to tap water and a salt block (sodium chloride).

The horses received 1.5 kg of meadow hay per 100 kg body weight per day as fed and crushed oats according to 1.2 g starch/kg body weight per day. Meals were equally offered 2 times a day (09.00 and 15.00 h). Six horses on the treatment group additionally received 0.15 g of inulin-type fructans (i.e., fructooligosaccharides + inulin) per kg body weight per day via Jerusalem artichoke meal (LIVEN GmbH, c/o Lienig Wildfrucht-Verarbeitung, Zossen, Germany). The other 6 horses on the control group received an equivalent quantity of corncob meal without grains. The horses were randomly allocated to the experimental groups. The supplements were offered as part of their daily meals. The nutrient composition of the offered feedstuffs, the supplements, and the whole diet is summarized in Table 1.

Table 1: Chemical composition of the feedstuffs and the whole diet offered during the experiment

Item[1, 2]	Hay	Oats	JAM[2, 3]	CMG[2]	Diet[4]
DM	944	912	939	927	940
Crude ash	52	29	136	26	50
Crude protein (CP)	90	124	63	36	93
pcdCP	40	100	57	16	47
pcdLYS	1.7	3.9	0.9	0.5	1.9
pcdMET+CYS	1.2	4.5	0.6	0.6	1.6
pcdTHR	1.7	3.5	1.0	0.6	1.9
AEE	9	49	6	8	14
Crude fiber	349	124	14	343	318
NDF	651	316	3	799	605
ADF	391	178	11	415	361
ADL	47	31	4	39	44
Starch	n.a.	498	n.a.	n.a.	65
Glucose	30	n.d.	14	9	26
Fructose	39	n.d.	63	10	34
Sucrose	6	11	122	16	8
Fructans	40	n.d.	466	14	38
ME	6.6	12.4	11.9	7.5	7.5

[1] All items are given in g/kg DM, except DM, given in g/kg, and ME, given in MJ/kg DM.

[2] Abbreviations: JAM = Jerusalem artichoke meal, CMG = corncob meal without grains, DM = dry matter, pcdCP = pre-cecal digestible CP, pcdLYS = pre-cecal digestible lysine, pcdMET+CYS = pre-cecal digestible methionine + pre-cecal digestible cysteine, pcdTHR = pre-cecal digestible threonine, AEE = acid ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, n.a. = not analyzed, n.d. = not detected, ME = metabolizable energy.

[3] The uncommon carbohydrate composition of JAM caused partly paradox results in detergent fiber analysis, which was not relevant for diet calculation because of the small quantities of the prebiotic supplement in the diets and cell wall carbohydrates in the supplement.

[4] Shows the nutrients' concentration in the whole diet that consisted of hay, oats, and one of the supplements (JAM or CMG).

The plant markers AIA, ADL, *n*-heptacosane, *n*-nonacosane, *n*-hentriacontane, and *n*-tritriacontane were received as parts of the diet. All 12 horses received the plant markers. Additionally, 6 horses (3 horses from the treatment group, and 3 horses from the control group) were orally dosed by hand with an external marker bolus (BACHMANN et al., 2016b) that contained 138 ± 7.64 g Cr_2O_3/kg DM, and 127 ± 7.37 g TiO_2/kg DM (*n* = 10; approximately 20 g DM per bolus), together with the meals, 2 times a day, for 12 d ante mortem.

The horses were euthanized at d 21, approximately 1 h after they received the morning meal, using pentobarbital (60 mg/kg body weight), following sedation with romifidine (0.12 mg/kg body weight), and anesthesia with diazepam (0.05 mg/kg body weight) and ketamine (2.2 mg/kg body weight).

Sampling and analyses

To estimate apparent digestibility along the digestive tract, representative chyme samples were taken immediately post mortem from the *pars nonglandularis* and *pars glandularis* of the stomach, the cecum, ventral colon ascendens, dorsal colon ascendens, and colon transversum. For this, individual gut compartments were clamped to avoid intermixture of chyme among adjacent compartments before they were opened for sampling. Unfortunately, there was too little material available for collection from the small intestine. Feces were sampled for the estimation of total tract apparent digestibility once a day during the final 5 d and were bulked per horse. The feedstuffs and the supplements were sampled at the beginning of the experiment and stored dry at room temperature. Chyme and feces were stored at –20 °C and lyophilized prior to analyses.

Dry samples were ground to pass a 0.5 mm (for alkanes) or 1.0 mm screen (for nutrients, AIA, ADL, and external markers) using a standard sample mill. Dry matter, crude ash, crude protein (CP; based on Kjeldahl nitrogen), acid ether extract (AEE), crude fiber, neutral detergent fiber (NDF; assayed with heat-stable amylase and expressed exclusive of residual ash), and acid detergent fiber (ADF; expressed exclusive of residual ash) were determined according to VDLUFA (2012; Methods No. 3.1, 4.1.1, 5.1.1 B, 6.1.1, 6.5.1, 6.5.2, and 8.1). The proteins in the feedstuffs were hydrolyzed with hydrochloric acid and essential amino acids were analyzed using ion exchange chromatography (Biochrom 30 with PEEK-Sodium Prewash Column, 100 × 4.6 mm, and PEEK-Oxidised Feedstuff Column, 200 × 4.6 mm; Biochrom Ltd., Cambridge, UK) according to VDLUFA (2012; Method No. 4.11.1). The metabolizable energy content of the feedstuffs was calculated on the basis of crude nutrient analyses according to KIENZLE and ZEYNER (2010) as recommended by the German key-book for nutrient requirements of horses (GFE, 2014). Neutral detergent insoluble CP was determined based on LICITRA et al. (1996) according to VDLUFA (2016; Method No. 4.13.1), and used to calculate the neutral detergent soluble and pre-cecal digestible contents of CP, lysine, methionine + cysteine, and threonine (GFE, 2014; ZEYNER et al., 2010; ZEYNER et al., 2015). Starch in oats and chyme was determined using the amyloglucosidase method (VDLUFA, 2012; Method No. 7.2.5). Glucose, fructose, sucrose, and fructans were determined in the feedstuffs and in chyme of gut compartments by high performance liquid chromatography (KONTRON Instruments HPLC; Tresser Instruments,

Rossdorf, Germany). A refractive index detector (Shodex RI-71; Showa Denko Europe GmbH, Shodex Business, Munich, Germany) and a 100 × 7.8 mm separation column (Rezex™ RPM-Monosaccharide Pb+2; Phenomenex Ltd. Deutschland, Aschaffenburg, Germany) were used. Settings were: 20 µL injection volume, 0.7 mL/min column flow, and 80 °C oven temperature.

Acid insoluble ash was determined according to VDLUFA (2012; Method No. 8.2 A). Ashed sample residues (550 °C) were boiled in 75 mL 4N hydrochloric acid for 15 min, and subsequently filtered. Ash-free filter circles were rinsed to adjust pH-neutrality, and again ashed to determine the AIA residual.

Acid detergent lignin was determined according to VDLUFA (2012; Method No. 6.5.3). Following extraction in 72%-sulphuric acid for 3 h, the filtrate was washed to neutral pH, dried (105 °C), and ashed (500 °C).

Plant alkanes were extracted from the feedstuffs, chyme, and feces and determined by gas chromatography (BACHMANN et al., 2016a). In brief, samples were saponified in ethanolic potassium hydroxide for 4 h at 90 °C, extracted by phase separation into *n*-heptane at 75 ˚C, and purified through silica-gel columns. Internal standards *n*-docosane and *n*-tetratriacontane (98% purity) were priorly added. A gas chromatograph (Shimadzu GC-2010; Shimadzu Corporation, Kyoto, Japan) with flame ionization detection at 315 °C, and a 30 m × 0.53 mm × 0.25 µm separation column (Rtx®-1 w/Integra-Guard; Restek Corporation, Bellefonte, PA, USA) was used. Settings were: on-column injection, 0.5 µL injection volume, injection temperature program: 80 °C hold for 0.1 min, 100 K/min to 310 °C, hold for 10 min, column oven temperature program: 80 °C hold for 0.1 min, 50 K/min to 240 °C, hold for 1 min, 6 K/min to 264 °C, 4 K/min to 284 °C, 2 K/min to 296 °C, hold for 10 min. Helium was used as carrier gas at 30.1 cm/s linear velocity (i.e., a column flow of 3.75 mL/min and pressure of 22.7 kPa), and makeup gas with a flow rate of 30 mL/min. A standard solution contained a homologous sequence of target alkanes (*n*-docosane to *n*-octatriacontane) and was run regularly to identify retention times and to determine the device-internal discrimination of alkanes with increasing molecular weight. Alkane contents were quantified on peak area basis in relation of target and internal standard alkanes, and corrected for any discrimination that might have occurred during solvent extraction (OLIVÁN and OSORO, 1999).

The Cr_2O_3 and TiO_2 external markers were extracted from bolus, chyme, and feces samples (BOGUHN et al., 2009; WILLIAMS et al., 1962), and analyzed using an

inductively coupled plasma optical emission spectrometer (Varian 715-ES; Agilent Technologies Inc., Palo Alto, CA, USA).

Calculations and statistical analysis

Apparent digestibility coefficients of DM, CP, AEE, NDF, ADF, starch, mono- and disaccharides (i.e., glucose + fructose + sucrose), and fructans were calculated at the end of each gut compartment and for the total tract of each horse as follows:

$$AD = 1 - \frac{h \times H_i + c \times C_i + s \times S_i}{F_i} \times \frac{F_n}{h \times H_n + c \times C_n + s \times S_n}$$

where, H_i, C_i, S_i, and F_i are the concentration of marker *i*, and H_n, C_n, S_n, and F_n are the concentration of nutrient *n* (g/kg DM) in hay, concentrate, supplement, and feces, respectively; and *h*, *c*, and *s* are the proportions of hay, concentrate, and supplement in the diets. These proportions were calculated as follows:

$$h = \frac{H}{H + C + S} \text{ , } c = \frac{C}{H + C + S} \text{ , and } s = \frac{S}{H + C + S}$$

where *H*, *C*, and *S* are the quantity (kg/day or kg/meal) of hay, concentrate, and supplement, respectively.

Statistical analysis was performed using SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA). Least squares means were estimated for apparent digestibility coefficients, obtained from the plant markers, separately for the stomach and hindgut compartments, respectively, and the differences among them were tested for significance. The significance level was at $P < 0.05$. The following model was used:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \underline{a}_l + \underline{e}_{ijkl}$$

where, y_{ijkl} is apparent digestibility; μ is the general mean; α_i is the fixed effect of compartment *i* (*i* = 1, 2; 1 = *pars nonglandularis*, 2 = *pars glandularis*; or *i* = 1, …, 5; 1 = cecum, 2 = ventral colon ascendens, 3 = dorsal colon ascendens, 4 = colon transversum, and 5 = feces); β_j is the fixed effect of marker *j* (*j* = 1, …, 6; 1 = AIA, 2 = ADL, 3 = *n*-heptacosane, 4 = *n*-nonacosane, 5 = *n*-hentriacontane, and 6 = *n*-tritriacontane); γ_k is the fixed effect of treatment *k* (*k* = 1, 2; 1 = Jerusalem artichoke meal, and 2 = corncob meal without grains); $\alpha\beta_{ij}$ is the interaction of fixed effects α_i and β_j; $\underline{a}_l$ is the random effect of horse *l* (*l* = 1, …, 12); and $\underline{e}_{ijkl}$ is the random residual effect. Apparent digestibility coefficients lower than zero were not considered. After excluding coefficients lower than zero, a total of 8-10 measurements per marker was

available from the stomach data, except for ADL, and 39-56 measurements per marker from the hindgut data. Apparent digestibility of mono- and disaccharides and fructans along the hindgut compartments was consistently 100% and was therefore not considered for statistical analysis.

Compartmental apparent digestibility coefficients estimated by the external markers Cr_2O_3 and TiO_2 showed a variation among the horses far outside a usual 0-100% range. Moreover, very few data remained available after removing estimates lower than zero. Therefore, only total tract apparent digestibility, based on the analysis of colon transversum chyme and feces, was considered. The following model was used:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \underline{a}_k + \underline{e}_{ijk}$$

where, y_{ijk} is apparent digestibility; μ is the general mean; α_i is the fixed effect of treatment i (i = 1, 2; 1 = Jerusalem artichoke meal, and 2 = corncob meal without grains); β_j is the fixed effect of marker j (j = 1, …, 8; 1 = AIA, 2 = ADL, 3 = *n*-heptacosane, 4 = *n*-nonacosane, 5 = *n*-hentriacontane, 6 = *n*-tritriacontane, 7 = Cr_2O_3, and 8 = TiO_2); $\alpha\beta_{ij}$ is the interaction of fixed effects α_i and β_j; $\underline{a}_k$ is the random effect of horse k (k = 1, …, 6); and $\underline{e}_{ijkl}$ is the random residual effect. After excluding coefficients lower than zero, a total of 24 measurements per marker (13-22 and 12 measurements per marker for AEE and starch apparent digestibility, respectively) was available in case of plant markers, and 12 measurements per marker (6 measurements per marker for starch apparent digestibility) in case of Cr_2O_3 and TiO_2.

3. Results

Apparent digestibility mostly increased until reaching the end of the *pars glandularis* (Table 2), but it mostly did not differ among the markers. Effect interactions did not exist. Apparent digestibility of WSC (i.e., starch, mono- and disaccharides, and fructans) was consistently great, both at the end of the *pars nonglandularis* (0.40-0.55 for starch, 0.24-0.53 for mono- and disaccharides, and 0.37-0.46 for fructans), and the *pars glandularis* (0.65-0.74 for starch, 0.72-0.78 for mono- and disaccharides, and 0.46-0.56 for fructans; Table 2). The Jerusalem artichoke meal supplementation did not affect apparent digestibilities at the end of the stomach.

Table 2: Least squares means of apparent digestibility coefficients of dry matter, crude protein, acid ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, mono- and disaccharides[1], and fructans along the stomach compartments, estimated using plant markers under near steady-state conditions.

Item[2]	Marker[3]	*Pars nonglandularis*	*Pars glandularis*
Dry matter	AIA	0.38 ± 0.07	0.47 ± 0.09^{AB}
	n-Heptacosane	0.29 ± 0.09	0.38 ± 0.06^{B}
	n-Nonacosane	0.33 ± 0.08^{b}	0.47 ± 0.07^{aAB}
	n-Hentriacontane	0.39 ± 0.08	0.52 ± 0.07^{A}
	n-Tritriacontane	0.44 ± 0.08	0.50 ± 0.07^{A}
Crude protein	AIA	0.37 ± 0.09	0.49 ± 0.10^{AB}
	n-Heptacosane	0.28 ± 0.11	0.41 ± 0.08^{B}
	n-Nonacosane	0.30 ± 0.10^{b}	0.51 ± 0.08^{aAB}
	n-Hentriacontane	0.36 ± 0.10^{b}	0.56 ± 0.08^{aA}
	n-Tritriacontane	0.43 ± 0.10	0.54 ± 0.08^{AB}
Acid ether extract	AIA	0.47 ± 0.10	0.58 ± 0.13
	n-Heptacosane	0.52 ± 0.13	0.60 ± 0.08
	n-Nonacosane	0.52 ± 0.12	0.69 ± 0.09
	n-Hentriacontane	0.57 ± 0.12	0.72 ± 0.09
	n-Tritriacontane	0.61 ± 0.12	0.70 ± 0.09
NDF	AIA	0.43 ± 0.07	0.41 ± 0.08^{A}
	n-Heptacosane	0.29 ± 0.08	0.24 ± 0.06^{B}
	n-Nonacosane	0.32 ± 0.07	0.34 ± 0.06^{AB}
	n-Hentriacontane	0.38 ± 0.07	0.41 ± 0.06^{A}
	n-Tritriacontane	0.44 ± 0.07	0.38 ± 0.06^{A}
ADF	AIA	0.41 ± 0.06	0.46 ± 0.08^{A}
	n-Heptacosane	0.28 ± 0.09	0.22 ± 0.05^{B}
	n-Nonacosane	0.30 ± 0.07	0.32 ± 0.06^{AB}
	n-Hentriacontane	0.37 ± 0.07	0.39 ± 0.06^{A}
	n-Tritriacontane	0.43 ± 0.07	0.35 ± 0.06^{A}
Starch	AIA	0.42 ± 0.12^{b}	0.65 ± 0.13^{a}
	n-Heptacosane	0.40 ± 0.14^{b}	0.65 ± 0.11^{a}
	n-Nonacosane	0.48 ± 0.13^{b}	0.71 ± 0.11^{a}
	n-Hentriacontane	0.53 ± 0.13^{b}	0.74 ± 0.11^{a}
	n-Tritriacontane	0.55 ± 0.13	0.72 ± 0.11
Mono- and disaccharides	AIA	0.53 ± 0.08^{bA}	0.74 ± 0.10^{a}
	n-Heptacosane	0.24 ± 0.10^{bB}	0.72 ± 0.07^{a}
	n-Nonacosane	0.38 ± 0.09^{bAB}	0.75 ± 0.07^{a}
	n-Hentriacontane	0.45 ± 0.09^{bAB}	0.78 ± 0.07^{a}
	n-Tritriacontane	0.47 ± 0.09^{bA}	0.78 ± 0.07^{a}
Fructans	AIA	0.37 ± 0.09	0.53 ± 0.10
	n-Heptacosane	0.37 ± 0.11	0.46 ± 0.09
	n-Nonacosane	0.38 ± 0.10	0.51 ± 0.09
	n-Hentriacontane	0.43 ± 0.10	0.56 ± 0.09
	n-Tritriacontane	0.46 ± 0.10	0.52 ± 0.09

[1] Equals the sum of glucose, fructose, and sucrose contents.
[2] NDF = neutral detergent fiber, ADF = acid detergent fiber.
[3] AIA = acid insoluble ash.
[a, b] Within a row, different superscripts show differences among compartments within same marker with $P < 0.05$.
[A, B] Within a column, different superscripts show differences among markers within same compartment with $P < 0.05$.

Along the hindgut compartments, apparent digestibility increased gradually from cecum to colon transversum ($P < 0.05$), except for AEE, where the greatest estimates were found at the end of the cecum ($P < 0.05$; Table 3). Apparent digestibility did largely differ among the markers ($P < 0.001$), except among the alkanes (Table 3). An interaction between the effects did not exist. The Jerusalem artichoke meal treatment had no effect on compartmental apparent digestibility along the hindgut. The apparent digestibility of starch did not differ among the hindgut compartments or the used markers, and was consistently above 0.90 (Table 3).

Table 3: Least squares means of compartmental[1] and total tract apparent digestibility coefficients of dry matter, crude protein, acid ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), and starch, estimated using plant markers under near steady-state conditions.

Item[2]	Marker[3]	CEC	VCA	DCA	CT	Total tract
Dry matter	AIA	0.50 ± 0.04^{cA}	0.69 ± 0.04^{bA}	0.64 ± 0.04^{bA}	0.79 ± 0.04^{aA}	0.79 ± 0.04^{aA}
	ADL	0.27 ± 0.04^{bD}	0.37 ± 0.04^{aC}	0.35 ± 0.04^{abC}	0.41 ± 0.04^{aC}	0.43 ± 0.05^{aC}
	n-Heptacosane	0.34 ± 0.04^{cC}	0.45 ± 0.04^{bB}	0.49 ± 0.04^{bB}	0.58 ± 0.04^{aB}	0.58 ± 0.04^{aB}
	n-Nonacosane	0.38 ± 0.04^{cBC}	0.45 ± 0.04^{bcB}	0.47 ± 0.04^{bB}	0.59 ± 0.04^{aB}	0.59 ± 0.04^{aB}
	n-Hentriacontane	0.43 ± 0.04^{cABC}	0.47 ± 0.04^{bcB}	0.54 ± 0.04^{abB}	0.61 ± 0.04^{aB}	0.60 ± 0.04^{aB}
	n-Tritriacontane	0.45 ± 0.04^{bAB}	0.47 ± 0.04^{bB}	0.52 ± 0.04^{abB}	0.58 ± 0.04^{aB}	0.56 ± 0.04^{aB}
Crude protein	AIA	0.52 ± 0.05^{cA}	0.70 ± 0.04^{bA}	0.64 ± 0.04^{bA}	0.82 ± 0.04^{aA}	0.79 ± 0.04^{aA}
	ADL	0.36 ± 0.05^{abB}	0.38 ± 0.05^{abB}	0.35 ± 0.05^{bC}	0.47 ± 0.05^{aC}	0.42 ± 0.06^{abC}
	n-Heptacosane	0.39 ± 0.04^{dB}	0.48 ± 0.04^{bdB}	0.50 ± 0.04^{bcB}	0.62 ± 0.04^{aB}	0.60 ± 0.04^{acB}
	n-Nonacosane	0.42 ± 0.05^{bAB}	0.49 ± 0.04^{bB}	0.47 ± 0.04^{bB}	0.64 ± 0.05^{aB}	0.61 ± 0.04^{aB}
	n-Hentriacontane	0.46 ± 0.05^{bAB}	0.49 ± 0.04^{bB}	0.56 ± 0.05^{abAB}	0.66 ± 0.05^{aB}	0.61 ± 0.04^{aB}
	n-Tritriacontane	0.47 ± 0.05^{bAB}	0.49 ± 0.05^{bB}	0.54 ± 0.05^{abAB}	0.63 ± 0.05^{aB}	0.58 ± 0.04^{abB}

Acid ether extract	AIA	0.62 ± 0.05	0.66 ± 0.05^{A}	0.59 ± 0.05^{A}	0.66 ± 0.05^{A}	0.61 ± 0.05^{A}
	ADL	0.52 ± 0.06^{a}	0.30 ± 0.05^{bB}	0.28 ± 0.06^{bC}	0.21 ± 0.06^{bcC}	0.06 ± 0.06^{cC}
	n-Heptacosane	0.52 ± 0.05^{a}	0.38 ± 0.05^{bB}	0.40 ± 0.05^{abB}	0.35 ± 0.05^{bB}	0.25 ± 0.05^{cB}
	n-Nonacosane	0.53 ± 0.05^{a}	0.39 ± 0.05^{bB}	0.39 ± 0.05^{bBC}	0.41 ± 0.05^{aBC}	0.27 ± 0.05^{cB}
	n-Hentriacontane	0.56 ± 0.06^{a}	0.41 ± 0.05^{bB}	0.44 ± 0.05^{abB}	0.44 ± 0.05^{abB}	0.28 ± 0.05^{cB}
	n-Tritriacontane	0.61 ± 0.06^{a}	0.40 ± 0.05^{bB}	0.44 ± 0.06^{bB}	0.40 ± 0.06^{bB}	0.21 ± 0.05^{cB}
NDF	AIA	0.43 ± 0.05^{cA}	0.65 ± 0.04^{bA}	0.60 ± 0.04^{bA}	0.76 ± 0.04^{aA}	0.75 ± 0.04^{aA}
	ADL	0.15 ± 0.05^{bCD}	0.28 ± 0.04^{aC}	0.27 ± 0.05^{aC}	0.30 ± 0.05^{aC}	0.34 ± 0.05^{aC}
	n-Heptacosane	0.25 ± 0.04^{cC}	0.39 ± 0.04^{abB}	0.44 ± 0.04^{aB}	0.50 ± 0.04^{aB}	0.51 ± 0.04^{aB}
	n-Nonacosane	0.29 ± 0.05^{cBC}	0.39 ± 0.04^{bcB}	0.42 ± 0.04^{bB}	0.52 ± 0.05^{aB}	0.52 ± 0.04^{aB}
	n-Hentriacontane	0.34 ± 0.05^{cABC}	0.40 ± 0.04^{bcB}	0.49 ± 0.05^{abB}	0.54 ± 0.05^{aB}	0.53 ± 0.04^{aB}
	n-Tritriacontane	0.37 ± 0.05^{bAB}	0.40 ± 0.05^{abB}	0.46 ± 0.05^{abB}	0.50 ± 0.05^{aB}	0.49 ± 0.04^{aB}
ADF	AIA	0.44 ± 0.05^{cA}	0.64 ± 0.04^{bA}	0.59 ± 0.04^{bA}	0.75 ± 0.04^{aA}	0.74 ± 0.04^{aA}
	ADL	0.14 ± 0.05^{bC}	0.26 ± 0.04^{aC}	0.26 ± 0.05^{aC}	0.29 ± 0.05^{aC}	0.32 ± 0.05^{aC}
	n-Heptacosane	0.27 ± 0.04^{cB}	0.37 ± 0.04^{bB}	0.42 ± 0.04^{abB}	0.49 ± 0.04^{aB}	0.49 ± 0.04^{aB}
	n-Nonacosane	0.28 ± 0.05^{cB}	0.38 ± 0.04^{bB}	0.41 ± 0.04^{bB}	0.51 ± 0.05^{aB}	0.50 ± 0.04^{aB}
	n-Hentriacontane	0.33 ± 0.05^{cAB}	0.39 ± 0.04^{bcB}	0.48 ± 0.05^{abB}	0.53 ± 0.05^{aB}	0.51 ± 0.04^{aB}
	n-Tritriacontane	0.36 ± 0.05^{bAB}	0.38 ± 0.05^{abB}	0.45 ± 0.05^{abB}	0.50 ± 0.05^{aB}	0.46 ± 0.04^{aB}
Starch[4]	AIA	0.96 ± 0.03	0.98 ± 0.02	0.98 ± 0.02	0.99 ± 0.02	n.a.
	ADL	0.94 ± 0.03	0.96 ± 0.02	0.97 ± 0.03	0.97 ± 0.03	n.a.
	n-Heptacosane	0.95 ± 0.02	0.96 ± 0.02	0.97 ± 0.02	0.98 ± 0.02	n.a.
	n-Nonacosane	0.95 ± 0.03	0.96 ± 0.02	0.96 ± 0.02	0.98 ± 0.03	n.a.
	n-Hentriacontane	0.95 ± 0.03	0.96 ± 0.02	0.98 ± 0.03	0.98 ± 0.03	n.a.
	n-Tritriacontane	0.94 ± 0.03	0.97 ± 0.03	0.97 ± 0.03	0.98 ± 0.03	n.a.

[1] CEC = cecum, VCA = ventral colon ascendens, DCA = dorsal colon ascendens, CT = colon transversum.

[2] NDF = neutral detergent fiber, ADF = acid detergent fiber.

[3] AIA = acid insoluble ash, ADL = acid detergent lignin.

[4] n.a. = not analyzed.

[a, b, c] Within a row, different superscripts show differences among compartments within same marker with $P < 0.05$.

[A, B, C, D] Within a column, different superscripts show differences among markers within same compartment with $P < 0.05$.

Between the 1.3-fold and 1.9-fold increased total tract apparent digestibility of DM and proximate nutrients was found in Jerusalem artichoke meal vs. corncob meal without grains treated horses when using Cr_2O_3 and TiO_2 as markers ($P < 0.05$), but not when using the plant markers (Table 4). Overall, there was no treatment effect. There was an effect of the marker ($P < 0.001$). No treatment × marker interaction was found.

Table 4: Least squares means of total tract apparent digestibility coefficients[1] of dry matter, crude protein, acid ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), and starch, estimated using external markers (Cr_2O_3 and TiO_2; offered by bolus 2 times a day) and plant markers under near steady-state conditions.

Item[2]	Treatment[3]	Cr_2O_3	TiO_2	AIA[4]	ADL[4]	*n*-Heptacosane	*n*-Nonacosane	*n*-Hentriacontane	*n*-Tritriacontane
Dry matter	CMG	0.47 ± 0.06^{bcB}	0.49 ± 0.06^{bcB}	0.80 ± 0.04^{a}	0.43 ± 0.04^{c}	0.57 ± 0.04^{b}	0.57 ± 0.04^{b}	0.59 ± 0.04^{b}	0.53 ± 0.04^{b}
	JAM	0.63 ± 0.05^{bA}	0.65 ± 0.05^{bA}	0.78 ± 0.04^{a}	0.43 ± 0.04^{d}	0.58 ± 0.04^{bc}	0.57 ± 0.04^{bc}	0.56 ± 0.04^{bc}	0.52 ± 0.04^{c}
Crude protein	CMG	0.51 ± 0.06^{bcB}	0.53 ± 0.06^{bcB}	0.81 ± 0.04^{a}	0.46 ± 0.04^{c}	0.60 ± 0.04^{b}	0.60 ± 0.04^{b}	0.61 ± 0.04^{b}	0.56 ± 0.04^{b}
	JAM	0.67 ± 0.04^{bA}	0.68 ± 0.04^{bA}	0.80 ± 0.04^{a}	0.49 ± 0.04^{d}	0.63 ± 0.04^{bc}	0.62 ± 0.04^{bc}	0.61 ± 0.04^{bc}	0.57 ± 0.04^{c}
Acid ether extract	CMG	0.19 ± 0.09^{bcd}	0.23 ± 0.09^{bc}	0.65 ± 0.06^{a}	0.15 ± 0.07^{c}	0.33 ± 0.06^{b}	0.34 ± 0.06^{b}	0.36 ± 0.06^{b}	0.29 ± 0.06^{b}
	JAM	0.36 ± 0.07^{b}	0.38 ± 0.07^{b}	0.62 ± 0.06^{a}	0.11 ± 0.08^{c}	0.28 ± 0.06^{b}	0.33 ± 0.06^{b}	0.34 ± 0.06^{b}	0.29 ± 0.06^{b}
NDF	CMG	0.39 ± 0.07^{bcB}	0.41 ± 0.07^{bcB}	0.77 ± 0.05^{a}	0.35 ± 0.05^{c}	0.51 ± 0.05^{b}	0.51 ± 0.05^{b}	0.53 ± 0.05^{b}	0.46 ± 0.05^{b}
	JAM	0.57 ± 0.05^{bcA}	0.59 ± 0.05^{bA}	0.74 ± 0.05^{a}	0.33 ± 0.05^{d}	0.51 ± 0.05^{bc}	0.49 ± 0.05^{bc}	0.48 ± 0.05^{bc}	0.43 ± 0.05^{c}
ADF	CMG	0.38 ± 0.07^{bcB}	0.40 ± 0.07^{bcB}	0.76 ± 0.05^{a}	0.33 ± 0.05^{c}	0.50 ± 0.05^{b}	0.50 ± 0.05^{b}	0.52 ± 0.05^{b}	0.45 ± 0.05^{b}
	JAM	0.55 ± 0.05^{bA}	0.57 ± 0.05^{bA}	0.72 ± 0.05^{a}	0.30 ± 0.05^{d}	0.49 ± 0.05^{bc}	0.47 ± 0.05^{bc}	0.46 ± 0.05^{bc}	0.41 ± 0.05^{c}
Starch	CMG	0.97 ± 0.01^{bc}	0.97 ± 0.01^{bc}	0.99 ± 0.01^{a}	0.97 ± 0.01^{c}	0.98 ± 0.01^{bc}	0.98 ± 0.01^{b}	0.98 ± 0.01^{b}	0.98 ± 0.01^{bc}
	JAM	0.98 ± 0.01^{bc}	0.98 ± 0.01^{b}	0.99 ± 0.01^{a}	0.97 ± 0.01^{c}	0.98 ± 0.01^{b}	0.98 ± 0.01^{b}	0.98 ± 0.01^{bc}	0.98 ± 0.01^{bc}

[1] Includes marker and nutrient information, respectively, from colon transversum chyme and feces.
[2] NDF = neutral detergent fiber, ADF = acid detergent fiber.
[3] CMG = corncob meal without grains, JAM = Jerusalem artichoke meal. JAM was dosed to achieve 0.15 g of fructooligosaccharides + inulin per kg body weight per day.
[4] AIA = acid insoluble ash, ADL = acid detergent lignin.
[a, b, c, d] Within a row, different superscripts show differences among markers within same treatment with $P < 0.05$.
[A, B] Within a column, different superscripts show differences among treatments within same marker with $P < 0.05$.

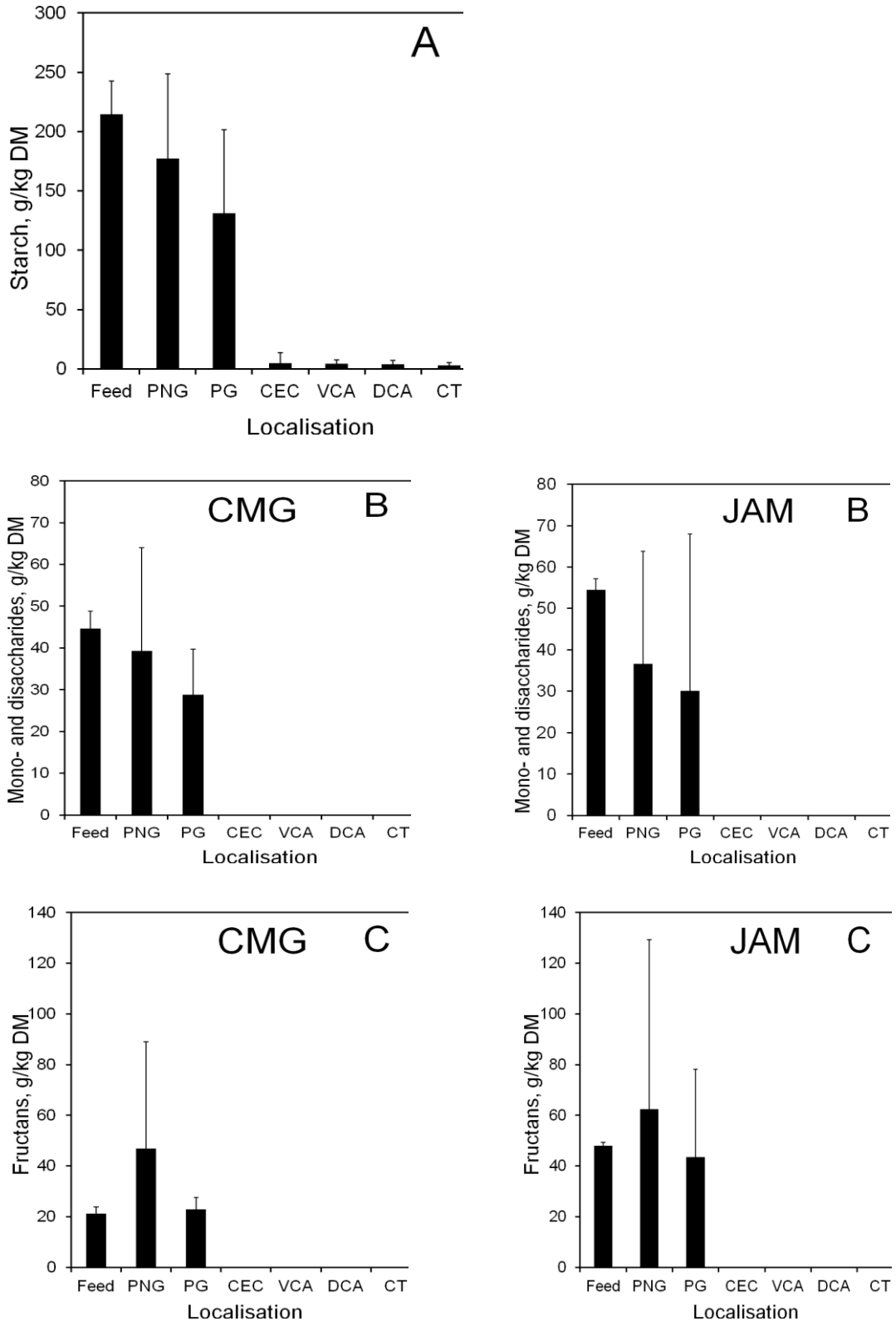

Fig. 1: Concentrations of starch (A), mono- and disaccharides (i.e., glucose + fructose + sucrose) (B), and fructans (C) in feed (i.e., the morning meal as specified in the text), and the chyme of stomach and hindgut compartments (means and standard deviations; n = 12 horses); mono- and disaccharides and fructans had systematically different concentrations in the diets according to CMG or JAM treatment; CEC = cecum, CMG = corncob meal without grains, CT = colon transversum, DCA = dorsal colon ascendens, DM = dry matter, JAM = Jerusalem artichoke meal, PG = *pars glandularis*, PNG = *pars nonglandularis*, VCA = ventral colon ascendens.

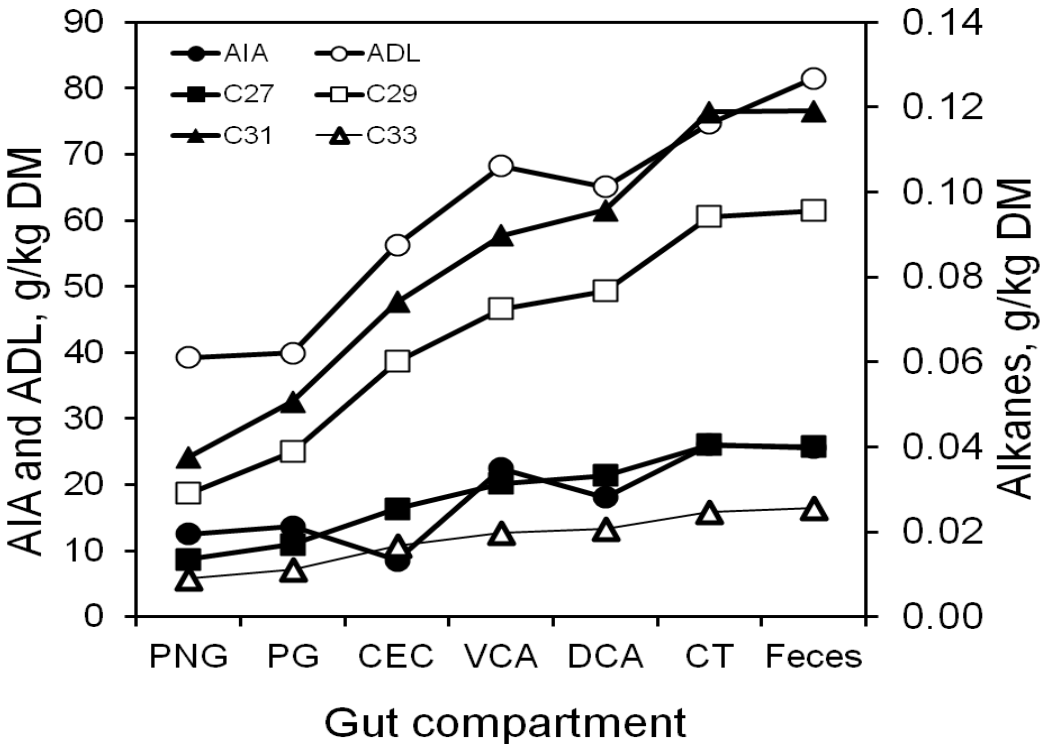

Fig. 2: Concentrations of acid insoluble ash (AIA), acid detergent lignin (ADL), and of plant alkanes *n*-heptacosane (C27), *n*-nonacosane (C29), *n*-hentriacontane (C31), and *n*-tritriacontane (C33) in chyme of stomach and hindgut compartments (means; n = 12 horses; for clarity, error bars are omitted); CEC = cecum, CT = colon transversum, DCA = dorsal colon ascendens, DM = dry matter, PG = *pars glandularis*, PNG = *pars nonglandularis*, VCA = ventral colon ascendens.

4. Discussion

The apparent digestibility of nutrients is defined as the relation of a nutrient's concentration in the diet and its concentration in chyme (i.e., compartmental apparent digestibility), or feces (i.e., total tract apparent digestibility). The part of the nutrient that is not recovered in chyme or feces is assumed to be degraded either by digestive enzymes, microbes, plant enzymes (from feed), or acid hydrolysis (in the stomach). In case of compartmental apparent digestibility, parts of nutrients can also disappear simply because of an outflow into the following compartment at a faster pace. The apparent digestibility is a crude measure and does not distinguish between undigested residues and endogenous excretion of the nutrient into the lumen (PAGAN, 1998).

The chyme available in the hindgut compartments at sampling did probably contain a mixture of nutrients descending from the morning meal and the meals until 2 d before, considering approximately 26 to 43 h total mean retention time of solid

digesta (PAGAN et al., 1998; ROSENFELD and AUSTBØ, 2009). For the hindgut compartments, apparent digestibility coefficients were therefore calculated on the basis of whole-diet nutrient and marker concentrations. However, the chyme available in the stomach likely contained nutrients only descending from the morning meal offered approximately 1 h ante mortem after overnight feed withdrawal. Therefore, apparent digestibility coefficients for the stomach were calculated on the basis of nutrient and marker concentrations from the morning meal. To calculate the correct proportions of the nutrient and marker concentrations that descend from hay, concentrate (oats), or supplements in the mixed morning meal, we considered the whole offered quantity of oats and supplements, respectively, but only 10% of the daily diet (i.e., 0.592 to 0.936 kg) in hay, calculated on the basis of a roughage intake time of approximately 39 min/kg (MEYER et al., 1975).

In the current study, especially the apparent digestibility of NDF and ADF seemed considerably great and probably overestimated along the stomach compartments. However, similar estimates were reported by VARLOUD et al. (2004). Pre-cecal fiber digestion also has been described by HINTZ et al. (1971). A partial degradation of fibers at the end of the stomach is, in our view, most likely conceivable through acid hydrolysis and microbial activity, but probably not to the estimated extent. The concentration of WSC decreased stepwise from the diet (i.e., the morning meal) until the end of the *pars glandularis* (Fig. 1). The apparent increase of the concentration of fructans in the *pars nonglandularis* was probably an issue of the large variation of measurements, but might also be explained as a relative increase when soluble starch and mono- and disaccharides more rapidly flowed out the stomach or have been degraded. Polymeric and oligomeric fructans, however, require more complex fermentation and hydrolysis, dependent on their type and degree of polymerization, before net degradation of total fructans becomes measurable (INCE et al., 2014). The disappearance of WSC we found at the end of the stomach is partly due to an outflow with the liquid digesta into the small intestine before a sample was taken. The determined apparent digestibility coefficients of DM, starch, mono- and disaccharides, and fructans, however, also confirmed an active microflora that primarily degrades readily soluble carbohydrates by the time reaching the end of the *pars nonglandularis* (COSTA et al., 2015; GLATTER et al., 2015, 2016; VARLOUD et al., 2004, 2007). Increased availability of WSC in the stomach led to increased production of volatile fatty acids, and to an elevated bacterial diversity (GLATTER et

al., 2015, 2016). In the current study, starch apparent digestibility at the end of the stomach based on ADL or AIA estimates ranged between 0.14 and 0.90 for individual horses, which is in line with VARLOUD et al. (2004) both in absolute terms and regarding the high variation. Particularly the apparent digestibility of starch, mono- and disaccharides, and fructans, but also the apparent digestibility of DM and the crude nutrients, increased until reaching the end of the *pars glandularis*. Apart from the outflow of dissolved nutrients and microbial degradation, we can also assume acting of plant hydrolases and acid hydrolysis in the stomach (MUELLER, 1995; STRAUCH et al., 2017). We suppose that liquid chyme, small particles, and dissolved components (e.g., WSC, peptides, and amino acids) pass the stomach past the layered solid chyme (MERRITT, 2003), which remains for a longer time to facilitate grounding (and partial degradation) also of fibrous materials. This would explain very fast postprandial blood glucose and amino acid responses on the one hand (BACHMANN et al., 2018a; BOCHNIA et al., 2017; GLATTER et al., 2017; VERVUERT et al., 2009; ZEYNER et al., 2017), but well filled stomachs that have been frequently observed, even after fasting periods, on the other hand. The apparent digestibility of CP and AEE up to 0.56 and 0.72, respectively, might reflect pepsin acting in the *pars glandularis*, and probably also acting of pre-duodenal lipases under acetic conditions (DE NIGIRS et al., 1988; EMBLETON and POUTON, 1997; MERRITT, 2003; STRAUCH et al., 2017).

As with the foregut, apparent digestibility coefficients estimated along the hindgut compartments may be very variable, particularly due to the feeding regimen. Moreover, approximately 90% of the equine microbiome is “instable” to environmental impacts such as the dietary composition and largely differs among individuals (BLACKMORE et al., 2013; DOUGAL et al., 2013). The opportunity to compare to individual published data is therefore limited. The comparison with a meta-analysis based on 287 digestibility trials revealed that current apparent digestibility estimates of proximate nutrients were in the range of what is known for the nutrient composition of the diet (ZEYNER and KIENZLE, 2002). Forage quality and forage quantity might affect ADL resistance against digestion (i.e., 89% recovery from straw, but 59% recovery from hay) (MIRAGLIA et al., 1999; VARLOUD et al., 2004), so that there is clear evidence that ADL underestimates apparent digestibility of DM and crude nutrients in mixed hay + concentrate diets to varying degrees. Acid detergent lignin seemed to get lost particularly in the dorsal colon ascendens, as

shown in Fig. 2. In horses, fecal recovery of long-chain alkanes, especially *n*-hentriacontane and *n*-tritriacontane, is mostly near complete (Bachmann et al., 2018b), and these markers' concentrations were consistently increasing in chyme, reflecting their relatively constant indigestibility (Fig. 2). As part of surface plant waxes, long-chain alkanes are located epi- and intracuticular, and especially intracuticular alkanes are strongly bound to the plants' cell walls, which is why the apparent digestibility of fibers seems better being estimated using these alkanes. Along the hindgut compartments, the determined apparent digestibility of starch, mono- and disaccharides, and fructans was consistently above 0.90, which actually indicates that these carbohydrates were almost absent in chyme after passing the stomach and the small intestine (Fig. 1).

In this study, external markers, which were dosed orally by bolus, failed to reliably estimate compartmental apparent digestibility in horses with mealtime feeding; only total tract apparent digestibility was estimated without excessive variation among the horses. Pre-ileal apparent digestibility of CP, AEE, and starch from oats and maize dominated diets were successfully obtained with Cr_2O_3 mixed into the diet (RADICKE, 1990; WILKE, 1992), but large variation among the horses, negative, and implausible apparent digestibility coefficients were likewise reported (WILKE, 1992). Moreover, the amount of coarse particles was small in these experimental diets. Both may led to a more uniform mixing of marker and gut contents. We administered Cr_2O_3 and TiO_2 as pulse dose by bolus 2 times a day. Marker intake can be better controlled with frequent administration. However, uniform mixing into the chyme was obviously not given. Then, the markers have shown pronounced diurnal cycling in chyme and feces driven by administration frequency (BACHMANN et al., 2016a); and this hindered representative spot sampling. External markers are known to easily migrate from coarse to fine feed particles or into liquids while passing the digestive tract (BACHMANN et al., 2018b). However, in horses, coarse and fine particles are more or less parallel passing, and consequentially, net selective retention of any particle class seems negligible (HUMMEL et al., 2018). Having a sufficient mixing of marker and chyme, marker migration thus should not considerably affect apparent digestibility estimation. We partially observed the adsorption of the green-colored Cr_2O_3 at the gastric mucosa, which remained even after thorough washing. It is currently not known whether such an adsorption may alter marker passage and is probably contributing to the high variation of apparent digestibility estimates.

In contrast to the plant marker based compartmental apparent digestibility estimates, Cr_2O_3 and TiO_2 revealed greater total tract apparent digestibility of DM and nutrients in favor of the Jerusalem artichoke meal supplementation. The provided dose of inulin-type fructans (0.15 g/kg body weight per day) was close to that assumed to be effective in a prebiotic scope (JULLIAND and ZEYNER, 2013). It did mainly led to an increase of acetic acid, *n*-butyric acid, ammonia, and lactate isomers in stomach contents of the same horses (GLATTER et al., 2016). However, this was not reflected by apparent digestibility coefficients because of a large variation among individual horses. The supplemented fructans were fermented in the stomach, hydrolyzed, and absorbed in the small intestine, and did rather not reach the hindgut. The observed greater apparent nutrient digestibilities in the horses that received the Jerusalem artichoke meal supplement were probably biased due the small number of available measurements.

5. Conclusions

The results have shown that starch, mono- and disaccharides, and fructans from the feed are largely disappeared in the chyme before reaching the cecum, which probably suggests a considerable degradation in the foregut. Already the stomach seems to have capacities to degrade WSC including soluble starch. This must be considered in the feeding practice to prevent gastric ulceration and related syndromes. Fructans, which are applied as prebiotics target to act in the hindgut (i.e., fructooligosaccharides + inulin at a dose of 0.15 g/kg body weight per day), might not completely reach it. This should be considered by manufacturers, distributors, veterinarians, and horse owners.

For partitioning of apparent digestibility estimation among different compartments of the digestive tract, ADL is not suitable. It mainly underestimates apparent digestibility because of its incomplete resistance against digestion. The AIA marker seemed suitable to estimate apparent digestibility of DM, CP, fiber, starch, mono- and disaccharides, and fructans at the end of the stomach, but it led to particularly high estimates along the hindgut compartments. Here, the nutrients' apparent digestibility, and especially the apparent digestibility of fiber fractions, might be better estimated by alkanes because of their cell wall associated nature. In this study, the use of external markers, applied repeatedly by bolus, was only accurate for the estimation of total tract apparent digestibility. A real validation of marker-based compartmental

estimates of apparent digestibility would be required (e.g., using the nylon bag method), but it is not feasible in every compartment of the digestive tract.

Conflict of interest statement

None of the authors have financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References

Bachmann, M., Wensch-Dorendorf, M., Bulang, M., Zeyner, A. (2016a): Impact of dynamics of faecal concentrations of plant and synthetic *n*-alkanes on their suitability for the estimation of dry matter intake and apparent digestibility in horses. *The Journal of Agricultural Sci*ence 154: 1291-1305.

Bachmann, M., Wensch-Dorendorf, M., Wulf, M., Glatter, M., Siebmann, M., Bierögel, C., Schumann, E., Bulang, M., Aurich, C., Zeyner, A. (2016b): Bolus matrix for administration of dietary markers in horses. *Livestock Science* 185: 43-49.

Bachmann, M., Czetö, A., Romanowski, K., Vernunft, A., Wensch-Dorendorf, M., Wolf, P., Metges, C.C., Zeyner, A., (2018a): Effects of grain species, genotype and starch quantity on the postprandial plasma amino acid response in horses. *Research in Veterinary Science* 118: 295-303.

Bachmann, M., Hepp, J., Zech, M., Bulang, M., Zeyner, A., (2018b): Application of natural wax markers in equine nutrition studies – current state, limitations and perspectives. *Livestock Science* 208: 77-89.

Blackmore, T.M., Dugdale, A., Argo, C.M.G., Curtis, G., Pinloche, E., Harris, P.A., Worgan, H.J., Girdwood, S.E., Dougal, K., Newbold, C.J., McEwan, N.R. (2013): Strong stability and host specific bacterial community in faeces of ponies. *PLoS ONE* 8: e75079, doi:10.1371/journal.pone.0075079.

Bochnia, M., Schürer, C., Gottschalk, J., Einspanier, A., Hillegeist, D., Wensch-Dorendorf, M., Greef, J.M., Glatter, M., Zeyner, A. (2017): Effects of isoenergetic quantities of a low-starch muesli feed high in fat and fiber vs. oat grains on the glycemic and insulinemic responses and feed intake patterns in sport ponies. *Journal of Animal Physiology and Animal Nutrition* 101: 43-50.

Boguhn, J., Baumgärtel, T., Dieckmann, A., Rodehutscord, M. (2009): Determination of titanium dioxide supplements in different matrices using two methods involving photometer and inductively coupled plasma optical emission spectrometer measurements. *Archives of Animal Nutrition* 63: 337-342.

Coenen, M., Mößeler, A., Vervuert, I. (2006): Fermentative gases in breath indicate that inulin and starch start to be degraded by microbial fermentation in the stomach and small intestine of the horse in contrast to pectin and cellulose. *The Journal of Nutrition* 136: 2108S-2110S.

Costa, M.C., Silva, G., Ramos, R.V., Staempfli, H.R., Arroyo, L.G., Kim, P., Weese, J.S. (2015): Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. *The Veterinary Journal* 205: 74-80.

De Nigris, S.J., Hamosh, M., Kasbekar, D.K., Lee, T.C., Hamosh, P. (1988): Lingual and gastric lipases; species differences in the origin of prepancreatic digestive lipases and in the localization of gastric lipase. *Biochimica et Biophysica Acta* 959: 38-45.

Dougal, K., de la Fuente, G., Harris, P.A., Girdwood, S.E., Pinloche, E., Newbold, C.J. (2013): Identification of a core bacterial community within the large intestine of the horse. *PLoS ONE 8* (10): e77660. https://doi.org/10.1371/journal.pone.0077660.

Embleton, J.K., Pouton, C.W. (1997): Structure and function of gastro-intestinal lipases. *Advanced Drug Delivery Reviews* 25: 15-32.

GfE, 2014. Empfehlungen zur Energie- und Nährstoffversorgung von Pferden. Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie (GfE), Mitteilung Nr. 11. DLG-Verlag, Frankfurt (Main), Germany.

Glatter, M., Borewicz, K., Wiedner, K., Mielenz, N., Bochnia, M., Bachmann, M., Smidt, H., Glaser, B., Breves, G., Zeyner, A., 2015. Feeding of Jerusalem artichoke meal alters the microbial community and fermentation products in the foregut of horses. In: *Proceedings of the 19th European Society of Veterinary and Comparative Nutrition Congress* Toulouse, France, 17-19 September 2015, p. 73.

Glatter, M., Wiedner, K., Hirche, F., Mielenz, N., Hillegeist, D., Bochnia, M., Cehak, A., Bachmann, M., Greef, J.M., Glaser, B., Wolf, P., Breves, G., Zeyner, A. (2016): Fermentation characteristics along the gastrointestinal tract after feeding of Jerusalem artichoke meal to adult healthy Warmblood horses. *Journal of Animal* Nutrition, doi:10.21767/2572-5459.100016.

Glatter, M., Bochnia, M., Goetz, F., Gottschalk, J., Koeller, G., Mielenz, N., Hillegeist, D., Greef, J.M., Einspanier, A., Zeyner, A., (2017): Glycaemic and insulinaemic responses of adult healthy warmblooded mares following feeding with Jerusalem artichoke meal. *Journal of Animal Physiology and Animal Nutrition* 101: 69-78.

Hintz, M.F., Hogue, D.E., Walker Jr., E.F., Lowe, J.E., Schryver, H.F. (1971): Apparent digestion in various segments of the digestive tract of ponies fed diets with varying roughage-grain ratios. *Journal of Animal Science* 32: 245-248.

Hummel, J., Scheurich, F., Ortmann, S., Crompton, L.A., Gerken, M., Clauss, M. (2018): Comparative selective retention of particle size classes in the gastrointestinal tract of ponies and goats. *Journal of Animal Physiology and Animal Nutrition* 102: 429-439.

Ince, J.C., Longland, A.C., Moore- Colyer, M.J.S., Harris, P.A. (2014): In vitro degradation of grass fructan by equid gastrointestinal digesta. *Grass and Forage Science* 69: 514-523.

Julliand, V., Zeyner, A. (2013): Dietary strategies for optimizing gastrointestinal health: an update on pre- and probiotics. In: *Proceedings of the 6th European Equine Health and Nutrition Congress,* Ghent, Belgium, 1-3 March 2013, pp. 75-84.

Kienzle, E., Zeyner, A. (2010): The development of a metabolizable energy system for horses. *Journal of Animal Physiology and Animal Nutrition* 94 (6): e231-e240.

Licitra, G., Hernandez, T.M., Van Soest, P.J. (1996): Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* 57: 347-358.

Merritt, A.M. (2003): The equine stomach: a personal perspective (1963-2003). In: *Proceedings of the 49th Annual Convention of the American Association of Equine Practitioners* (AAEP), New Orleans, LA, USA, 21-25 November 2003, pp.75-102.

Meyer, H., Ahlswede, L., Reinhardt, H.J. (1975): Untersuchungen über Freßdauer, Kaufrequenz und Futterzerkleinerung beim Pferd. *Deutsche Tierärztliche Wochenschrift* 82: 54-58.

Miraglia, N., Bergero, D., Bassano, B., Tarantola, M., Ladetto, G. (1999): Studies of apparent digestibility in horses and the use of internal markers. *Livestock Science* 60: 21-25.

Miyaji, M., Ueda, K., Kobayashi, Y., Hata, H., Kondo, S. (2008): Fiber digestion in various segments of the hindgut of horses fed grass hay or silage. *Animal Science Journal* 79: 339-346.

Müller, M. (1995): Fructanspaltung durch Enzyme pflanzlicher Herkunft. In: Seyfarth, W., Müller, T. (Eds.) Epiphytenbesatz und Fructangehalt von Futtergräsern, *ZALFF-Bericht, vol. 21. Zentrum für Agrarlandschafts- und Landnutzungsforschung*, Müncheberg, Germany, pp. 121-127.

Oliván, M., Osoro, K. (1999): Effect of temperature on alkane extraction from faeces and herbage. *The Journal of Agricultural Science* 132 (3): 305-312.

Pagan, J.D. (1998): Nutrient digestibility in horses. In: Pagan, J.D. (Ed.) *Advances in Equine Nutrition, vol. 1*. Nottingham University Press, Nottingham, UK, pp. 77-84.

Pagan, J.D., Harris, P.A., Brewster-Barnes, T., Duren, S.E., Jackson, S.G. (1998): Exercise affects digestibility and rate of passage of all-forage and mixed diets in Thoroughbred horses. *The Journal of Nutrition* 128: 2704S-2707S.

Radicke, S. (1990): Untersuchungen zur Verdauung von Mais- und Haferstärke beim Pferd. *Dissertation thesis* University of Veterinary Medicine, Hannover, Foundation, Germany.

Rosenfeld, I., Austbø, D. (2009): Effect of type of grain and feed processing on gastrointestinal retention times in horses. *Journal of Animal Sci*ence 87: 3991-3996.

Strauch, S., Wichert, B., Greef, J.M., Hillegeist, D., Zeyner, A., Liesegang, A. (2017): Evaluation of an in vitro system to simulate equine foregut digestion and the influence of acidity on protein and fructan degradation in the horse′s stomach. *Journal of Animal Physiology and Animal Nutrition* 101: 51-58.

Varloud, M., de Fombelle, A., Goachet, A.G., Drogoul, C., Julliand, V. (2004): Partial and total apparent digestibility of dietary carbohydrates in horses as affected by the diet. *Animal Science* 79: 61-72.

Varloud, M., Fonty, G., Roussel, A., Guyonvarch, A., Julliand, V. (2007): Postprandial kinetics of some biotic and abiotic characteristics of the gastric ecosystem of horses fed a pelleted concentrate meal. *Journal of Animal Science* 85 (10): 2508-2516.

VDLUFA (2012): Band III. VDLUFA-Verlag, Darmstadt, Germany.

VDLUFA (2016): Methode 4.13.1, Bestimmung des Neutral-Detergenzien-löslichen Rohproteins (NDLXP). Band III. VDLUFA-Verlag, Darmstadt, Germany.

Vervuert, I., Voigt, K., Hollands, T., Cuddeford, D., Coenen, M. (2009): Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. *The Veterinary Journal* 182: 67-72.

Wilke, S. (1992): Zur praeilealen Verdaulichkeit von Hafer und Mais verschiedener Zubereitungen beim Pferd. *Dissertation thesis* University of Veterinary Medicine, Hannover, Foundation, Germany.

Williams, C.H., David, D.J., Iismaa, O. (1962): The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *The Journal of Agricultural Science* 59 (3): 381-385.

Zeyner, A., Kienzle, E. (2002): A method to estimate digestible energy in horse feed. *The Journal of Nutrition* 132 (6): 1771S-1773S.

Zeyner, A., Kirchhof, S., Susenbeth, A., Südekum, K.-H., Kienzle, E. (2010): Protein evaluation in horse feed: a novel concept. In: Ellis, A.D., Longland, A.C., Coenen, M., Miraglia, N. (eds.). The impact of nutrition on the health and welfare of horses. *EAAP publication no. 128*. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 40-42.

Zeyner, A., Kirchhof, S., Susenbeth, A., Südekum, K.-H., Kienzle, E. (2015): A new protein evaluation system for horse feed from literature data. *Journal of Nutritional Science* 4: e4, doi:10.1017/jns.2014.66.

Zeyner, A., Romanowski, K., Orgis, A., Vernunft, A., Gottschalk, J., Einspanier, A., Koeller, G., Wensch-Dorendorf, M. (2017): Feed intake patterns and immediate glycaemic and insulinaemic responses of horses following ingestion of different quantities of starch from oat, barley and maize grains. *The Open Nutrition Journal* 11: 39-51.

5 General discussion

The following chapter deals with the impact of feeding a natural prebiotic compound (Jerusalem artichoke meal) on the gastrointestinal as well as extraintestinal system in horses (Fig. 8).

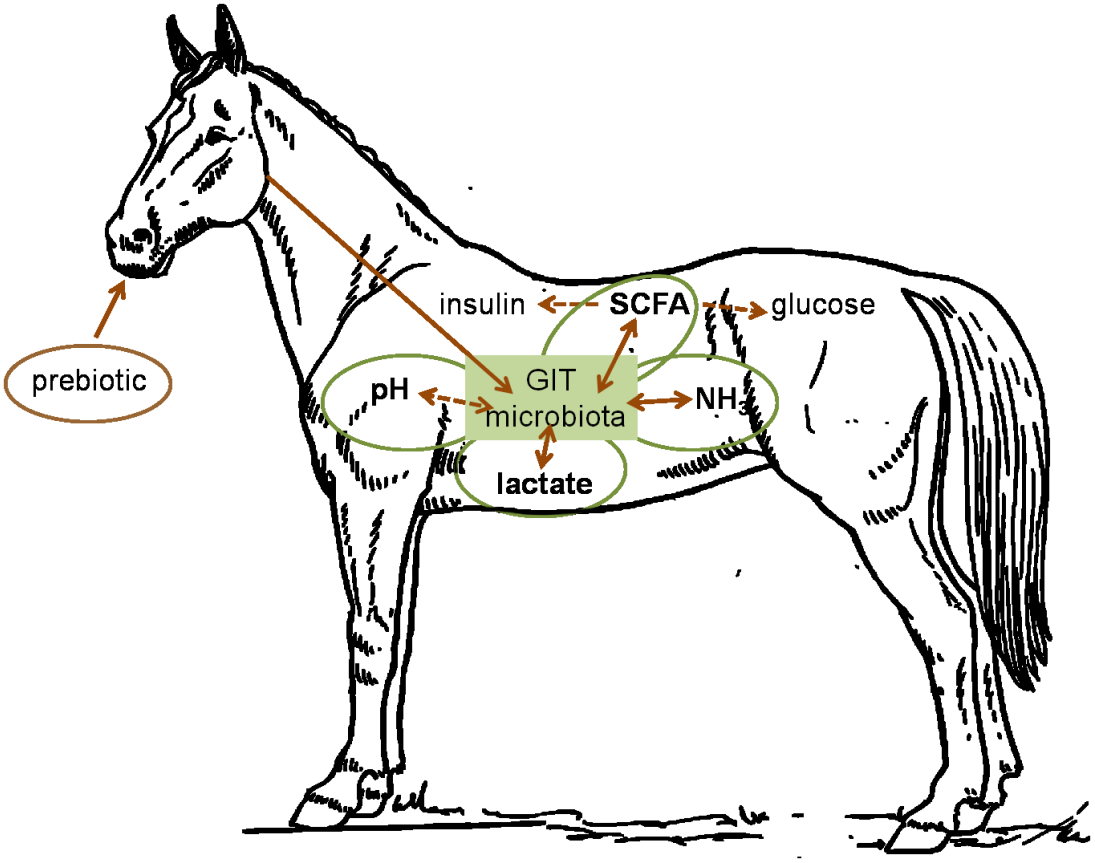

Fig. 8: Overview of possible direct (⟶) intestinal and indirect (⋯⋯▸) extraintestinal impacts of feeding prebiotics

First, the methods of investigation are discussed regarding of specific features and influences of the animals as well as the execution of the different studies. Afterwards, the composition of the natural prebiotic and the concomitant impact on the target microbial species are described. Furthermore, the direct impact on the intestinal microbiota (concerning the relative abundance) as well as the consequent indirect effect on the fermentative parameters (i.a. SCFA, pH value) is stated. Subsequently, the indirect modification of extraintestinal parameters (here: the glycaemic and insulinaemic response) via the microbial metabolism after feeding the natural prebiotic is summarized. Finally, the effect of feeding a prebiotic compound on the nutrient digestibility is delineated.

5.1 Critique of method

The scientific research aims to objectively investigate the hypothesis and to perform a profound study. Nevertheless, some aspects of the study requirement and execution need to be critically seen and discussed.

The usage of horses as study objects is associated with a high effort for the provision, the handling and the execution. **Paper I**, **paper II** and **paper IV** used horses differing in age, breed, origin, physical condition and former housing condition which led to an inhomogeneous animal material. Due to their use as animals in a superior project, the number of horses was predefined and there was no opportunity to get a homogeneous testing group. As a result, mostly elderly horses were used in the investigation but there were also some younger ones included (3 respectively 5 years old). **Paper III** used a testing group consisting exclusively of mares with a varying age from 6-13 years showing also animal individual differences. Furthermore, the number of animals per study was small compared to other animal studies but in the range of other horse investigations.

The collection of the chyme from the different parts of the intestinal tract represents a snapshot from the current state in the digestive tract. Consequently, the examined fermentative parameters (**paper I**) mirror only the status at the sample collection time. Moreover, digesta from the small intestine was not separated according to their anatomy but rather used as aggregate samples. The horses were euthanized approximately 1 hour after the morning meal which was chosen to guarantee a well-filled gastrointestinal tract, especially the foregut. Unfortunately, there could be no data collected to investigate the water consumption during respectively after the morning meal which might have influenced the viscosity and mean retention time of the digesta (**paper I and paper IV**).

The collection of feces from the rectum was not possible during the study for **paper I** and **II** because the horses have defecated already before the euthanasia. Therefore, the colon transversum was used instead but in this part of the GIT, the water absorption is not yet completed which has to be taken into consideration during data analysis and interpretation. Fecal samples were exclusively taken for the digestibility analysis in **paper IV** (d1-5 prior to the slaughtering).

The DNA extraction method (**paper II**) enables no conclusion to the metabolic activity of the detected microbiota because the original material might have contained live as

well as not alive organism. Furthermore, the concentration of bacteria was only given in relative abundance and there were no information concerning the concentration of e.g., colony forming units in the specific digestive compartments. Moreover, there was no differentiation between the luminal and mucosal bacterial composition in the gastrointestinal tract.

Meanwhile the collection of blood samples in **paper III**, the horses had no opportunity for feed consumption (here: continuously intake of hay) but were able to consume water. This might have interfered with the measured blood parameters because of an possibly altered blood volume.

The calculation of the digestibility coefficients in **paper IV** was conducted according to the digestive physiology in the equine GIT. Therefore, the “foregut” (here: stomach) was assumed to contain the morning meal whereas the “hindgut” digestibility coefficients were calculated based on the whole diet. This might not reflect the digestive process in total because the mean retention time of the solid chyme is assumed to range from 26 – 43h. Furthermore, the application of external markers using bolus feeding requires the total consumption without any refusals. This might not be usable for all horses in general because the feeding behaviour is very selective.

5.2 Application of natural prebiotic compounds

At the feeding of natural prebiotic active substances (like Jerusalem artichoke meal), the composition of the compound should be known to guarantee a health-promoting effect. Concerning the kind of prebiotic active compound used (e.g inulin-type fructans), the origin or the plant species is important. In Fig. 9, the chromatograms of two natural sources for inulin showed that the amount of the compounds with a higher DP is very variable. The chicory derived product (A) contained a higher amount of longer molecules in comparison to the Jerusalem artichoke meal (B) used in the above-mentioned studies (**paper I – IV**).

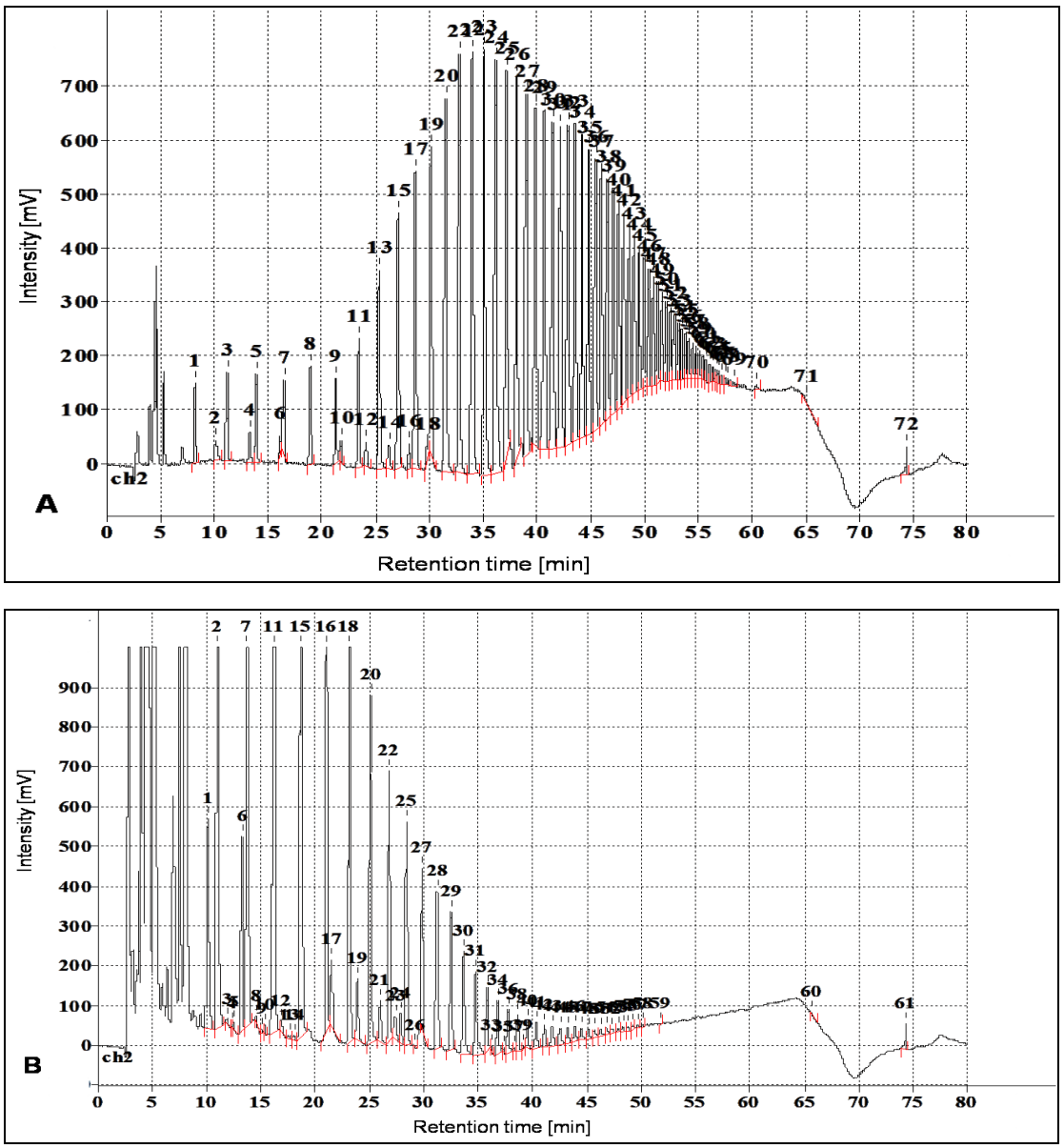

Fig. 9: Chromatogram of a commercial chicory derived product (A) and the Jerusalem artichoke meal (B) (according to HILLEGEIST, personal communication)

Generally, the amount of prebiotic active compounds (here: inulin) varied in the plant in addiction to their stage of development (LI et al., 2015) as well as the time of day (CAIRNS et al., 2002) and the season of the year (BACON and LOXLEY, 1952). LI et al. (2015) examined the amount of inulin in fresh Jerusalem artichoke tuber before and after flowering of the plant. The inulin content varied from 3.5 % 10 d before flowering to 12.21 % 40 d after flowering. Therefore, the harvesting time highly influences the amount of prebiotic active compounds in the natural product. The content of prebiotic active substances in the Jerusalem artichoke meal used in the

present investigation (**paper I, II, III** and **IV**) was declared with 6.3 % inulin + 56.4 % FOS. According to LI et al. (2015), the tuber could have been harvested until less than 10 d after flowering of the plant. The examination of the Jerusalem artichoke meal after the study revealed only an amount of 46.6 % inulin + FOS (**paper I, II, III** and **IV**). Therefore, the intended dosage of 0.2 g inulin + FOS/kg bwt x d^{-1} was not achieved in the above-mentioned studies. Instead, the horses ingested presumably 0.15 g/kg bwt x d^{-1}. Furthermore, the amount of fructans can vary during daytime (CAIRNS et al., 2002). Unfortunately, there is no information available when the Jerusalem artichoke meal in the current study was harvested.

Assuming that inulin is described with a DP > 10, the amount of inulin in the Jerusalem artichoke meal used in the present study was around 34.8 % (+ 11.8 % FOS). Therefore, the total content of prebiotic active substances in the natural product was lower than declared but the proportion of inulin itself was higher. Furthermore, the DP could vary during the different stages of development of the plant (LI et al., 2015) but also during osmotic adaptation processes like sustainment of petal expansion (daylily), in drought, cold tolerance and sink regulation (GUPTA and KAUR, 2000). The mean DP in the Jerusalem artichoke meal (see Fig. 9) was around 16 - 20 which might indicate a developmental stage of 40 - 50 d after flowering (according to LI et al., 2015). Consequently, the highest content of inulin should be stored in the tubers during this developmental stage but the analyzed content contradicts this assumption. The fructans which were synthesized from plants (here: dicotyledonous) contain a mix of different DP (HEYER et al., 1998). LI et al. (2015) investigated exclusively the inulin content and DP in fresh tubers whereas in the current study, the amount of FOS + inulin from dried Jerusalem artichoke tuber meal was analyzed. This might explain the differences in the inulin content as well as the DP concerning the current study compared to the literature. In other studies which used scFOS/FOS and/or inulin in horses (e.g., COENEN et al., 2006; RESPONDEK et al., 2007, 2008), the accuracy of the applied dosage is not clear because there is no analysis given from the used products/compounds.

Furthermore, the ratio of longer and shorter molecules (respectively long or short DP) in the prebiotic compound determines the effect in the organism. The Jerusalem artichoke meal used in the above-mentioned studies (**paper I, II, III** and **IV**), contained mostly shorter molecules (DP 16 - 20) in comparison to the chicory derived product (see Fig. 8; ~ 65 % for DP 21 - 40). Inulin type fructans with a longer DP had

a more pronounced impact on the bacterial metabolism in the distal parts of the digestive tract in comparison to oligosaccharides or inulin with a shorter DP (VAN DE WIELE et al., 2007; AZORÍN- ORTUÑO et al., 2009; HAN et al., 2013; MUELLER et al., 2016). However, the results from **paper I** and **II** indicated that the fermentation of the Jerusalem artichoke meal already started in the foregut and that the impact on the distal parts of the digestive tract was less pronounced. The degradation of fructans in the foregut might be due to acid hydrolyisis and microbial fermentation (former already assumed in COENEN et al., 2006; INCE et al., 2014; STRAUCH et al., 2017). Compounds with a DP until 20 molecules are more easily available for the saccharolytic fermentation of the intestinal bacteria than longer compounds (VAN DE WIELE et al., 2007). Therefore, the degradation of e.g. the Jerusalem artichoke meal proceeded in the more proximal parts of the digestive tract. As mentioned above, further studies using scFOS/FOS or inulin in the nutrition of horses declared no DP of the used compounds (e.g. RESPONDEK et al., 2007, 2008). Thus, the dosage of prebiotic active compounds which might achieve the hindgut is not known.

5.3 Prebiotic impact on the equine intra- and extraintestinal metabolism

5.3.1 Influence on the bacterial composition and metabolism in the gastrointestinal tract

The feedstuffs ingested by horses are not only digested by host enzymes, but a considerable amount is also fermented by the intestinal microbiota which in turn provides substrates for the host metabolism. Prebiotics are defined to be exclusively fermented by bacteria in the digestive tract and are resistant to mammalian enzymes (ROBERFROID et al., 2010). *In vitro* studies assume that inulin-type fructans might be pre-digested by the gastric acid in the foregut (stomach) of horses and so are more easily available for the intestinal autochthonous microbiota (INCE et al., 2014; STRAUCH et al., 2017). The equine GIT harbours an abundant microbial community which is i.a. specialized on the fermentation of plant fibres (SANTOS et al., 2011) but similarly prone to disturbances due to abiotic or biotic stressors RESPONDEK et al., 2008; DE FOMBELLE et al., 2001). Currently, the existence of a so-called core microbiome is also assumed for herbivores especially equines (DOUGAL et al., 2013; O´DONNELL et al., 2017). The knowledge of a key microbial community (present in

all or at least the majority of the individual animals within the population) in healthy animals might be basic to prevent or intervene, if the system (here: the GIT) is disturbed or stressed. Fig. 10 presents the core microbial community in the CON feeding group (Fig. 10 A) in comparison to the JAM group (Fig. 10 B; data from **paper II**). Detailed information about the core community members are presented in Table S2 (appendix).

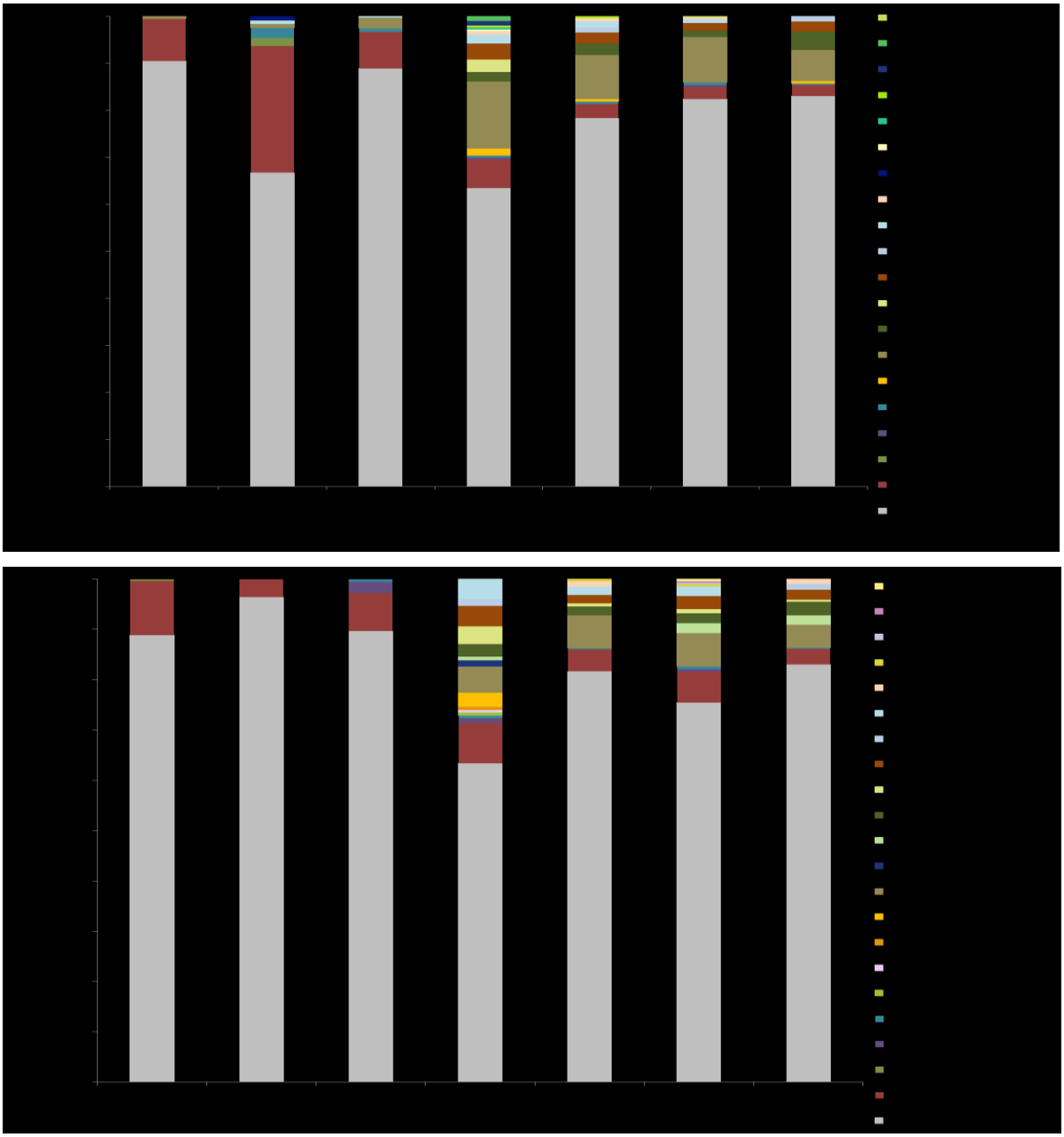

Fig. 10: Proportion of shared (= core microbiome; coloured) and not shared sequences (grey) in the equine GIT of the CON group (A) and the JAM feeding group (B) [PN = *pars nonglandularis*, PG = *pars glandularis*, SI = small intestine, CAE = caecum, CV = colon ventrale, CD = colon dorsale, CT = colon transversum]

The obvious difference in the prebiotic feeding group compared to the control is the lower amount of shared sequences in the stomach (here: *pars glandularis*) and colon ventrale, whereas in the colon dorsale, the proportion of the core microbiota was higher (Fig. 10, Table S2). In the stomach (also: *pars glandularis*) of the JAM feeding group, the core community consists only of the genera *Lactobacilli* whereas in the CON feeding group, there are 6 different genera (**paper I**, Table S2). The genus *Lactobacilli* primarily produces lactate acid (more precisely the enantiomer D-lactate; EWASCHUK et al., 2002). The concentration of D-lactate in the PG was 3-fold higher in the JAM group compared to the CON group (**paper I**) which coincides with the higher relative abundance of that genus (**paper II)**. Concerning the hindgut of the CON feeding group, the number of core genera in each part of the GIT is identical to DOUGAL et al. (2013): colon ventrale with 10 and colon dorsale with 9 core genera (Fig. 10, Table S2). Feeding of the natural prebiotic resulted in a lesser number of core genera in the colon ventrale (here: 9) whereas the number increased in the colon dorsale (here: 13) compared to the CON feeding group as well as to the results of DOUGAL et al. (2013). Moreover, the diversity increased in all parts of the GIT after feeding of the prebiotic (**paper II**). Hence, a higher microbial diversity in association with a core community consisting of many genera might lead to a more stable intestinal microbial community which is further less susceptible to disturbances (Mc CANN, 2000; DOUGAL et al., 2013). In accordance with the results from DOUGAL et al. (2013), the equine core microbiota consisted of many low abundant genera (or OTU´s) and made up not more than maximal ~ 37 % of the entire microbiota in the specific parts of the GIT. Furthermore, the size of the core microbiota in the CT (~ 17 % for both feeding groups; **paper II)** is identical to horses fed a hay diet (DOUGAL et al., 2014). Horses fed with a starch-rich concentrate or a complementary feed high in oil (0.69 % of bwt x d^{-1}) showed a smaller size of the core microbiome (~ 6 % respectively ~ 11 %; DOUGAL et al., 2014). Compared to other herbivore animals (here: cow; JAMI and MIZRAHI, 2012) the equine core community consists of a lesser number of genera and was not dominated by high abundant microbial species/genera which might explain the existing susceptibility to abiotic and/or biotic stressors.

According to their chemical structure, the prebiotic compounds are degraded faster (low DP, e.g. FOS/scFOS) or slower (high DP, e.g. inulin) in the digestive tract (VAN DE WIELE et al., 2007). Furthermore, the concentration of fermentation products

(here: SCFA) also depends on the chemical composition, in which e.g. lactate and *n*-butyric acid are produced at higher concentration if low DP fructans are used (KOENEN et al., 2016). The feeding of JAM in the current study (**paper I**) resulted in a higher concentration of SCFA (i.a. acetic and *n*-butyric acid) as well as lactate (both isomers) chiefly in the foregut. This coincided with the above-mentioned chemical structure description because the used natural prebiotic consisted of low DP inulin-type fructans (see Fig. 9) and might therefore be fast degraded already in the foregut of the horses. In a study with pigs, specific bacterial species in the foregut (here: mucosa associated *Lactobacillus* spp.) showed a higher relative abundance, if low DP inulin was used whereas the relative abundance of specific bacterial species (here: *Bifidobacterium* spp., *Bacteroides* and *Clostridia*) in the hindgut increased, if high DP inulin was fed (PATTERSON et al., 2010). Furthermore, the authors described a connection between chemical structure of the prebiotic (low or high DP) and oxygen-sensitivity of several bacteria. Equally, the equine foregut harbours more aerotolerant species (in some literature also described as anaerobic) like e.g. *Lactobacillus* whereas the hindgut harbours more anaerobic species (e.g. *Bacteroides, Clostridia*; DICKS et al., 2014). The genus *Lactobacillus* i.a. inhabit the stomach (YUKI et al., 2000, PERKINS et al., 2012) and showed, as already mentioned, a higher relative abundance after feeding the natural prebiotic JAM (**paper II**) which coincides with a higher concentration of lactate (D- and L- isomer) in this part of the gastrointestinal tract (**paper I**). Taking the relative abundance of *Lactobacillus* and their fermentation end product lactate acid together (both isomers; **paper I** and **II**), a high correlation is noticed (Fig. 11 A; $R^2 = 0.867$).

The fermentation of inulin-type fructans by bacteria proceeds either extracellular or intracellular. The last-named intracellular fermentation is preferred because exclusively the target species are able to use the formed mono- and disaccharides whereas in the other case (extracellular fermentation), each bacterium (in the surrounding environment) is able to utilize the formed substrates (MUELLER et al., 2016). Extracellular degradation is proved for the genus *Bacteroides* and some species from the genus *Lactobacillus*, whereas the intracellular degradation take place in *Bifidobacteria* and is also assumed for some *Lactobacillus* species (VAN DER MEULEN et al., 2006; TSUJIKAWA, NOMOTO and OSAWA, 2013). Related to the supplemented natural prebiotic in the current study (**paper I** and **II**), it is not clear, to what extent the *Lactobacilli* metabolized the JAM intra- or extracellular and

therefore might also interact with the other microorganisms in the foregut (and hindgut) by providing substrates for their metabolism.

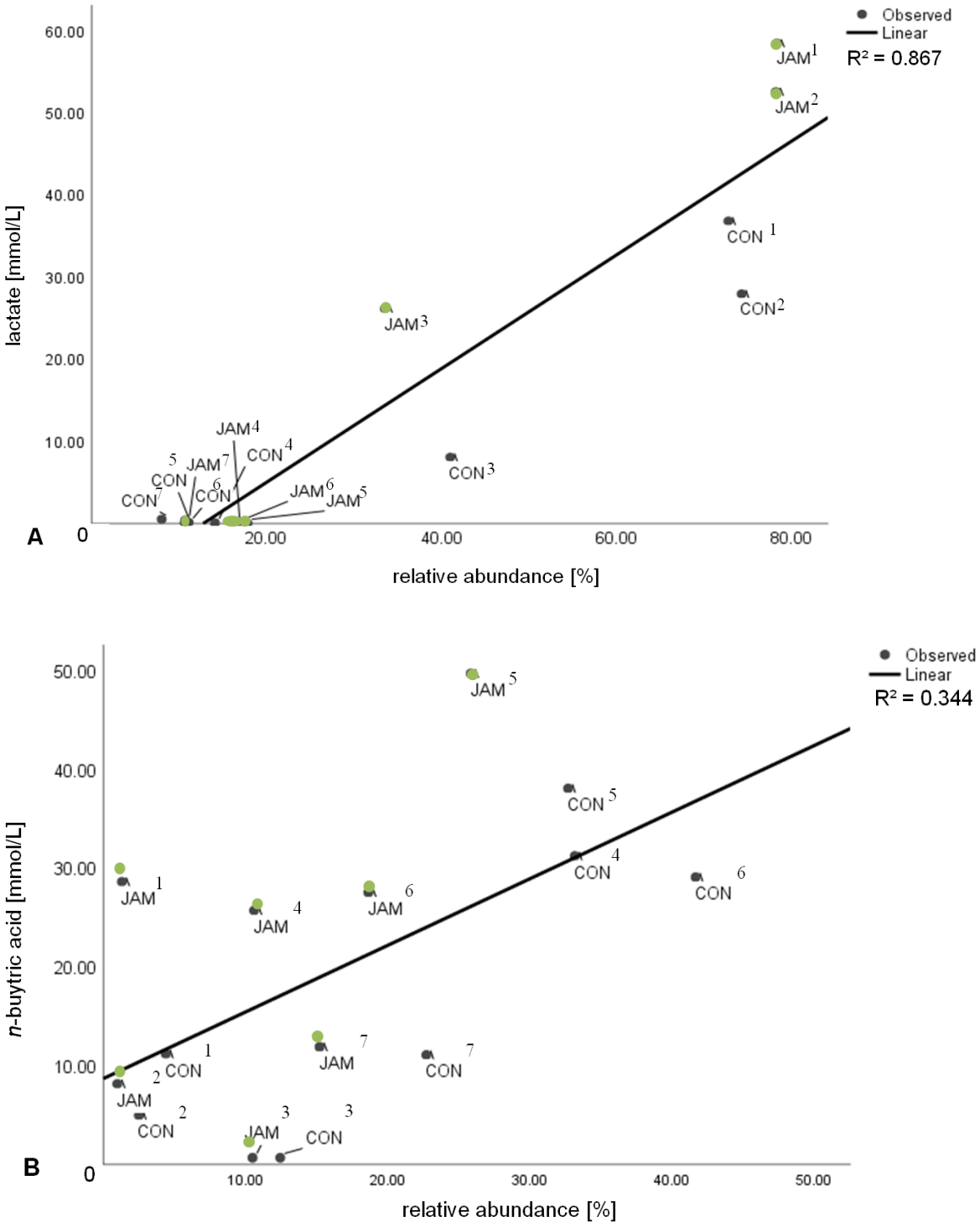

Fig. 11: Theoretical assumption of a correlation selected SCFA with the genus Lactobacillus (A) and the family Lachnospiraceae (B) via regression analysis troughout the entire GIT [JAM = Jerusalem artichoke meal, CON = placebo, 1 = *pars nonglandularis*, 2 = *pars glandularis*, 3 = small intestine, 4 = caecum, 5 = colon ventrale, 6 = colon dorsale, 7 = colon transversum]

The genus *Lactobacillus* is able to influence the digestive tract positively and/or negatively. YUKI et al. (2000) described the ability of *Lactobacilli* to adhere to the intestinal epithelium (*pars nonglandularis*) of the horse stomach and therefore to occupy possible binding sites for pathogens which is desirable. Furthermore, *Lactobacilli* are able to protect the digestive tract from pathogens by lowering the pH value (due to the production of i.a. lactic acid) and/or production of antimicrobial compounds like hydrogen peroxide or bacteriocins (KABUKI et al., 1997; SERVIN and COCONNIER, 2003). Consequently, a higher relative abundance of this genus after feeding of the natural prebiotic (**paper I** and **II**) might be positively related to the prevention of gastrointestinal diseases derived from pathogens in horses. Apart from that, a low pH might also have negative consequences for the equine GIT like e.g. development of gastric ulcers (COENEN, 1992; NADAEU et al., 2000) or acidosis in the hindgut (AL JASSIM and ANDREWS, 2009). The pH value in the foregut (especially stomach) after feeding of JAM (**paper I**) was in the normal range (COENEN, 1992), so that the higher relative abundance of the genus *Lactobacillus* (**paper II**) might have to assess positively. Concerning the hindgut, the increasing relative abundance of the *Lactobacilli* (**paper II**) is critically seen because some authors assume a connection between the development of laminitis and the abundance of *Lactobacilli* (BAILEY et al., 2003). The intestinal milieu parameter (here: pH value, lactic acid concentration; **paper I**) indicated no significant impact of JAM (fed in the above-mentioned dosage; **paper I** and **II**), so that a negative impact on the hindgut might be improbable.

Prebiotic feeding intends to increase the production of SCFA, from which *n*-butyric acid has a key role concerning the barrier function of the intestinal epithelium in the hindgut (PLOEGER et al., 2012). The feeding of the natural prebiotic JAM increased the concentration of *n*-butyric acid primarily in the stomach (*pars nonglandularis* and *pars glandularis*) and the colon ventrale (**paper I**). Concerning the hindgut, the increment is preferred because of the above-mentioned positive impact on the intestinal epithelial barrier function (PLOEGER et al., 2012). In the stomach, the increased concentration of *n*-butyric acid might have converse effect. *In vitro* studies assumed, that high *n*-butyric acid concentrations in combination with a low pH value might have negative impacts concerning the barrier function in the stomach (NADEAU et al., 2003; CEHAK et al., 2019). Furthermore, histopathological changes were described in the *pars glandularis* after incubation with an *n*-butyric acid

concentration equivalent to the results in **paper I** after feeding of the natural prebiotic JAM (CEHAK et al., 2019). However, the effect on the glandular mucosa was numerically but not significantly more pronounced at pH 3.0 compared to pH 5.4 (CEHAK et al., 2019). The measured pH in **paper I** was both in the CON and the JAM feeding group at the lower value (CON: 3.45; JAM: 3.27). This supports the assumption, that low DP prebiotics might already be digested in the equine stomach which could lead to the development of health problems like gastric ulcers. As already mentioned, *in vitro* investigations assume a predigestion of inulin-type fructans via gastric acid in the stomach of horses and therefore made the substrate easier available for microbial fermentation (INCE et al., 2014; STRAUCH et al., 2017). There might be two possible bacterial species in the stomach which are involved in the fermentation of the low DP prebiotic i.a. to *n*-butyric acid: the family *Lachnospiraceae* and *Clostridiaceae* (here: the genus *Sarcina*), both were determined in **paper II**. The family *Lachnospiraceae* is known to be a dominant butyric acid producer in the digestive tract (BIDDLE et al., 2013). In contrast to the genus *Lactobacillus*, there is no clear correlation between *n*-butyric acid concentration in the GIT and relative abundance of *Lachnospiraceae* (Fig. 11 B; R^2 = 0.344) after feeding of JAM. Especially in the stomach, the higher *n*-butyric acid concentration in the JAM group is not connected in any way with an increased relative abundance of this family. Another presumption for the higher concentration is the conversion of acetic acid and lactate to *n*-butyric acid by e.g. the genus *Sarcina* (MORRISON et al., 2006). The higher relative abundance of *Lactobacilli* in the stomach (**paper II**) together with the simultaneously higher production of lactate (**paper I**) might forces other surrounding bacteria (like e.g. *Sarcina*) to convert them to SCFA (primarily *n-butyric* acid).

The hindgut is described as target region for the prebiotic feeding (ROBERFROID et al., 2010). Thereby, an increment of the bacterial metabolism and likewise production of SCFA (i.a. *n*-butyric acid) is intended. Feeding of JAM resulted in an increased *n*-butyric acid concentration solely in the colon ventrale (**paper I**) whereas in the other parts of the hindgut, the concentration was either reduced (caecum) or equal to the CON group (colon dorsale, colon transversum). Especially in the colon ventrale, the relative abundance of the genus *Lactobacillus* (**paper II**) but not the concentration of lactate (D/L-lactate, **paper I**) was increased. Instead of this, the concentration of acetic acid and ammonia increased in the JAM feeding group (**paper I**). VAN DE

WIELE et al. (2007) described the possibility of other bacterial species (like e.g. *Lachnospiraceae*) to convert acetate to *n*-butyric acid. In the JAM feeding group, the relative abundance of species from an uncultured *Lachnospiraceae* family member decreased but the relative abundance of another member with *incertae sedis* in the family *Lachnospiraceae* increased (**paper II**). This might explain the increment of *n*-butyric acid in this part of the GIT. Nevertheless, after feeding of JAM, the increase of this specific SCFA in the hindgut in general and the colon ventrale in particular is not as high as in the foregut (primarily the *pars nonglandularis*). RESPONDEK et al. (2008) described a tendentially higher concentration of SCFA (in total) and *n*-butyric acid in particular after feeding of scFOS at ~ 0.07 g/kg bwt x d^{-1} (which is nearly half of the dosage used in **paper I** and **II**) to 4 crossbred geldings (bwt: 425 ± 27 kg) whereas the concentration (log_{10} CFU/ml) of *Lactobacilli* and lactate-utilizing bacteria decreased. This is controversial to the results from **paper II**, whereby a higher relative abundance of e.g. *Lactobacilli* was detected. An explanation might be e.g. the different investigation methods: culture technique (RESPONDEK et al., 2008; selective) vs. sequencing (**paper II;** widespread), usage of prebiotic (scFOS vs. inulin-type fructan), former housing conditions as well as breed and age of the used horses. Furthermore, the above-mentioned investigation from RESPONDEK et al. (2008) targeted on the possible positively interaction with feeding of prebiotics during biotic stress whereas the current study (**paper I** and **II**) not included any stressors.
Overall, feeding of the natural prebiotic Jerusalem artichoke meal resulted in a modification of the microbial community in the equine GIT (**paper II**), in which specific parts showed marked altered fermentation parameters (**paper I**). The varied bacterial metabolic end products might have in addition to it an impact on the metabolic processes in the animal (by way of comparison see in introduction Fig. 3).

5.3.2 Influence on the extraintestinal metabolism

Prebiotics are able to interact respectively modify the extraintestinal metabolism (like e.g. the glycaemic and insulinaemic response) by an accelerated production of SCFA in the digestive tract (see Fig. S2, supplementary material). The effects are diverse and depend primarily on the formation of acetate, propionate as well as *n*-butyrate whereby the impact is specific concerning the target (tissue or viscera). The feeding

of the natural prebiotic Jerusalem artichoke meal increased the production of SCFA in the equine GIT, formerly in the foregut (**paper I**) but also modified the glycaemic and insulinaemic response (**paper III**). In comparison to the control group, the postprandial (PP) blood glucose concentration was reduced and returned faster to baseline whereas the PP insulin concentration was higher but also declined faster compared to the control (**paper III**). The microbial breakdown of prebiotic compounds (like inulin-type fructans) led to the production of primarily fructose (ROBERFROID, 2007) which causes minor changes in the glycaemic response in comparison to glucose (BORER et al., 2012; LI et al., 2015). Results from **paper I** and **II** confirmed the assumption (COENEN et al., 2006, INCE et al., 2014; STRAUCH et al., 2017) that the prebiotic is, in part, already degraded in the foregut, primarily in the stomach. This is accompanied by a slightly higher amount of fructose formerly in the stomach (see Table S3), a higher concentration of SCFA (**paper I**) and a modified bacterial composition (**paper II**). Feeding of JAM resulted in a higher relative abundance of the genus *Lactobacillus* and an unknown member of the family *Lachnospiraceae* as well as *Christenellaceae* (**paper II**) in the foregut. All of the mentioned bacteria are able to ferment the prebiotic but it is not known, to what extent especially these bacteria produce and/or metabolize fructose. The fast return of the blood glucose concentration to the baseline is positively to see, whereas the high PP blood insulin concentration is critically to note (**paper III**). Metabolic disorders like laminitis are often a consequence of permanent or frequently high PP insulin concentrations which lead to a reduced insulin sensitivity in the target tissues (TREIBER et al., 2006; GEOR, 2008). Nevertheless, the more pronounced decrease of the PP glucose concentration in comparison to the control, which is associated with the slightly higher PP insulin secretion, might a positive consequence from the long-term supply of the prebiotic. Furthermore, BAMFORD et al. (2016 a, b) reported, that a diminished insulin sensitivity is not achieved by a once-daily high glycaemic meal but rather after chronic stimulation of the pancreatic insulin secretion with several high glycaemic meals per day. The horses in the current investigation (**paper III**) were fed twice daily and not exceeded the recommended starch intake of 1 g starch/kg bwt x d^{-1} (VERVUERT et al., 2009b; GfE, 2014) so that a high glycaemic response might be excluded. A higher starch intake per day (> 1 g/kg bwt x d^{-1}) significantly elevated the risk for the development of gastric ulcers EGUS grade ≥ 2 (LUTHERSSON et al., 2009). Furthermore, prebiotics are able to enhance the secretion of incretin

hormones like GLP-1, GIP and PYY (DELZENNE et al., 2007) which further interacts in the PP insulin stimulated glucose removal from the bloodstream (KAZAKOS, 2011). This is achieved by a stimulation of the G-protein coupled receptors FFAR 2 and FFAR 3 (former GPR 41 and 43) via SCFA (KASABUCHI et al., 2015). The receptors are located in the intestinal L-cells and stimulated by propionate and (*n*-) butyrate (FFAR 3) as well as acetate (FFAR 2) to release i.a. GLP-1. In rats, the feeding of inulin-type fructans increased the PP GLP-1 concentration (DELZENNE et al., 2005, 2007; CANI et al., 2009). Unfortunately, the PP concentration of GLP-1 was not determined in the current investigation (**paper III**) but the higher concentration of SCFA in the foregut (**paper I**) might have functioned positively concerning the PP release of incretin hormones like GLP-1.

In addition, SCFA are able to affect the glucose homeostasis indirectly via the metabolization in the target tissues/viscera. Hereby, the conversion of propionate to glucose in the liver is already proven for ruminants, whereby it is only assumed for horses (HUNTINGTON, 1990). Unfortunately, the results from **paper III** in association with **paper I** not provide any indication to the contribution of propionate concerning the blood glucose level. Furthermore, the basal blood glucose concentration in **paper III** indicates only marginal differences between the two feeding groups.

Moreover, the size of the adipocytes is linked with insulin sensitivity and further susceptible to SCFA (ROBERTSON, 2007). Hereby, SCFA are able to bind to specific G-protein coupled receptors (as aforementioned FFAR 2/3, formerly GPR 41/43) and suppresses adipose specific insulin signalling which lead to an inhibited fat accumulation in the adipose tissues and further on to an increased insulin sensitivity (KASABUCHI et al., 2015). The higher concentration of SCFA (**paper I**) after feeding the natural prebiotic might interfere in the above-mentioned way and thereby might lead to an improved insulin sensitivity but this aspect was, unfortunately, not determined in **paper III** by specific tests (like e.g. oral glucose tolerance test or frequently sampled intravenous glucose tolerance test [FSIGT]; FIRSHMAN and VALBERG, 2007). Further, the stimulatory impact of SCFA to the size of adipocytes is limited to a critical size, by which the metabolism switches to a dysregulation and finally lead to the development (or even enhancement) of an insulin resistance (GEOR, 2008).

The impact of feeding prebiotics concerning the extraintestinal metabolism in horses is further addicted to the initial state of the animals (e.g. obese, predisposed to

laminitis, breed, age). In **paper III**, the warmblood type horses were clinically healthy with no known history of metabolic disorders like laminitis. Ponies, predisposed to laminitis, exhibited a higher PP serum insulin concentration after feeding of inulin in comparison to control ponies (BAILEY et al., 2007 [3 g inulin/kg bwt x d^{-1}]; BORER et al., 2012 [1 g inulin/kg bwt x d^{-1}]). Furthermore, RESPONEDEK et al. (2011) described an improved insulin sensitivity and reduced resting serum insulin concentration after feeding of 45 g scFOS/d (~ 0.09 g/kg bwt x d^{-1}) to obese Arabian geldings. A recent study from JACOB et al. (2018) indicated higher insulin response to glucose in aged horses (~ 21 years) compared to adult ones (~ 9 years). The authors declared, that it is not known whether these results are a consequence of a higher pancreatic insulin secretion or a minor ability of the target tissues/viscera to clear the circulating insulin concentration from the bloodstream. Nevertheless, the mares in the current study (**paper III**) had a mean age of 9.5 years indicating no age-related change concerning the PP insulin release. Finally, also the breed might affect the PP glycaemic and insulinaemic response whereby Standardbred horses might have a higher insulin sensitivity compared to Andalusian-cross or mixed-breed ponies (BAMFORD et al., 2014). The horses in **paper III** consisted of warmblood type horses (4 trotter, 1Trakehner and 1 Mecklenburger). Unfortunately, the individual insulin sensitivity was not tested before/during the study so that a possible breeding impact might not be excluded. Further investigations might include different starting conditions to describe the impact of prebiotics in the extraintestinal metabolism in detail.

5.4 Nutrient digestibility at prebiotic supplementation

The calculation of segmental nutrient digestibility coefficients in horses requires the knowledge of digesta passage rates and the mean retention time of the chyme in the different parts of the GIT. **Paper IV** used two different approaches to estimate digestibility coefficients in the foregut (respectively stomach) and the hindgut. In the hindgut, the mean retention time of the solid chyme is believed to range from 26 to 43h (ROSENFELD and AUSTBØ, 2009). Therefore, the estimation of the nutrient digestibility is based on the whole diet the horses received. In contrast to this, the calculation of the foregut (here: stomach) digestibility coefficients is solely based on

the morning meal or more specifically on the morning concentrate meal and 10 % of the daily roughage intake. Unfortunately, the hay intake was not measured for **paper I, II** and **IV** so that, according to the literature (VERVUERT et al., 2013), a hay intake of 10 % from the daily ration was assumed. This approach is in the range of other digestibility trials in horses (VARLOUD et al., 2004), in which the calculation was also adapted to the correlating part of the GIT and the assumed ingested meal amount.

The calculated starch and sugar digestibility coefficients in the stomach were in the range of other literature data using the internal marker AIA and ADL (VARLOUD et al., 2003). In **Paper IV**, the nutrient digestibility coefficients calculated for starch and mono-/disaccharides in the PG were 0.65 respectively 0.74 whereas in the PN, there were 0.42 and 0.53. VARLOUD et al. (2003) estimated the starch and sugar digestibility using the whole stomach whereas in **paper IV**, the 2 anatomical parts were separately investigated. Nonetheless, the calculated coefficients were in the range of the PG (VARLOUD et al., 2003: 0.69 and 0.60). Furthermore, the concentrate meal in **paper IV** was semi-crushed oat grains supplemented with either the JAM or CON whereas in the study of VARLOUD et al. (2003), pelleted meals were used. The pelleting of grains is assumed to extend the transit time in the GIT so that this might had an influence of the nutrient digestibility in this part (ROSENFELD and AUSTBØ, 2009).

The hindgut digestibility coefficients for starch, fructans and mono-/disaccharides were also in the range of the literature (VARLOUD et al., 2003). Furthermore, the nutrient digestibility of 0.99 for fructans supported the assumption, that a great amount is already degraded pre-cecally and not reaching the hindgut (COENEN et al., 2006; INCE et al., 2014; STRAUCH et al., 2017). Therefore, the prebiotic had only a slight influence on the microbiota (**paper II**) and their metabolism (**paper I)** as well as the digestibility of the nutrients (**paper IV**) in the equine hindgut.

The estimated total tract digestibility coefficients for dry matter, crude protein and acid ether extract were in the range of the literature (RESPONDEK et al., 2007). RESPONDEK et al. (2007) fed scFOS for 21 d (0.06 g/kg bwt x d^{-1}) in comparison to **paper IV**, in which FOS + inulin (0.15 g/kg bwt x d-1) were fed. Nevertheless, both studies showed no impact of prebiotic feeding on the total tract nutrient digestibility although the acid ether extract digestibility was numerically higher in the prebiotic feeding group which might be negligible.

5.5 Perspective for further applications and investigations

The feeding of prebiotics to horses is a currently proceeding field of interest. Unfortunately, the overall as well as detailed impact is not known yet but in contrast to this, a considerable quantity of products with a supposed prebiotic impact are available on the market. The results from the current investigations (**paper I** to **IV**) are the first beginnings to determine the possible impacts to the horse. Further studies might start to evaluate a dose response curve in which the effective dosage is described. The difference between prebiotic (see JULLIAND and ZEYNER, 2013; ~ 0.2 g/kg bwt x d^{-1}) and harmful dosage (see VAN EPS and POLLIT, 2006; ~ 20 g/kg bwt x d^{-1}) is quite high. Based on the results from **paper I - IV**, the possible degradation of prebiotics (here: inulin-type fructans) in the foregut (primarily the stomach) has to be taken into consideration to get a possible health-promoting effect. Therefore, the prebiotic compound might be administered in a galenic treated way so that the degradation only begins in the hindgut. As a consequence, this involves possibly a correction of the recommended prebiotic dosage because a starting decomposition in the foregut might be excluded.

Furthermore, the composition of the ration should be investigated in detail because there are possible components which might enhance or even diminished the prebiotic effect. Concerning the horses per se, the impact of several influencing factors like e.g. age, breed, gender, developing status (here: foal/weanling, adult, elderly), predisposition, health condition (e.g. insulin resistance or reduced insulin sensitivity) or weight (obese, non-obese, anorexiant) has to be taken into consideration and investigated in detail. Literature data suggest the assumption, that the above-mentioned factors have a great impact on the health-promoting effect of prebiotics.

Results dealing with the extraintestinal impact of feeding prebiotics to horses are scarce. Here, a detailed investigation of additional parameters (e.g. plasma cholesterol concentration, immunglobulines, hormones [i.a. leptin, GLP-1]) might give a better understanding of the systemic impacts of prebiotics to horses.

6 Conclusion

Feeding of prebiotics or prebiotic active compounds is a potential way to promote health in equines. The scientific based evidence for a positive impact concerning the entire gastrointestinal tract and further on the systemic metabolism is scarce. Therefore, the current examinations were conducted to investigate some aspects of prebiotic feeding to horses in detail.

First hypothesis: The fermentative degradation of prebiotic substances by horses already starts in the stomach.

The first hypothesis is confirmed. The microbial breakdown and consequently elevated concentration of fermentation end products like SCFA and lactate was affirmed but the impact concerning the stomach´ health is critically seen.

Second hypothesis: Water-soluble carbohydrates, including fructans, are degraded to a large extent in the foregut.

Furthermore, the disappearance of the prebiotic already in the foregut involved a minor concentration reaching the hindgut so that the second hypothesis is also partly approved.

Fourth hypothesis: The concentration of *n*-butyric acid will be elevated in a particular degree, formerly in the hindgut.

Prebiotic feeding intends to interact chiefly with the hindgut microbiota. In contrast to this, the natural Jerusalem artichoke meal fed supplementary to horses was even degraded in the upper digestive tract and not reaching the target area in considerable concentrations. Moreover, the intended increment of microbial metabolic end products (here: SCFA respectively *n*-butyric acid) was achieved but, in contrast to the literature data, already in the foregut. Hence, the fourth hypothesis is partly confirmed.

Third hypothesis: The feeding of FOS + inulin as prebiotic active compounds has an impact on the microbiota of the entire gastrointestinal tract.

The third hypothesis was approved because the feeding of the natural prebiotic had an impact on the microbial composition in the entire gastrointestinal tract whereby the effect concerning the foregut was more pronounced.

Fifth hypothesis: Due to the disappearance of prebiotic active substances in the foregut, the impact on the microbiota in the hindgut dependent on the dosage is comparatively low.

Moreover, the fifth hypothesis is also approved because the impact on the hindgut microbiota was according to the starting degradation in the foregut and the resulting minor dosage reaching the hindgut comparatively low.

Sixth hypothesis: Daily prebiotic doses of FOS + inulin improves the insulin sensitivity and thus postprandial glucose clearance in several animals and the benefit is also assumed for clinically normal, non-obese horses following a hay-concentrate meal.

Non-obese clinically healthy horses might benefit from the long-term supply of the natural prebiotic consisting of FOS + inulin by an accelerated postprandial glucose clearance from the bloodstream after ingestion of a hay-concentrate meal. Therefore, the sixth hypothesis is approved. Nevertheless, the impact of specific initial state conditions (e.g. age, breed, health status) has not been taken into consideration during this investigation but this might modify the outcomes.

In conclusion, the supplemental feeding of natural prebiotics or prebiotic active compounds have distinct impacts concerning the equine gastrointestinal tract as well as the glycaemic and insulinaemic response but some aspects had to be discussed critically. The effect regarding the upper digestive tract respectively the stomach is not completely evaluated so that a harmful impact might not be excluded. Consequently, the supplementation with prebiotics to achieve a health-promoting effect needs to be investigated further.

7 Summary

Horses are often used as sport animals which were fed with starch-rich but low-fibre diets. Due to their adaptation to fibre-rich feedstuff, the modified diet in combination with abiotic/biotic stressors represents a risk factor for the development of gastrointestinal derived diseases. The concept of feeding prebiotics intends to promote the autochthonous microbiota predominantly in the hindgut and therefore to counteract possible disturbances. Several health-promoting products are available but the scientific based evidence is scarce. The current investigations aimed to evaluate the impact of feeding a natural prebiotic compound (Jerusalem artichoke meal) on the microbial metabolism and composition in the entire gastrointestinal tract as well as the systemic effect on the glycaemic and insulinaemic response and the total respectively compartmental tract digestibility of nutrients.

During a 3 weeks adaptation period, 12 adult horses (10 mares, 1 stallion, 1 gelding; mean bwt 534 ± 64.5 kg; mean age 14 ± 7.5 years) were divided into two feeding groups. Additionally to their basal diet consisting of crushed oat grains (1.2 g starch/kg bwt * d^{-1}) and meadow hay (as fed basis: 1.5 kg/100 kg bwt * d^{-1}), they were supplementary fed either with Jerusalem artichoke meal (JAM; recommended prebiotic dosage of 0.2 g FOS + inulin/kg bwt * d^{-1}) or maize cob meal without grains as control (CON; equivalent amount). On d 21 of the adaptation period, the horses were euthanized by a veterinarian and digesta samples were taken from 7 different parts in the gastrointestinal tract (stomach: *pars nonglandularis* [PN], *pars glandularis* [PG], small intestine [SI], colon: ventrale [CV], dorsale [CD] and transversum [CT]). Therein, the concentration of specific metabolic end products (SCFA, ammonia, lactate), the pH value and the relative abundance of the microbiota was determined. Furthermore, the total and compartmental tract nutrient digestibility coefficients were calculated according to the usage of internal or external (via bolus) applied markers. The feeding of JAM resulted in an elevated concentration of SCFA especially in the stomach with a concomitant modification of the microbiota. Particularly, the concentration of *n*-butyric acid as well as the relative abundance of the genus *Lactobacillus* was markedly increased. Furthermore, the impact on the hindgut was marginal with only a slightly elevated concentration of *n*-butyric acid exclusively in the colon ventrale and an increased relative abundance of an unknown member of the family *Lachnospiraceae*. The nutrient digestibility was not affected by the prebiotic

feeding but the partly degradation of fructans already in the stomachs was proven. In a second investigation, 6 adult clinically healthy mares (4 trotter, 1 Trakehner, 1 Mecklenburger; mean bwt 529 ± 38.7 kg; mean age 9.5 ± 2.9 years; mean BCS 5.1 ± 0.49/9) were used to evaluate the glycaemic and insulinaemic response after feeding the natural prebiotic Jerusalem artichoke meal. The mares were fed during a 2 x 3 weeks trial in 2 equal meals per day a basal ration consisting of crushed oat grains (1 g starch/kg bwt * d^{-1}) and meadow hay (1.5 kg/100 kg bwt * d^{-1}). Additionally, the horses intended to receive the prebiotic Jerusalem artichoke meal (prebiotic compound FOS + inulin in a dosage of 0.2 g/kg bwt * d^{-1}) or maize cob meal without grains as control (equivalent amount). At the end of each trial, blood was sampled to different time points ante- as well as postprandial (PP) and the concentration of glucose and insulin was determined. After feeding the prebiotic, the PP glucose concentration reached a lower peak compared with the control but the PP concentration of insulin was higher. Furthermore, the PP glucose clearance from the bloodstream was achieved faster in the prebiotic feeding group compared to the control and even the PP insulin concentration declined faster. The results indicate a positive impact of the long-term prebiotic supply in clinically healthy horses whereas the higher PP insulin concentration needs to be discussed critically. Summarizing, the results from the above-mentioned investigations indicate an impact of the natural prebiotic compound in the entire equine gastrointestinal tract as well as regarding the glycaemic and insulinaemic response. Nevertheless, the degradation of the inulin-type fructan already in the foregut needs to be seen critically because this might imply negative impacts concerning the stomach´s health. Furthermore, the autochthonous microbiota in the hindgut, which is actually the target area for the prebiotic, showed only marginal variations because of the lesser content of prebiotic active substances reaching this part. Future use might apply a galenic treated prebiotic but this might involve a correction of the recommended prebiotic dosage. Moreover, the systemic impact of feeding prebiotics needs to be evaluated in detail because the above-mentioned investigation included only clinically healthy, warmblood type mares. Horses have high intra-individual differences. Therefore, the transfer of results concerning the feeding of prebiotics is not recommended because dependent on the initial state of the horse, the outcome might be different. Further investigations need to be conducted including diverse starting conditions to get a more detailed view on the effects of feeding prebiotics to horses.

8 Zusammenfassung

Pferde werden aufgrund ihres Einsatzes als Sportpartner häufig mit stärkereichen aber gleichermaßen faserarmen Rationen versorgt. Die Fütterung von leistungsgerechten, nährstoffreichen Rationen stehen im Gegensatz zu ihrer natürlichen Adaptation an faserreiche Futterkomponenten und können, in Verbindung mit abiotischen oder biotischen Stressfaktoren, ein erhöhtes Risiko für die Entwicklung gastrointestinaler Erkrankungen darstellen. Die Fütterung von Präbiotika zielt dabei auf eine Stabilisierung der autochthonen Mikroflora im Dickdarmbereich ab und könnte somit der Ausbildung dieser intestinalen Störungen entgegenwirken. Derzeit sind etwaige „gesundheitsfördernde“ Produkte auf dem Markt, doch wissenschaftlich fundierte Erkenntnisse bezüglich der positiven Wirkungen dieser sind nur marginal vorhanden. Die Studien der vorliegenden Arbeit haben das Ziel, den Einfluss der Fütterung eines natürlichen Präbiotikums („Jerusalem artichocke meal“ = Topinambur) auf den mikrobiellen Stoffwechsel und deren Zusammensetzung im gesamten Verdauungstrakt zu beschreiben, einen systemischen Effekt bezüglich der glykämischen und insulinämischen Response zu evaluieren sowie die Gesamttraktverdaulichkeit beziehungsweise die Nährstoffverdaulichkeit in den einzelnen Kompartimenten des Verdauungstraktes zu untersuchen.

Während einer 3-wöchigen Adaptationsperiode wurden 12 adulte Pferde (10 Stuten, 1 Hengst sowie 1 Wallach; mittlere LM: 534 ± 64,5 kg; mittleres Alter: 14 ± 7,5 Jahre) randomisiert in 2 Fütterungsgruppen aufgeteilt. Zusätzlich zu einer Basalration bestehend aus gequetschtem Hafer (1,2 g Stärke/kg LM * d^{-1}) und Wiesenheu (OS; 1,5 kg/100 kg LM * d^{-1}) erhielten die Pferde entweder Topinambur (JAM; empfohlene präbiotische Dosierung: 0,2 g FOS + Inulin/kg LM * d^{-1}) oder Maisspindelmehl als Kontrolle (CON; masseäquivalent). Die Pferde wurden am 21. Tag der Adaptationsperiode von einem Tierarzt euthanisiert und es wurden Chymus Proben aus 7 verschiedenen Lokalitäten des Verdauungstraktes entnommen (Magen: *pars nonglandularis* [PN], *pars glandularis* [PG], Dünndarm [SI], Colon: ventrale [CV], dorsale [CD] und transversum [CT]). Darin wurden die Konzentration an spezifischen metabolischen Endprodukten (SCFA, Ammoniak, Laktat), der pH-Wert sowie die relativen Abundanzen der Mikrobiota bestimmt. Die Fütterung des JAM führte zu einer erhöhten Konzentration an SCFA vornehmlich im Bereich des Magen, welches mit einer Modifikation der dort ansässigen Mikrobiota einherging. Vor allem die

Konzentration an *n*-Buttersäure sowie die relative Abundanz der Gattung *Lactobacillus* waren deutlich erhöht. Des Weiteren zeigte sich im Dickdarmbereich nur ein marginaler Einfluss der Fütterung von JAM, wobei hier eine leichte Zunahme der Konzentration an *n*-Buttersäure sowie der vermehrten relativen Abundanz eines unbekannten Vertreters der Familie *Lachnospiraceae* lokal auf das Colon ventrale begrenzt war. Die Nährstoffverdaulichkeit wurde durch die Supplementierung des Präbiotikums nicht beeinflusst, wobei ein partieller Abbau von Fruktanen bereits im Magen nachgewiesen werden konnte. In einer zweiten Untersuchung wurden 6 adulte, klinisch unauffällige, gesunde Stuten (4 Traber, 1 Trakehner sowie 1 Mecklenburger; mittlere LM: 529 ± 38,7 kg; mittleres Alter: 9,5 ± 2,9 Jahre; mittlerer BCS: 5,1 ± 0,49/9) zur Beurteilung der glykämischen sowie insulinämischen Response nach der Fütterung des natürlichen Präbiotikums (JAM) verwendet. Die Stuten erhielten während einer 2 x 3-wöchigen Versuchsperiode in 2 identischen Mahlzeiten pro Tag eine Basalration bestehend aus gequetschtem Hafer (1 g Stärke/kg LM * d^{-1}) sowie Wiesenheu (OS; 1,5 kg/100 kg LM * d^{-1}) vorgelegt. Des Weiteren wurde angestrebt, den Stuten zusätzlich das Präbiotikum (JAM; Dosierung der präbiotischen Komponenten FOS + Inulin von 0,2 g/kg LM * d^{-1}) oder Maisspindelmehl als Kontrollvariante (masseäquivalent) vorzulegen. Am Ende jeder Versuchsperiode wurde Blut zu verschiedenen Zeitpunkten ante- sowie postprandial entnommen und darin die Konzentration an Glukose sowie Insulin bestimmt. Im Vergleich zur Kontrollfütterung zeigte sich nach der Supplementierung des Präbiotikums ein niedrigerer postprandialer Glukosepeak, aber die postprandiale Insulinkonzentration war deutlich höher. Weiterhin wurde durch die Präbiotikasupplementierung eine schnellere Entfernung der postprandial angefluteten Glukose aus dem Blutstrom erreicht und sogar die postprandiale Insulinkonzentration zeigte einen schnelleren Abfall im Vergleich zur Kontrollgruppe. Die Ergebnisse deuten auf einen positiven Effekt einer Langzeit-Präbiotikasupplementation bei klinisch unauffälligen, gesunden Pferden hin, wobei die hohe postprandiale Insulinkonzentration kritisch diskutiert werden muss.

Zusammenfassend zeigen die Ergebnisse der oben genannten Untersuchungen einen Einfluss der Fütterung eines natürlichen Präbiotikums auf den gesamten equinen Verdauungstrakt sowie auch auf die glykämische und insulinämische Response. Dennoch sollte der bereits im vorderen Gastrointestinaltrakt beginnende Abbau der Inulin-Typ-Fruktane kritisch diskutiert werden, weil dies negative Folgen

für die Gesundheit der Magenschleimhaut nach sich ziehen könnte. Die autochthone Mikroflora im Dickdarmbereich, welche vornehmlich das Ziel der Präbiotikafütterung ist, zeigte hierbei nur marginale Veränderungen. Aufgrund des deutlich geringen Anteiles der Wirksubstanz im zugefütterten Präbiotikum erreichte auch nur eine geringere Dosis diesen Abschnitt des Gastrointestinaltraktes. Die zukünftige Applikation von Präbiotika impliziert möglicherweise eine galenische Behandlung der Substanz. Dies würde gleichermaßen eine Korrektur der empfohlenen präbiotischen Dosierung nach sich ziehen, da nun unter Ausschluss einer Vorverdauung im Magen eine höhere Wirkdosis den Dickdarm erreichen könnte. Des Weiteren sollte der systemische Einfluss einer Fütterung von Präbiotika im Detail untersucht werden, da in der oben genannten Studie lediglich gesunde, klinisch unauffällige Warmblut-Stuten verwendet wurden. Generell zeigen Pferde hohe intra-individuelle Differenzen. Demzufolge ist eine Übertragung der Ergebnisse zur Anwendung von Präbiotika bei Pferden nicht ohne Weiteres zu empfehlen, da, aufgrund der Abhängigkeit vom Ausgangszustand der Tiere (hier gemeint: Gewicht, Gesundheitsstatus o.ä.), die Resultate differieren könnten. Zukünftige Studien sollten mit unterschiedlichen Anfangsbedingungen durchgeführt werden, sodass ein detaillierter Einblick in die Wirkung der Präbiotikasupplementierung bei Pferden erreicht werden kann.

9 References

Al Jassim, R. A. M.; Andrews, F. M. (2009): The bacterial community of the horse gastrointestinal tract and its relation to fermentative acidosis, laminitis, colic, and stomach ulcers. *The Veterinary Clinics of North America. Equine Practice* 25 (2): 199–215.

Asplin, K. E.; Sillence, M. N.; Pollitt, C. C.; McGowan, C. M. (2007): Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *The Veterinary Journal* 174 (3): 530–535.

Azorín-Ortuño, M.; Urbán, C.; Cerón, J. J.; Tecles, F.; Allende, A.; Tomás-Barberán, F. A.; Espín, J. C. (2009): Effect of low inulin doses with different polymerisation degree on lipid metabolism, mineral absorption, and intestinal microbiota in rats with fat-supplemented diet. *Food Chemistry* 113 (4): 1058–1065.

Bach Knudsen, K. E. (2012): Prebiotic with special emphasis on butyrogenic carbohydrates and the effects on gut function. In: H. Wilhelm Schaumann Stiftung (Ed.): 24. Hülsenberger Gespräche. Lübeck, 06.-08.06.2012: 84–90.

Bachmann, M.; Wensch-Dorendorf, M.; Bulang, M.; Zeyner, A. (2016): Impact of dynamics of faecal concentrations of plant and synthetic n-alkanes on their suitability for the estimation of dry matter intake and apparent digestibility in horses. *Journal of Agricultural Science* 154 (7): 1291–1305.

Bachmann, M.; Wensch-Dorendorf, M.; Wulf, M.; Glatter, M.; Siebmann, M.; Bierögel, C. et al. (2016b): Bolus matrix for administration of dietary markers in horses. *Livestock Science* 185: 43–49.

Bacon, J. S. D.; Loxley, R. (1952): Seasonal changes in the carbohydrates of the Jerusalem artichoke tuber. *Biochemical Journal* 51 (2): 208–213.

Bailey, S. R.; Baillon, M.-L.; Rycroft, A. N.; Harris, P. A.; Elliot, J. (2003): Identification of equine cecal bacteria producing amines in an *in vitro* model of carbohydrate overload. *Applied and Environmental Microbiology* 69 (4): 2087–2093.

Bailey, S. R.; Menzies-Gow, N.; Harris, P. A.; Habershon-Butcher, J. L.; Crawford, C.; Berhane, Y.; Boston, R. C.; Elliot, J. (2007): Effect of dietary fructans and dexamethasone administration on the insulin response of ponies predisposed to laminitis. *Journal of the American Veterinary Medical Association* 231 (9): 1365–1373.

Bamford, N. J.; Baskerville, C. L.; Harris, P. A.; Bailey, S. R. (2015): Postprandial glucose, insulin, and glucagon-like peptide-1 responses of different equine breeds adapted to meals containing micronized maize. *Journal of Animal Science* 93 (7): 3377–3383.

Bamford, N. J.; Potter, S. J.; Baskerville, C. L.; Harris, P. A.; Bailey, S. R. (2016b): Effect of increased adiposity on insulin sensitivity and adipokine concentrations in different equine breeds adapted to cereal-rich or fat-rich meals. *The Veterinary Journal* 214: 14–20.

Bamford, N. J.; Potter, S. J.; Harris, P. A.; Bailey, S. R. (2016a): Effect of increased adiposity onoinsulin sensitivity and adipokine concentrations in horses and ponies fed a high fat diet, with or without a once daily high glycaemic meal. *Equine Veterinary Journal* 48 (3): 368–373.

Bamford, N. J.; Potter, S. J.; Harris, P. A.; Bailey, S. R. (2014): Breed differences in insulin sensitivity and insulinemic responses to oral glucose in horses and ponies of moderate body condition score. *Domestic Animal Endocrinology* 47: 101–107.

Barrangou, R.; Altermann, E.; Hutkins, R.; Cano, R.; Klaenhammer, T. R. (2003): Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. *Proceedings of the National Academy of Sciences of the United States of America* 100 (15): 8957–8962.

Barry, K. A.; Vester, B. M.; Fahey, G. C. (jr.) (2009): Prebiotics in companion and livestock animal. In: D. Charalampopoulos und R. A. Rastall (Ed.): Prebiotics and Probiotics Science Technology. New York: Springer New York: 443–447.

Berg, E. L.; Fu, C. J.; Porter, J. H.; Kerley, M. S. (2005): Fructooligosaccharide supplementation in the yearling horse: Effects on fecal pH, microbial content and volatile fatty acid concentration. *Journal of Animal Science* (83): 1549–1553.

Bergmann, E. N. (1990): Energy Contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70 (2): 567–590.

Bernet, M.-F.; Brassart, D.; Neeser, J.-R.; Servin, A. L. (1993): Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. *Applied and Environmental Microbiology* 59 (12): 4121–4128.

Bessesen, D. H. (2001): The role of carbohydrates in insulin resistance. *The Journal of Nutrition* 131: 2782S–2786S.

Biddle, A.; Stewart, L.; Blanchard, J.; Leschine, S. (2013): Untangling the genetic basis of fibrolytic specialization by *Lachnospiraceae* and *Ruminococcaceae* in diverse gut communities. *Diversity* 5 (3): 627–640.

Blaut, M. (2002): Relationship of prebiotics and food to intestinal microflora. *European Journal of Nutrition* 41 (Suppl 1): I/11-I/16.

Bochnia, M.; Schürer, C.; Gottschalk, J.; Einspanier, A.; Hillegeist, D.; Wensch-Dorendorf, M.; Greef, J. M.; Glatter, M.; Zeyner, A. (2017): Effects of isoenergetic quantities of a low-starch muesli feed high in fat and fibre vs. oat grains on the glycemic and insulinemic responses and feed intake patterns in sport ponies. *Journal of Animal Physiology and Animal Nutrition* 101 (Suppl 1): 43–50.

Boehmer, B. M.; Branner, G. R.; Roth-Maier, D. A. (2005): Precaecal and faecal digestibility of inulin (DP 10-12) or an inulin/*Enterococcus faecium* mix and effects on nutrient digestibility and microbial gut flora. *Journal of Animal Physiology and Animal Nutrition* 89 (11-12): 388–396.

Borer, K. E.; Bailey, S. R.; Menzies-Gow, N. J.; Harris, P. A.; Elliott, J. (2012): Effect of feeding glucose, fructose, and inulin on blood glucose and insulin concentrations in normal ponies and those predisposed to laminitis. *Journal of Animal Science* 90 (9): 3003–3011.

Brøkner, C.; Austbo, D.; Naesset, J. A.; Blache, D.; Bach Knudsen, K. E.; Tauson, A. H. (2016): Metabolic response to dietary fibre composition in horses. *Animal* 10 (7): 1155–1163.

Brown, G. D.; Gordon, S. (2001): A new receptor for beta-glucans. *Nature* (413): 36–37.

Cairns, A. J.; Cookson, A.; Thomas, B. J.; Turner, L. B. (2002): Starch metabolism in the fructan-grasses. Patterns of starch accumulation in excised leaves of *Lolium temulentum* L. *Journal of Plant Physiology* 159 (3): 293–305.

Cani, P. D.; Dewever, C.; Delzenne, N. M. (2004): Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *British Journal of Nutrition* 92: 521–526.

Cani, P. D.; Lecourt, E.; Dewulf, E. M.; Sohet, F. M.; Pachikian, B. D.; Naslain, D.; Backer, F. de; Neyrinck, A. M.; Delzenne, N. M. (2009): Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *The American Journal of Clinical Nutrition* 90 (5): 1236–1243.

Cann, A. J.; Fandrich, S. E.; Heaphy, S. (2005): Analysis of the virus population present in equine faeces indicates the presence of hundreds of uncharacterized virus genomes. *Virus genes* 30 (2): 151–156.

Cehak, A.; Burmester, M.; Geburek, F.; Feige, K.; Breves, G. (2009): Electrophysiological characterization of electrolyte and nutrient transport across the small intestine in horses. *Journal of Animal Physiology and Animal Nutrition* 93 (3): 287–294.

Cehak, A.; Marholt, L.; Krägeloh, T.; Zuraw, A.; Kershaw, O.; Brehm, R.; Breves, G. (2019) Does prebiotic feeding affect equine gastric health? A Study on the effects of prebiotic-induced gastric butyric acid production on mucosal integrity of the equine stomach. *Research in Veterinary Science* 124: 303-309.

Cehak, A.; Schröder, B.; Feige, K.; Breves, G. (2013): *In vitro* studies on intestinal peptide transport in horses. *Journal of Animal Science* 91 (11): 5220–5228.

Cehak, A.; Wilkens, M. R.; Guschlbauer, M.; Mrochen, N.; Schröder, B.; Feige, K.; Breves, G. (2012): *In vitro* studies on intestinal calcium and phosphate transport in horses. *Comparative Biochemistry and Physiology Part A* 161 (2): 259–264.

Chameroy, K. A.; Frank, N.; Elliott, S. B.; Boston, R. C. (2016): Comparison of plasma active glucagon-like peptide 1 concentrations in normal horses and those with equine metabolic syndrome and in horses placed on a high-grain diet. *Journal of Equine Veterinary Science* 40: 16–25.

Cherbut, C. (2002): Inulin and oligofructose in the dietary fibre concept. *British Journal of Nutrition* 87 (S2): S159-S162.

Coenen, M. (1992): Beobachtungen zum Vorkommen von Magenulcera beim Pferd. *Pferdeheilkunde*: 188–191.

Coenen, M.; Moeßeler, A.; Vervuert, I. (2006): Fermentative gases in breath indicate that inulin and starch start to be degraded by microbial fermentation in the stomach and small intestine of the horse in contrast to pectin and cellulose. *The Journal of Nutritition* (136): 2108S-2110S.

Costa, M. C.; Silva, G.; Ramos, R. V.; Staempfli, H. R.; Arroyo, L. G.; Kim, P.; Weese, J. S. (2015): Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. *The Veterinary journal* 205 (1): 74–80.

Crawford, C.; Sepulveda, M. F.; Elliott, J.; Harris, P. A.; Bailey, S. R. (2007): Dietary fructan carbohydrate increases amine production in the equine large intestine: implications for pasture-associated laminitis. *Journal of Animal Science* 85 (11): 2949–2958.

D´Mello, J.P.F. (2000): Farm animal metabolism and nutrition. Wallingford, Oxon: CABI Publ.

Daly, K.; Al-Rammahi, M.; Arora, D. K.; Moran, A. W.; Proudman, C. J.; Ninomiya, Y.; Shirazi-Beechey, S. P. (2012b): Expression of sweet receptor components in equine small intestine. Relevance to intestinal glucose transport. *American Journal of Physiology. Regulatory,Integrative and Comparative Physiology* 303 (2): R199-208.

Daly, K.; Proudman, C. J.; Duncan, S. H.; Flint, H. J.; Dyer, J.; Shirazi-Beechey, S. P. (2012a): Alterations in microbiota and fermentation products in equine large intestine in response to dietary variation and intestinal disease. *The British Journal of Nutrition* 107 (7): 989–995.

De Fombelle, A., Julliand, V.; Drogoul, C.; Jacotot, E. (2001): Feeding and microbial disorders in horses: 1-effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities. *Journal of Equine Veterinary Science* 21 (9): 439–445.

De Fombelle, A.; Varloud, M.; Goachet, A. G.; Jacotot, E.; Philippeau, C.; Drogoul, C.; Julliand, V. (2003): Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets. *Animal Science* (77): 293–304.

De Laat, M. A., McGowan, C. M.; Sillence, M. N.; Pollitt, C. C. (2010): Equine laminitis: induced by 48 h hyperinsulinaemia in Standardbred horses. *Equine Veterinary Journal* 42 (2): 129–135.

De Laat, M. A.; McGree, J. M.; Sillence, M. N. (2016): Equine hyperinsulinemia: investigation of the enteroinsular axis during insulin dysregulation. *American Journal of Physiology-Endocrinology and Metabolism* 310 (1): E61-72.

De La Corte, F. D.; Valberg, S. J.; Mickelson, J. R.; Hower-Moritz, M. (1999): Blood glucose clearance after feeding and exercise in polysaccharide storage myopathy. *Equine Veterinary Journal, Supplement* (30): 324–328.

Deacon, C. F.; Ahrén, B. (2011): Physiology of incretins in health and disease. *The Review of Diabetic Studies* 8 (3): 293–306.

Delzenne, N. M.; Cani, P. D. (2010): Nutritional modulation of gut microbiota in the context of obesity and insulin resistance. Potential interest of prebiotics. *International Dairy Journal* 20 (4): 277–280.

Delzenne, N. M.; Cani, P. D.; Daubioul, C.; Neyrinck, A. M. (2005): Impact of inulin and oligofructose on gastrointestinal peptides. *British Journal of Nutrition* 93 (S1): S157-S161.

Delzenne, N. M.; Cani, P. D.; Neyrinck, A. M. (2007): Modulation of glucagon-like peptide 1 and energy metabolism by inulin and oligofructose: experimental data. *Journal of Nutrition* (137): 2547S-2551S.

Delzenne, N. M.; Neyrinck, A. M.; Cani, P. D. (2013): Gut microbiota and metabolic disorders: How prebiotic can work? *The British Journal of Nutrition* 109 (Suppl 2): S81-S85.

Delzenne, N. M.; Williams, C. M. (2002): Prebiotics and lipid metabolism. *Current Opinion in Lipidology* 13 (1): 61–67.

Dengler, F.; Rackwitz, R.; Pfannkuche, H.; Gäbel, G. (2018): Coping with hypoxia. Adaptation of glucose transport mechanisms across equine jejunum epithelium. *Journal of Equine Veterinary Science* 69: 1–10.

Dicks, L.M.T.; Botha, M.; Dicks, E.; Botes, M. (2014): The equine gastro-intestinal tract: An overview of the microbiota, disease and treatment. *Livestock Science* 160: 69–81.

Dougal, K.; de la Fuente, G.; Harris, P. A.; Girdwood, S. E.; Pinloche, E.; Newbold, C. J.; Schuch, R. (2013): Identification of a core bacterial community within the large intestine of the horse. *PLoS ONE* 8 (10): e77660.

Dougal, K.; de la Fuente, G.; Harris, P. A.; Girdwood, S. E.; Pinloche, E.; Geor, R. J.; Nielsen, B. D.; Schott, H. C.; Elzinga, S.; Newbold, C. J., Ibekwe, A. M. (2014): Characterisation of the Faecal Bacterial Community in Adult and Elderly Horses Fed a High Fibre, High Oil or High Starch Diet Using 454 Pyrosequencing. *PLoS ONE* 9 (2): e87424.

Duehlmeier, R.; Deegen, E.; Fuhrmann, H.; Widdel, A.; Sallmann, H.-P. (2001): Glucose-dependent insulinotropic polypeptide (GIP) and the enteroinsular axis in equines. *Comparative Biochemistry and Physiology Part A* (129): 563–575.

Dyer, J.; Al-Rammahi, M.; Waterfall, L.; Salmon, K. S. H.; Geor, R. J.; Bouré, L.; Edwards, G. B.; Proudman, C. J.; Shirazi-Beechey, S. P. (2009): Adaptive response of equine intestinal Na+/glucose co-transporter (SGLT1) to an increase in dietary soluble carbohydrate. *Pflügers Archiv: European Journal of Physiology* 458 (2): 419–430.

Dyer, J.; Fernandez-Castano Merediz, E.; Salmon, K. S.; Proudman, C. J.; Edwards, G. B.; Shirazi-Beechey, S. P. (2002): Molecular characterisation of carbohydrate digestion and absorption in equine small intestine. *Equine Veterinary Journal* (34): 349–358.

Ebner, F. (2017): Naturstoffe und Biochemie: Ein Überblick für Chemiker und Biotechnologen. Springer Fachmedien Wiesbaden.

Elliott, J.; Bailey, S. R. (2006): Gastrointestinal derived factors are potential triggers for the development of acute equine laminitis. *Journal of Nutrition* 136 (7): 2103S-2017S.

Ewaschuk, J. B.; Zello, G. A.; Naylor, J. M.; Brocks, D. R. (2002): Metabolic acidosis: separation methods and biological relevance of organic acids and lactic acid enantiomers. *Journal of Chromatography B* (781): 39–56.

Fernandes, K. A.; Kittelmann, S.; Rogers, C. W.; Gee, E. K.; Bolwell, C. F.; Bermingham, E. N.; Thomas, D. G.; Forster, R. J. (2014): Faecal microbiota of forage-fed horses in New Zealand and the population dynamics of microbial communities following dietary change. *PLoS ONE* 9 (11): e112846.

Fernandez-Castaño Merediz, E.; Dyer, J.; Salmon, K. S. H.; Shirazi-Beechey, S. P. (2004): Molecular characterisation of fructose transport in equine small intestine. *Equine Veterinary Journal* 36 (6): 532–538.

Finnie, I. A.; Dwarakanath, A. D.; Taylor, B. A.; Rhodes J.M. (1995): Colonic mucin synthesis is increased by sodium butyrate. *Gut* (36): 93–99.

Fiordaliso, M.; Kok, N.; Desager, J.-P.; Goethals, F.; Deboyser, D.; Roberfroid, M.; Delzenne, N. (1995): Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids* 30 (2): 163–167.

Firshman, A. M.; Valberg, S. J. (2007): Factors affecting clinical assessment of insulin sensitivity in horses. *Equine Veterinary Journal* 39 (6): 567–575.

Fitzgerald, D. M.; Walsh, D. M.; Sillence, M. N.; Pollit, C. C.; Laat, M. A. de (2019): Insulin and incretin responses to grazing in sulin-dysregulated and health ponies. *Journal of Veterinary Internal Medicine* (33): 225–232.

Flickinger, E. A.; Fahey, G. C. (2002): Pet food and feed applications of inulin, oligofructose and other oligosaccharides. *British Journal of Nutrition* 87 (S2): S297-S300.

Gaenzle, M. G.; Follador, R. (2012): Metabolism of oligosaccharides and starch in lactobacilli: a review. *Frontiers in Microbiology* 3: 340.

Geor, R. J. (2008): Metabolic predispositions to laminitis in horses and ponies: obesity, insulin resistance and metabolic syndromes. *Journal of Equine Veterinary Science* 28 (12): 753–759.

Gesellschaft für Ernährungsphysiologie [Society for Nutrition Physiology] (Ed.) (2014): Empfehlungen zur Energie- und Nährstoffversorgung von Pferden: DLG-Verlag (Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere, 11).

Gibson, G. R.; Hutkins, R.; Sanders, M. E.; Prescott, S. L.; Reimer, R. A.; Salminen, S. J.; Scott, K.; Stanton, C.; Swanson, K. S.; Cani, P. D.; Verbeke, K.; Reid, G. (2017): Expert consensus document. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature reviews. Gastroenterology & Hepatology* 14 (8): 491–502.

Gibson, G. R.; Roberfroid, M. E. (1995): Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *The Journal of Nutritition* (125): 1401–1412.

Gibson, G. R.; Scott, K. P.; Rastall, R. A.; Tuohy, K. M.; Hotchkiss, A.; Dubert-Ferrandon, A.; Gareau, M.; Murphy, E. F:, Saulnier, D.; Loh, G.; Macfarlane, S.; Delzenne, N. M.; Ringel, Y.; Kozianowski, G.;

Dickmann, R.; Lenoir-Wijnkoop, I.; Walker, C.; Buddington, R. (2010): Dietary prebiotics. Current status and new definition. *Food Science & Technology Bulletin: Functional Foods* 7 (1): 1–19.

Grodsky, G. M. (1972): A treshold distribution hypothesis for packet storage of insulin and its mathematical modelling. *The Journal of Clinical Investigation* (51): 2047–2059.

Guilloteau, P.; Martin, L.; Eeckhaut, V.; Ducatelle, R.; Zabielski, R.; van Immerseel, F. (2010): From the gut to the peripheral tissues. The multiple effects of butyrate. *Nutrition Research Reviews* 23 (2): 366–384.

Gupta, A. K.; Kaur, N. (2000): Fructan metabolism in Jerusalem artichoke and chicory. In: A. K. Gupta and N. Kaur (Eds.): Carbohydrate reserves in plant synthesis and regulation, Vol. 26: Elsevier Science B.V.: 223–248.

Guerbuez, E.; Inal, F.; Ata, S. Ü.; Citil, Ö. B.; Kav, K.; Kücükkaya, F. (2010): Effects of supplemental fructo-oligosaccharide and mannan-oligosaccharide on nutrient digestibilities, volatile fatty acid concentrations and immune function in horses. *Turkish Journal of Veterinary & Animal Science* 34 (1): 39–44.

Haensel, R.; Sticher, O., Steinegger, E. (2010): Pharmakognosie-Phytopharmazie. Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg: p. 500.

Han, K.-H.; Tsuchihira, H.; Nakamura, Y.; Shimada, K.; Ohba, K.; Aritsuka, T.; Uchino, H.; Kikuchi, H.; Fukushima, M. (2013): Inulin-type fructans with different degrees of polymerization improve lipid metabolism but not glucose metabolism in rats fed a high-fat diet under energy restriction. *Digestive Diseases and Sciences* 58 (8): 2177–2186.

Harlow, B. E.; Lawrence, L. M.; Kagan, I. A.; Flythe, M. D. (2014): Inhibition of fructan-fermenting equine faecal bacteria and *Streptococcus bovis* by hops (*Humulus lupulus* L.) β-acid. *Journal of Applied Microbiology* 117 (2): 329–339.

Harmsen, H.J.M.; Raangs, G. C.; Franks, A. H.; Wildeboer-Veloo, A.C.M.; Welling, G. W. (2002): The effect of the prebiotic inulin and probiotic *Bifidobacterium longum* on the fecal microflora of healthy volunteers measured by FISH and DGGE. *Microbial Ecology in Health and Disease* (14): 211–219.

Harris, J. I.; Sanger, F.; Naughton, M. A. (1956): Species differences in insulin. *Archives of Biochemistry and Biophysics* (65): 427–438.

Hedemann, M. S.; Knudsen, K. E. B. (2010): Dried chicory root has minor effects on the digestibility of nutrients and the composition of the microflora at the terminal ileum and in faeces of growing pigs. *Livestock Science* (134): 53–55.

Heppes, C. (2003): Blutglukose-, Insulin- und Glukagonkonzentration bei Vielseitigkeitspferden unter Belastung. PhD-thesis. Tierärztliche Hochschule Hannover, Hannover.

Herrmann, J.; Hermes, R.; Breves, G. (2011): Transepithelial transport and intraepithelial metabolism of short-chain fatty acids (SCFA) in the porcine proximal colon are influenced by SCFA concentration and luminal pH. *Comparative Biochemistry and Physiology Part A* 158 (1): 169–176.

Hesta, M.; Janssens, G. P. J.; Millet, S.; Wilde, R. de (2003): Prebiotics affect nutrient digestibility but not faecal ammonia in dogs fed increased dietary protein levels. *British Journal of Nutrition* 90: 1007-1014.

Heyer, A.G; Schroeer, B.; Radosta, S.; Wolff, D.; Czapla, S.; Springer, J. (1998): Structure of the enzymatically synthesized fructan inulin. *Carbohydrate Research* 313 (3-4): 165–174.

Hoffman, R. M.; Boston, R. C.; Stefanovski, D.; Kronfeld, D. S.; Harris, P. A. (2003): Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. *Journal of Animal Science* 81 (9): 2333–2342.

Horton, H. R.; Moran, L. A.; Scrimgeour, K. G.; Perry, M. D.; Rawn, J. D. (2008): Biochemie. Pearson Studium.

Huntington, G. B. (1990): Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. *Reproduction Nutrition Development* (30): 35–47.

Ince, J. C.; Longland, A. C.; Moore-Colyer, M. J. S.; Harris, P. A. (2014): *In vitro* degradation of grass fructan by equid gastrointestinal digesta. *Grass and Forage Science* 69 (3): 514–523.

Jacob, S. I.; Geor, R. J.; Weber, P. S. D.; Harris, P. A.; McCue, M. E. (2018): Effect of age and dietary carbohydrate profiles on glucose and insulin dynamics in horses. *Equine Veterinary Journal* 50 (2): 249–254.

Jami, E.; Mizrahi, I. (2012): Composition and Similarity of Bovine Rumen Microbiota across Individual Animals. *PLOS ONE* 7 (3): e33306.

Janczyk, P.; Pieper, R.; Smidt, H.; Souffrant, W. B. (2010): Effect of alginate and inulin on intestinal microbial ecology of weanling pigs reared under different husbandry conditions. *FEMS Microbiology Ecology* 72 (1): 132–142.

Johnson, R. J.; Rivard, C.; Lanaspa, M. A.; Otabachian-Smith, S.; Ishimoto, T.; Cicerchi, C.; Cheeke, P. R.; Macintosh, B.; Hess, T. (2013): Fructokinase, fructans, intestinal permeability, and metabolic syndrome: An equine connection? *Journal of Equine Veterinary Science* 33 (2): 120–126.

Julliand, V. (1992): Microbiology in the equine hindgut. *1. Europäische Konferenz über die Ernährung des Pferdes, Pferdeheilkunde, Sonderheft*: 42–47.

Julliand, V.; Fombelle, A. de; Drogoul, C.; Jacotot, E. (2001): Feeding and microbial disorders in horses: Part 3—Effects of three hay:grain ratios on microbial profile and activities. *Journal of Equine Veterinary Science* 21 (11): 543–546.

Julliand, V.; Grimm, P. (2016): HORSE SPECIES SYMPOSIUM: The microbiome of the horse: History and current knowledge. *Journal of Animal Science* (94): 2262–2274.

Julliand, V.; Grimm, P. (2017): The impact of diet on the hindgut microbiome. *Journal of Equine Veterinary Science* 52: 23–28.

Julliand, V.; Zeyner, A. (2013): Dietary strategies for optimizing gastro intestinal health: an update on pre- and probiotics. In: Horse Health Nutrition, Vol. 6. European Equine Health & Nutrition Congress. Ghent, 01.03.-02.03.2013: 75–84.

Kabuki, T.; Saito, T.; Kawai, Y.; Uemura, J.; Itoh, T. (1997): Production, purification and characterization of reutericin 6, a bacteriocin with lytic activity produced by *Lactobacillus reuteri* LA6. *International Journal of Food Microbiology* (34): 145–156.

Karasu, G. K.; Krabbenborg, R.; Einspanier, A.; Vervuert, I. (2015): Insulinaemic and glycaemic responses to a second meal of a fibre- or starch-enriched compound feed in healthy horses. *The Veterinary Journal* (204): 220–222.

Kashimura, J.; Kimura, M.; Jtokawa, Y. (1996): The effects of isomaltulose, isomalt, and isomaltulose-based oligomers on mineral absorption and retention. *Biological Trace Elementa Research* (54): 239–250.

Kasubuchi, M.; Hasegawa, S.; Hiramatsu, T.; Ichimura, A.; Kimura, I. (2015): Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients* 7 (4): 2839–2849.

Kazakos, K. (2011): Incretin effect. GLP-1, GIP, DPP4. *Diabetes Research and Clinical Practice* 93: S32-S36.

Kirchgeßner, M.; Stangl, G. I.; Schwarz, F. J.; Roth, F. X.; Südekum, K.-H.; Eder, K. (2014): Tierernährung: Leitfaden für Studium und Praxis. Frankfurt am Main: DLG-Verlag.

Kleessen, B.; Hartmann, L.; Blaut, M. (2001): Oligofructose and long-chain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora. *British Journal of Nutrition* 86 (2): 291–300.

Kleine, B.; Rossmanith, W. (2014): Hormone und Hormonsystem: Lehrbuch der Endokrinologie. 3. Aufl. Berlin: Springer Spektrum.

Koeller, G.; Bassewitz, K.; Schusser, G. F. (2016): Referenzbereiche von Insulin, insulinahnlichem Wachstumsfaktor 1 (IGF-1) und adrenokortikotropem Hormon bei Ponys. *Tierärztliche Praxis. Ausgabe G, Großtiere/Nutztiere* 44 (1): 19–25.

Koenen, M. E.; Cruz Rubio, J. M.; Mueller, M.; Venema, K. (2016): The effect of agave fructan products on the activity and composition of the microbiota determined in a dynamic *in vitro* model of the human proximal large intestine. *Journal of Functional Foods* 22: 201–210.

Kronfeld, D. S.; Treiber, K. H.; Hess, T. M.; Boston, R. C. (2005): Insulin resistance in the horse: Definition, detection, and dietetics. *Journal of Animal Science* 83: E22-E31.

Kulikov, E. E.; Isaeva, A. S.; Rotkina, A. S.; Manykin, A. A.; Letarov, A. V. (2007): Diversity and dynamics of bacteriophages in horse feces. *Microbiology* 76 (2): 236–242.

Le Bourgot, C.; Ferret-Bernard, S.; Le Normand, L.; Savary, G.; Menendez-Aparicio, E.; Blat, S.; Appert-Bossard, E.; Respondek, F.; Le Huërou-Luron, I. (2014): Maternal short-chain fructooligosaccharide supplementation influences intestinal immune system maturation in piglets. *PLoS ONE* 9 (9): e107508.

Li, W.; Zhang, J.; Yu; Li, Q.; Dong, F.; Wang, G.; Gu, G.; Guo, Z. (2015): Extraction, degree of polymerization determination and prebiotic effect evaluation of inulin from Jerusalem artichoke. *Carbohydrate Polymers* 121: 315–319.

Lindåse, S.; Müller, C.; Nostell, K.; Bröjer, J. (2018): Evaluation of glucose and insulin response to haylage diets with different content of nonstructural carbohydrates in 2 breeds of horses. *Domestic animal endocrinology* 64: 49–58.

Liggenstoffer, A. S.; Youssef, N. H.; Couger, M. B.; Elshahed, M. S. (2010): Phylogenetic diversity and community structure of anaerobic gut fungi (phylum *Neocallimastigomycota*) in ruminant and non-ruminant herbivores. *The ISME Journal* 4 (10): 1225–1235.

Loh, G.; Eberhard, M.; Brunner, R. M.; Henning, U.; Kuhla, S.; Kleessen, B.; Metges, C. C. (2006): Inulin alters the intestinal microbiota and short-chain fatty acid concentrations in growing pigs regardless of their basal diet. *The Journal of Nutritition* (136): 1198–1202.

Longland, A. C.; Byrd, B. M. (2006): Pasture nonstructural carbohydrates and equine laminitis. *Journal of Nutrition* 136 (7): 2099S–2102S.

Luthersson, N.; Nielsen, K. H.; Harris, P.; Parkin, T. D. H. (2009): Risk factors associated with equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. *Equine Veterinary Journal* 41 (7): 625–630.

Lwin, K.-O.; Matsui, H. (2014): Comparative analysis of the methanogen diversity in horse and pony by using mcrA gene and archaeal 16s rRNA gene clone libraries. *Archaea* (http://dx.doi.org/10.1155/2014/483574)

Mackie, R. I.; Wilkins, C. A. (1988): Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. *Applied and Environmental Microbiology* 54: 2155–2160.

Masanetz, S.; Wimmer, N.; Plitzner, C.; Limbeck, E.; Preißinger, W.; Pfaffl, M. W. (2010): Effects of inulin and lactulose on the intestinal morphology of calves. *Animal* 4 (5): 739–744.

Mc Cann, K. S. (2000): The diversity-stability debate. *Nature* 405 (6783): 228–233.

Mc Cowen, K. C.; Abel, E. D. (2000): The contribution of defects in insulin signalling in skeletal muscle to insulin resistance and type 2 diabetes. In: Advances in structural biology (Malhotra, S. K. (Ed.)), JAI press; Greenwich, London: pp. 43-44.

Mc Nabney, S. M.; Henagan, T. M. (2017): Short chain fatty acids in the colon and peripheral tissues. A focus on butyrate, colon cancer, obesity and insulin resistance. *Nutrients* 9 (12): 1348

Milinovich, G. J.; Trott, D. J.; Burrell, P. C.; Eps, A. W. van; Thoefner, M. B.; Blackall, L. L.; Al Jassim, R A. M.; Morton, J. M. P.; Pollit, C. C. (2006): Changes in equine hindgut bacterial populations during oligofructose-induced laminitis. *Environmental Microbiology* 8 (5): 885–898.

Miyaji, M.; Ueda, K.; Nakatsuji, H.; Tomioka, T.; Kobayashi, Y.; Hata, H.; Kondo, S. (2008): Mean retention time of digesta in the different segments of the equine hindgut. *Animal Science Journal* 79 (1): 89–96.

Morrison, D. J.; Preston, T. (2016): Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut microbes* 7 (3): 189–200.

Morrison D. J.; Mackay, W. G.; Edwards, C. A.; Preston, T.; Dodson, B.; Weaver, L. T. (2006): Butyrate production from oligofructose fermentation by the human faecal flora: what is the contribution of extracellular acetate and lactate? *British Journal of Nutrition* 96: 570–577.

Mueller, M.; Reiner, J.; Fleischhacker, L.; Viernstein, H.; Loeppert, R.; Praznik, W. (2016): Growth of selected probiotic strains with fructans from different sources relating to degree of polymerization and structure. *Journal of Functional Foods* 24: 264–275.

Nadeau, J. A.; Andrews, F. M.; Mathew, A. G.; Argenzio, R. A.; Blackford, J. T.; Sohtell, M.; Saxton, A.M. (2000): Evaluation of diet as cause of gastric ulcers in horses. *American Journal of Veterinary Research* 61 (7): 784–790.

Nadeau, J. A.; Andrews, F. M.; Patton, C. S.; Argenzio, R. A.; Mathew, A. G.; Saxton, A. M. (2003): Effects of hydrochloric, acetic, butyric, and propionic acids on pathogenesis of ulcers in the nonglandular portion of the stomach of horses. *American Journal of Veterinary Research* 64 (4): 404–412.

Nedjadi, T.; Moran, A. W.; Al-Rammahi, M. A.; Shirazi-Beechey, S. P. (2014): Characterization of butyrate transport across the luminal membranes of equine large intestine. *Experimental Physiology* 99 (10): 1335–1347.

O' Donnell, M. M.; Harris, H. M. B.; Ross, R. P.; O'Toole, P. W. (2017): Core fecal microbiota of domesticated herbivorous ruminant, hindgut fermenters, and monogastric animals. *Microbiology Open* 6 (5): e509.

Onishi, J. C.; Park, J.-W.; Prado, J.; Eades, S. C.; Mirza, M. H.; Fugaro, M. N.; Haeggblom, M. M.; Reinemeyer, C. R. (2012): Intestinal bacterial overgrowth includes potential pathogens in the carbohydrate overload models of equine acute laminitis. *Veterinary Microbiology* 159 (3-4): 354–363.

Ozeki, K.; Imai, S.; Katsuno, M. (1973): On the distribution of the ciliated protozoa in the large intestine of horse. *Tohoku Journal of Agricultural Science* 24 (2): 86–101.

Parnell, J. A.; Reimer, R. A. (2009): Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *The American Journal of Clinical Nutrition* 89 (6): 1751–1759.

Patterson, J. K.; Yasuda, K.; Welch, R. M.; Miller, D. D.; Lei, X. G. (2010): Supplemental dietary inulin of variable chain lengths alters intestinal bacterial populations in young pigs. *The Journal of Nutrition* 140 (12): 2158–2161.

Perkins, G. A.; den Bakker, H. C.; Burton, A. J.; Erb, H. N.; McDonough, S. P.; McDonough, P. L.; Parker, J.; Rosenthal, R. L.; Wiedmann, M.; Dowd, S. E.; Simpson, K. W. (2012): Equine stomachs harbor an abundant and diverse mucosal microbiota. *Applied and Environmental Microbiology* 78 (8): 2522–2532.

Pierce, K. M.; Sweeney, T.; Brophy, P. O.; Callan, J. J.; Fitzpatrick, E.; McCarthy, P.; O'Doherty, J. V. (2006): The effect of lactose and inulin on intestinal morphology, selected microbial populations and volatile fatty acid concentrations in the gastro-intestinal tract of the weanling pig. *Animal Science* 82 (3): 311–318.

Ploeger, S.; Stumpff, F.; Penner, G. B.; Schulzke, J.-D.; Gäbel, G.; Martens, H.; Shen, Z.; Günzel, D.; Aschenbach, J. R. (2012): Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Annals of the New York Academy of Sciences* 1258 (1): 52–59.

Pollock, C.; Cairns, A. J. (1991): Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology* 42 (1): 77–101.

Pradhan, S. K.; Das, A.; Kullu, S. S.; Saini, M.; Pattanaik, A. K.; Dutta, N.; Sharma, A. K. (2015): Effect of feeding Jerusalem artichoke (*Helianthus tuberosus*) root as prebiotic on nutrient utilization, fecal characteristics and serum metabolite profile of captive Indian leopard (*Panthera pardus fusca*) fed a meat-on-bone diet. *Zoo biology* 34 (2): 153–162.

Pratt-Phillips, S.; Kutzner-Mulligan, J.; Marvin, R.; Brown, H.; Sykes, C.; Federico, J. (2014): The effect of feeding two or three meals per day of either low or high nonstructural carbohydrate concentrates on postprandial glucose and insulin concentrations in horses. *Journal of Equine Veterinary Science* 34 (11-12): 1251–1256.

Rassow, J. (2016): Biochemie. Stuttgart: Thieme.

Reischer, N. (2012): Dynamik der Serumkonzentration und Ausscheidung von D-Laktat bei jungen Kälbern mit Durchfall. PhD-thesis. Ludwig-Maximillians-Universität, München.

Respondek, F.; Goachet, A. G.; Julliand, V. (2008): Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. *Journal of Animal Science* 86 (2): 316–323.

Respondek, F.; Goachet, A. G.; Rudeaux, F.; Julliand, V. (2007): Effects of short-chain fructo-oligosaccharides on the microbial and biochemical profile of different segments of the gastro-intestinal tract in horses. *Pferdeheilkunde* 23 (2): 146–150.

Respondek, F.; Myers, K.; Smith, T. L.; Wagner, A.; Geor, R. J. (2011): Dietary supplementation with short-chain fructo-oligosaccharides improves insulin sensitivity in obese horses. *Journal of Animal Science* 89 (1): 77–83.

Roberfroid, M. (2007): Prebiotics: The concept revisited. *Journal of Nutrition* (137): 830S-837S.

Roberfroid, M.; Gibson, G. R.; Hoyles, L.; McCartney, A. L.; Rastall, R.; Rowland, I.; Wolvers, D.; Watzl, B.; Szajewska, H.; Stahl, B.; Guarner, F.; Respondek, F.; Whelan, K.; Coxam, V.; Davicco, M.-J.; Léotoing, L.; Wittrant, Y.; Delzenne, N. M.; Cani, P. D.; Neyrinck, A. M.; Meheust, A. (2010): Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition* 104 (S2): S1-S62.

Roberfroid, M. B.; Delzenne, N. M. (1998): Dietary fructans. *Annual Review of Nutrition* 18: 117–143.

Robertson, M. D. (2007): Metabolic cross talk between the colon and the periphery: implications for insulin sensitivity. *The Proceedings of the Nutrition Society* 66 (3): 351–361.

Roller, M.; Rechkemmer G.; Watzl, B. (2004): Prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* modulates intestinal immune functions in rats. *Journal of Nutrition* 134 (1): 153–156.

Rosenfeld, I.; Austbø, D. (2009): Effect of type of grain and feed processing on gastrointestinal retention times in horses. *Journal of Animal Science* 87 (12): 3991–3996.

Sadet-Bourgeteau, S.; Julliand, V. (2010): Equine microbial gastro-intestinal health. The impact of nutrition on health and welfare of horses. In: Wageningen Academic Publishers (Ed.): EAAP Scientific Series (128): 161–182.

Saltiel, A. R.; Kahn, C. R. (2001): Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414 (6865): 799–806.

Samanta, A. K.; Jayapal, N.; Senani, S.; Kolte, A. P.; Sridhar, M. (2013): Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. *Sociedade Brasileira de Microbiologia* 44 (1): 1–14.

Samanta, A. K.; Jayapal, Natasha; Jayaram, C.; Roy, Sohini; Kolte, A. P.; Senani, S.; Sridhar, Manpal (2015): Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. *Bioactive Carbohydrates and Dietary Fibre* 5 (1): 62–71.

Santos, A. S.; Rodrigues, M. A. M.; Bessa, R. J. B.; Ferreira, L. M.; Martin-Rosset, W. (2011): Understanding the equine cecum-colon ecosystem: current knowledge and future perspectives. *Animal* 5 (1): 48–56.

Scheepers, A.; Joost, H.-G.; Schürmann, A (2004): The glucose transporter families SGLT and GLUT: Molecular basis of normal and aberrant function. *Journal of Parenteral and Enteral Nutrition* 28 (5): 364–371.

Scheppach, W. (1994): Effects of short chain fatty acids on gut morphology and function. *Gut* (Suppl. 1): S35-S38.

Seifert, S.; Watzl, B. (2007): Inulin and oligofructose: Review of experimental data on immune modulation. *The Journal of Nutritition* (137): 2563S-2567S.

Servin, A. L.; Coconnier, M.-H. (2003): Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. *Best Practice & Research Clinical Gastroenterology* 17 (5): 741–754.

Sevane, N.; Bialade, F.; Velasco, S.; Rebolé, A.; Rodríguez, M. L.; Ortiz, L. T.; Cañón, J.; Dunner, S. (2014): Dietary inulin supplementation modifies significantly the liver transcriptomic profile of broiler chickens. *PLoS ONE* 9 (6): e98942.

Shepherd, M. L.; Swecker, W. S.; Jensen, R. V.; Ponder, M. A. (2012): Characterization of the fecal bacteria communities of forage-fed horses by pyrosequencing of 16S rRNA V4 gene amplicons. *FEMS Microbiology Letters* 326 (1): 62–68.

Shibata, N.; Suzuki, A.; Kobayashi, H.; Okawa, Y. (2007): Chemical structure of the cell-wall mannan of *Candida albicans* serotype A and its difference in yeast and hyphal forms. *The Biochemical Journal* 404 (3): 365–372.

Soenksen, P. H.; Tompkins, C. V.; Srivastava, M. C.; Nabarro, J. D. N. (1973): A comparative study on the metabolism of human insulin and porcine proinsulin in man. *Clinical Science and Molecular Medicine* 45 (5): 633–654.

Strauch, S.; Wichert, B.; Greef, J. M.; Hillegeist, D.; Zeyner, A.; Liesegang, A. (2017): Evaluation of an *in vitro* system to simulate equine foregut digestion and the influence of acidity on protein and fructan degradation in the horse's stomach. *Journal of Animal Physiology and Animal Nutrition* 101 (Suppl 1): 51–58.

Stull, C. L.; Rodiek, A. V. (1988): Responses of blood glucose, insulin and cortisol concentration to common equine diets. *The Journal of Nutrition* 118: 206–213.

Suagee, J. K.; Corl, B. A.; Hulver, M. W.; McCutcheon, L. J.; Geor, R. J. (2011): Effects of hyperinsulinemia on glucose and lipid transporter expression in insulin-sensitive horses. *Domestic Animal Endocrinology* 40 (3): 173–181.

Świątkiewicz, S.; Koreleski, J.; Arczewska-Włosek, A. (2010): Effect of prebiotic fructans and organic acids on mineral retention in laying hens. *Acta Agriculturae Scandinavica, Section A - Animal Science* 60 (2): 125–128.

Tako, E.; Glahn, R. P.; Welch, R. M.; Lei, X.; Yasuda, K.; Miller, D. D. (2008): Dietary inulin affects the expression of intestinal enterocyte iron transporters, receptors and storage protein and alters the microbiota in the pig intestine. *British Journal of Nutrition* 99 (3): 472–480.

Tarini, J.; Wolever, T. M. S. (2010): The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Applied Physiology, Nutrition, and Metabolism* 35 (1): 9–16.

Treiber; K. H.; Boston, R. C.; Kronfeld, D. S.; Staniar; W. B.; Harris, P. A. (2005a): Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycemic meals. *Journal of Animal Science* (83): 2357–2364.

Treiber, K. H.; Kronfeld, D. S.; Hess, T. M.; Byrd, B. M.; Splan, R. K. (2005b): Pre-laminitic metabolic syndrome in genetically predisposed ponies involves compensated insulin resistance. *Journal of Animal Physiology and Animal Nutrition* (89): 430–431.

Treiber, K. H.; Kronfeld, D. S.; Geor, R. J. (2006): Insulin resistance in equids: Possible role in laminitis. *Journal of Nutrition* 136 (7): 2094S–2098S.

Tsujikawa, Y.; Nomoto, R.; Osawa, R. (2013): Difference in degradation patterns on inulin-type fructans among strains of *Lactobacillus delbrueckii* and *Lactobacillus paracasei*. *Bioscience of Microbiota, Food and Health* 32 (4): 157–165.

Uyeno, Y.; Shigemori, S.; Shimosato, T. (2015): Effect of probiotics/prebiotics on cattle health and productivity. *Microbes and Environments* 30 (2): 126–132.

Van de Wiele, T.; Boon, N.; Possemiers, S.; Jacobs, H.; Verstraete, W. (2007): Inulin-type fructans of longer degree of polymerization exert more pronounced *in vitro* prebiotic effects. *Journal of Applied Microbiology* 102 (2): 452–460.

Van der Meulen, R.; Makras, L.; Verbrugghe, K.; Adriany, T.; Vuyst, L. de (2006): *In vitro* kinetic analysis of oligofructose consumption by *Bacteroides* and *Bifidobacterium spp*. indicates different degradation mechanisms. *Applied and Environmental Microbiology* 72 (2): 1006–1012.

Van Eps, A. W.; Pollit, C. C. (2006): Equine laminitis induced with oligofructose. *Equine Veterinary Journal* 38 (3): 203–208.

Van Eps, A. W.; Pollit, C. C. (2009): Equine laminitis model: Lamellar histopathology seven days after induction with oligofructose. *Equine Veterinary Journal* 41 (8): 735–740.

Van Hijum, S. A. F. T.; Kralj, S.; Ozimek, L. K.; Dijkhuizen, L.; van Geel-Schutten, I. G. H. (2006): Structure-function relationship of glucansucrase and fructansucrase enzymes from lactic acid bacteria. *Microbiology and Molecular Biological Reviews* 70 (1): 157–176.

Van Weyenberg, S.; Sales, J.; Janssens, G.P.J. (2006): Passage rate of digesta through the equine gastrointestinal tract. A review. *Livestock Science* 99 (1): 3–12.

Varloud, M.; Fombelle, A. de; Goachet, A. G.; Drogoul, C.; Julliand, V. (2004): Partial and total apparent digestibility of dietary carbohydrates in horses as affected by the diet. *Animal Science* (79): 61–72.

Velasco, S.; Ortiz, L. T.; Alzueta, C.; Rebolé, A.; Treviño, J.; Rodríguez, M. L. (2010): Effect of inulin supplementation and dietary fat source on performance, blood serum metabolites, liver lipids, abdominal fat deposition, and tissue fatty acid composition in broiler chickens. *Poultry Science* 89 (8): 1651–1662.

Vendrig, J. C.; Coffeng, L. E.; Fink-Gremmels, J. (2014): Effects of orally administered galacto-oligosaccharides on immunological parameters in foals: a pilot study. *Bio Med Central Veterinary Research* 10 (1): 367.

Vervuert, I.; Coenen, M.; Bothe, C. (2003): Effects of oat processing on the glycaemic and insulin responses in horses. *Journal of Animal Physiology and Animal Nutrition* 87: 96–104.

Vervuert, I.; Voigt, K.; Hollands, T.; Cuddeford, D.; Coenen, M. (2009a): The effect of mixing and changing the order of feeding oats and chopped alfalfa to horses on glycaemic and insulinaemic responses, and breath hydrogen and methane production. *Journal of Animal Physiology and Animal Nutrition* 93 (5): 631–638.

Vervuert, I.; Voigt, K.; Hollands, T.; Cuddeford, D.; Coenen, M. (2009b): Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. *The Veterinary Journal* 182 (1): 67–72.

Vervuert, I.; Brüssow, N.; Bochnia, M.; Cuddeford, D.; Coenen, M. (2013): Electromyographic evaluation of masseter muscle activity in horses fed (i) different types of roughage and (ii) maize after different hay allocations. *Journal of Animal Physiology and Animal Nutrition* 97 (3): 515–521.

Vhile, S. G.; Kjos, N. P.; Sørum, H.; Overland, M. (2012): Feeding Jerusalem artichoke reduced skatole level and changed intestinal microbiota in the gut of entire male pigs. *Animal* 6 (5): 807–814.

Wähler, R. (2015): Präbiotika und die Behandlung des metabolischen Syndroms. *OM & Ernährung* 152: 2–8.

Walter, J.; Schwab, C.; Loach, D. M.; Ganzle, M. G.; Tannock, G. W. (2008): Glucosyltransferase A (GtfA) and inulosucrase (Inu) of *Lactobacillus reuteri* TMW1.106 contribute to cell aggregation, *in vitro* biofilm formation, and colonization of the mouse gastrointestinal tract. *Microbiology* 154: 72–80.

Wicksteed, B.; Alarcon, C.; Briaud, I.; Lingohr, M. K.; Rhodes, C. J. (2003): Glucose-induced translational control of proinsulin biosynthesis is proportional to preproinsulin mRNA levels in islet beta-cells but not regulated via a positive feedback of secreted insulin. *The Journal of Biological Chemistry* 278 (43): 42080–42090.

Wicksteed, B.; Herbert, T. P.; Alarcon, C.; Lingohr, M. K.; Moss, L. G.; Rhodes, C. J. (2001): Cooperativity between the preproinsulin mRNA untranslated regions is necessary for glucose-stimulated translation. *The Journal of Biological Chemistry* 276 (25): 22553–22558.

Yuki, N.; Shimazaki, T.; Kushiro, A.; Watanabe, K.; Uchida, K.; Yuyama, T.; Morotomi, M. (2000): Colonization of the stratified squamous epithelium of the nonsecreting area of horse stomach by *Lactobacilli*. *Applied and Environmental Microbiology* 66 (11): 5030–5034.

Yun, H.; Lee, J. H.; Park, C. E.; Kim, M.-J.; Min, B.-I.; Bae, H.; Choe, W.; Kang, I.; Kim, S.-S.; Ha, J. (2009): Inulin increases glucose transport in C2C12 myotubes and HepG2 cells via activation of AMP-activated protein kinase and phosphatidylinositol 3-kinase pathways. *Journal of Medicinal Food* 12 (5): 1023–1028.

Zeyner, A.; Hoffmeister, C.; Einspanier, A.; Gottschalk, J.; Lengwenat, O.; Illies, M. (2006): Glycaemic and insulinaemic response of quarter horses to concentrates high in fat and low in soluble carbohydrates. *Equine Veterinary Journal Suppl.* (36): 643–647.

Zeyner, A.; Romanowski, K.; Orgis, A.; Vernunft, A.; Gottschalk, J.; Einspanier, A.; Koeller, G.; Wensch-Dorendorf, M. (2017): Feed Intake Patterns and Immediate Glycaemic and Insulinaemic Responses of Horses Following Ingestion of Different Quantities of Starch From Oat, Barley and Grains. *The Open Nutrition Journal* 11 (1): 39–51.

Zhang, W.; Li, D.; Lu, W.; Yi, G. (2003): Effects of isomalto-oligosaccharides on broiler performance and intestinal microflora. *Poultry Science* 82 (4): 657–663.

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11 Appendix

Review article: Anwendung von Präbiotika in der Ernährung von Pferden

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Aus der Professur für Tierernährung der Martin-Luther-Universität Halle-Wittenberg in Zusammenarbeit mit dem Physiologischen Institut der Tierärztlichen Hochschule Hannover

Anwendung von Präbiotika in der Ernährung von Pferden

von Maren Glatter, Gerhard Breves und Annette Zeyner

(5 Abbildungen, 3 Tabellen, 88 Literaturangaben)

Kurztitel: *Präbiotika in der Pferdeernährung*

Stichworte: *Präbiotika, Darmgesundheit*

Zusammenfassung

Derzeit sind verschiedene Produkte auf dem Markt die mit präbiotischen Effekten für Pferde beworben werden. Experimentell basierte Aussagen zur Wirksamkeit liegen jedoch nur in sehr begrenztem Umfang vor. Mit präbiotischem Ziel kommen in der Regel oligo- und polymere Inulin-Typ-Fruktane zum Einsatz. Diese sollen im Dickdarm erwünschte Mikroorganismen selektiv stimulieren. In diesem Fall würden kurzkettige Fettsäuren, primär n-Butyrat, in höheren Konzentrationen gebildet werden, welche sowohl direkt (über eine Modifikation von Resorption und Intermediärstoffwechsel) als auch indirekt (über systemische Effekte) das Wohlbefinden des Tieres beeinflussen können. Wesentliche Ziele sind eine stabile mikrobielle Homöostase im Darm mit positiven Auswirkungen auf die epitheliale Integrität, den Immunstatus und die Stoffwechselgesundheit des Tieres. In Abhängigkeit von Lokalisation und Umfang der Fermentation im Gastrointestinaltrakt müssen jedoch auch kritische Effekte in Betracht gezogen werden.

Abstract

Currently several products claiming prebiotic effects in horses are on the market. Experimentally based evidence to substantiate that is however scarce. With prebiotic purposes, oligo- and polymeric inulin-type fructans are commonly recommended. These prebiotics are supposed to stimulate health-promoting microorganism in the hindgut. In this case short chain fatty acids, primarily *n*-butyrate, would be formed in

elevated concentrations which in turn might influence the animal`s well-being directly (*via* modification of absorption and intermediary metabolism) as well as indirectly (*via* systemic effects). Particularly important aims are to stabilize the microbial homeostasis of the digestive tract with positive effects on epithelial integrity, immune status and the metabolic health of the animal in question. Nevertheless, probably critical effects of prebiotic substances must also be considered, depending on the localization and extent of being fermented within the gastrointestinal tract.

1 Einleitung

Präbiotika können das Wohlbefinden und die Gesundheit der Tiere positiv beeinflussen und mögliche Krankheitsrisiken wie gastrointestinale Störungen durch Begünstigung der Homöostase der autochthonen Mikrobiota reduzieren. Derzeit sind verschiedene Präparate auf dem Markt, welche gesundheitsfördernde Effekte bewerben. Wissenschaftlich fundierte Ergebnisse zur Wirkung im Pferd liegen allerdings nur in geringer Anzahl vor.

2 Definition und Klassifizierung

Das Konzept der Präbiotika wurde erstmals *1995* von *Gibson* und *Roberfroid* eingeführt und seither sowohl in der Human- als auch in der Tierernährung stetig weiterentwickelt. Laut Definition der „International Scientific Association for Probiotics and Prebiotics“ (ISAPP, 6th Meeting of ISAPP, London, Ontario) im Jahre 2008 ist „ein diätetisches Präbiotikum ein selektiv fermentierbarer Futterinhaltsstoff, welcher spezifische Veränderungen in der Zusammensetzung und/oder der Aktivität der gastrointestinalen Mikroflora bewirkt und somit der Gesundheit des Wirtes Vorteile bringt“. Erweitert wurde die Definition von *Roberfroid et al. (2010)* als „die selektive Stimulation des Wachstums und/oder der Aktivität einer oder einer begrenzten Anzahl an mikrobiellen Gattungen/Arten innerhalb der Darmflora, welche die Gesundheit des Wirtes positiv beeinflussen“.
Laut *Roberfroid (2007)* sollte ein Präbiotikum folgende Kriterien erfüllen:

1) Resistenz gegenüber Magensäure sowie Hydrolyse durch körpereigene Enzyme und gastrointestinaler Absorption,
2) Fermentation durch die gastrointestinale Mikroflora,
3) Selektive Stimulation von Wachstum und/oder Aktivität derjenigen intestinalen Bakterien, welche die Gesundheit und das Wohlbefinden des Wirtes fördern.

In der Pferdeernährung eingesetzte Präbiotika entstammen in der Regel der Gruppe der Fruktane. Dies sind in der Grundstruktur zumeist oligo- und polymere Verbindungen aus Fruktoseeinheiten. Fruktane werden aufgrund ihrer chemischen Zusammensetzung als entweder Inulin-Typ-Fruktane (ITF) oder Phlein-Typ-Fruktane bezeichnet (Tab. 1). Sie ersetzen bzw. ergänzen in einigen Pflanzentaxa die Stärke

als Speicherkohlenhydrat. Phlein-Typ-Fruktane sind vornehmlich in Gräsern (*Poales*) der gemäßigten Breiten zu finden. Im Gegensatz dazu sind die Inulin-Typ-Fruktane (ITF) hauptsächlich in Korbblütlern (*Compositae*) wie Chicoree, Dahlie oder Topinambur aber auch Löwenzahn enthalten. Bei sehr hoher Aufnahme vornehmlich durch nicht oder unzureichend adaptierte Pferde können sowohl Phlein-Typ-Fruktane (*Longland and Byrd*, *2006*) als auch ITF *(van Eps and Pollitt, 2006, 2009)* Hufrehe auslösen. Grundsätzlich können kritische und wohl auch positive Wirkungen von beiden Fruktan-Typen ausgehen. Dies hängt neben Adaptation und Dosis (bzw. bei handelsüblichen Präbiotika vom tatsächlichen Anteil der Wirksubstanz im Präparat) vom Polymeristaionsgrad (PG) und der Art der intramolekularen Bindung ab und wird möglicherweise durch die nur in Phlein-Typ-Fruktanen vorkommenden Seitenverknüpfungen weiter modifiziert. Dennoch werden als Präbiotika derzeit nach Kenntnis der Autoren nur ITF (Tab. 1) genutzt, was auf technologische Vorteile hinsichtlich Anbau, Ertragssicherheit und großtechnische Gewinnung zurückzuführen ist. ITF sind charakterisiert durch eine lineare ß-2,1-Verknüpfung mehrerer Fruktosylreste mit einer meist terminalen Glukoseeinheit. Je nach Kettenlänge (dem sog. Polymerisationsgrad, PG) innerhalb der ITF werden kurzkettige Fruktooligosaccharide (scFOS; PG ~ 3,6), Fruktooligosaccharide (FOS; PG ~ 4) und Inulin (PG im Mittel 12) unterschieden. Wie Abbildung 1 anhand eines Dahlienextraktes zeigt enthält ein präbiotisches Präparat in der Regel ein Gemisch aus Fruktanen unterschiedlicher Kettenlänge, mit gehäuftem Auftreten innerhalb jeweils spezifischer PG-Bereiche. Von Interesse für den Einsatz bei Pferden sind in unterschiedlicher Menge auftretende niedermolekulare Begleitkohlenhydrate (v. a. Glukose, Fruktose, Saccharose). Mit zunehmendem PG steigt die Wahrscheinlichkeit, dass das Präbiotikum den Dickdarm intakt erreicht und dort der residenten Mikrobiota als Substrat dient *(van de Wiehle et al., 2007; Azorín-Ortuño et al., 2009; Li et al., 2015)*. Bei der Interpretation produktbegleitender Informationen empfiehlt es sich zu beachten, dass Fruktane als Speicherkohlenhydrat von Pflanzen diurnal und sogar circadian erheblichen Schwankungen unterliegen und daher nicht zuletzt der Erntezeitpunkt eine wesentliche Variable darstellt *(Bacon und Loxeley, 1952; Bonnett et al., 1997; Dahlhoff, 2003)*. Neben den Fruktanen wird auch den Galaktooligosacchariden (*Roberfroid, 2007*), den Xylooligosacchariden (*Samanta et al., 2015*), den Mannanoligosacchariden sowie Laktulose (Hund: *Zentek et al., 2002;* Pferd: *Zeyner et al., 2003ab*) eine präbiotische Wirkung zugesprochen. In der Pferdeernährung ist deren Anwendung bisher erst anfänglich untersucht und noch nicht verbreitet.

Tab. 1: Klassifikation von Substanzen mit präbiotischer Wirkung (nach *Roberfroid et al., 2010*)

Biochemischer Oberbegriff	**Biochemische Bezeichnung und strukturelle Charakteristika**	**Allgemeine Bezeichnung und mittlerer Polymerisationsgrad (PG_M)**
Inulin-Typ-Fruktane (ITF)	*Inulin-Typ-Fruktane (ITF)* Linear, β (2→) Fruktosyl-Fruktose $G_{py}F_n$ und/oder $F_{py}F_n$	Inulin
	Kurze bis lange Polymere PG 2 - 60 ITF mit PG_M 12	Inulin PG_M 12
	Kurze Oligomere PG 2 - 8 ITF mit PG_M 3-4	Fruktooligosaccharide (FOS) Kurzkettige FOS (scFOS) PG_M 3,6 Oligofruktose PG_M 4
	Lange Polymere PG 10 - 60 ITF mit PG_M 25	Hochmolekulares Inulin PG_M 25 Langkettige FOS (lcFOS)
	Gemisch PG (2 - 8) + PG (10 - 60) ITF_{mix}	Mischung aus oligomeren und langen Polymeren
Galaktane (GOS)	Mischung aus β (1→), β (1→ 3), β (1→) Galaktosyl-Galaktose Gal_n-Gal und/oder Gal_n-Glc PG 2 - 8	Galaktooligosaccharide, *trans*-Galaktooligosaccharide
Mischung aus GOS und ITF	GOS-FOS	Galaktooligosaccharide und hochmolekulares Inulin bekannt als: GOS-FOS oder scGOS-lcFOS

PG = Polymerisationsgrad; G_{py} = Glukopyranosyl; F_n = Fruktofuranosid; F_{py} = Fruktofuranosyl; GOS = Galakto-oligosaccharide; Gal = Galaktose; Glc = Glukose

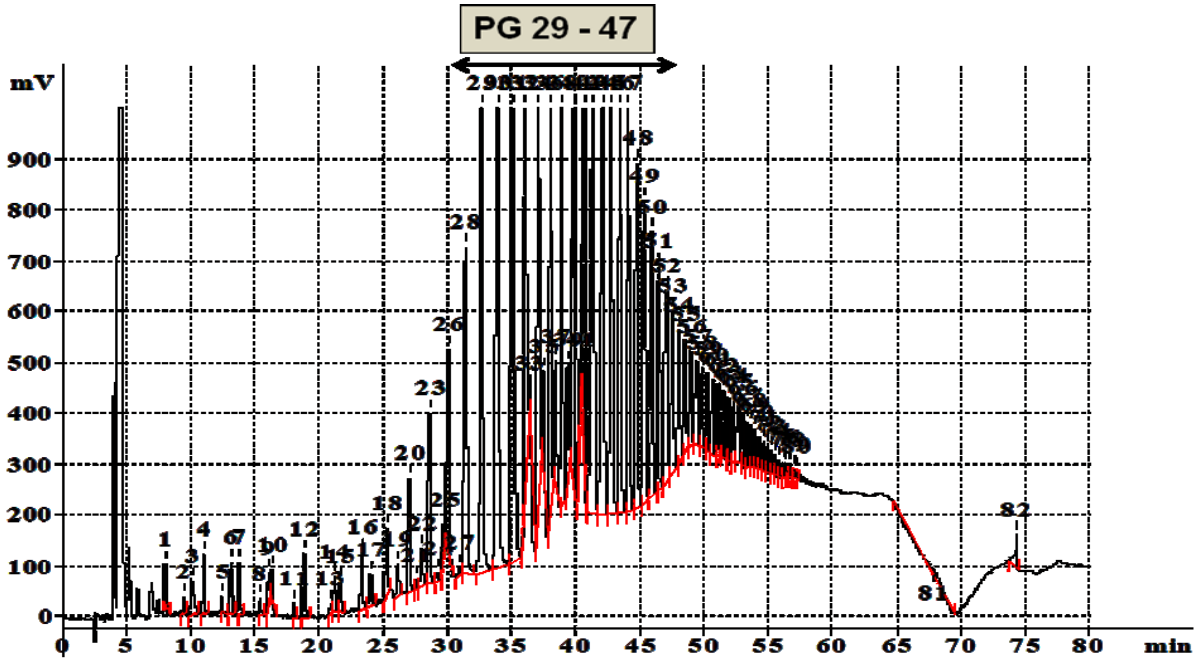

Abb. 1: Auftrennung eines kommerziellen Dahlia-Extraktes in einzelne Kohlenhydrat-Ketten (Polymerisationsgrad, PG) mittels HPLC (nach Hillegeist und Greef)

3 Wirkung von Präbiotika beim Menschen und anderen Spezies

Ein signifikanter Anteil des zugeführten Präbiotikums soll den Magen nahezu unverdaut passieren und in den Dickdarmbereich gelangen. Da Präbiotika nicht von körpereigenen Enzymen hydrolysiert werden können, stehen diese somit den dort ansässigen Mikroorganismen als Substrat für deren Metabolismus zur Verfügung. Mikrobielle Fermentationsprodukte, wie z.B. kurzkettige Fettsäuren (SCFA) können auf unterschiedlichen Wirkebenen einen positiven und/oder stimulierenden Einfluss nehmen, z. B. durch Regulation von Stoffwechselprozessen, durch die Aufrechterhaltung sowie die Verbesserung des Immunstatus oder durch Beeinflussung von Resorptionsprozessen (Abb. 2).

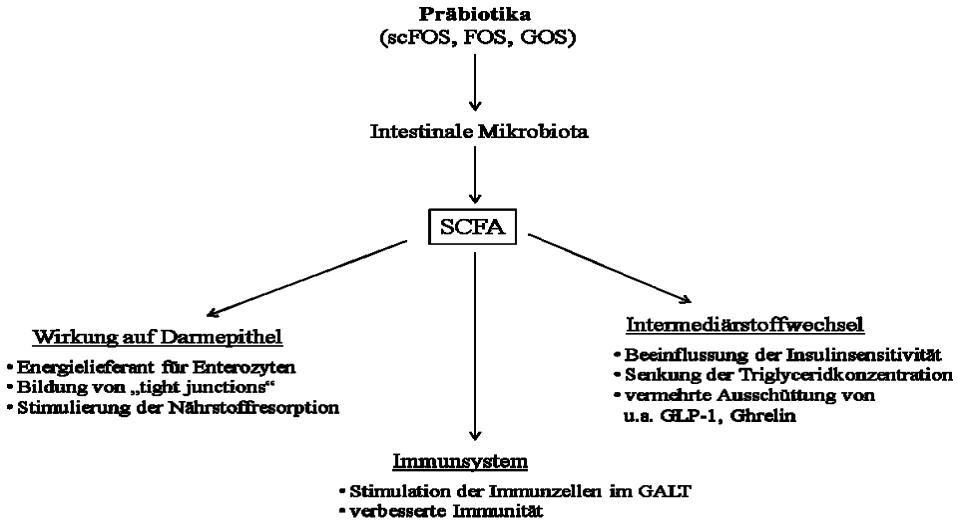

Abb. 2: Wirkungswege von Präbiotika; scFOS = kurzkettige Fruktooligosaccharide; FOS = Fruktooligosaccharide; GOS = Galaktooligosaccharide; SCFA = „short chain fatty acids"; GALT = „gut associated lymphoid tissue" (nach Wähler, 2015).

3.1 Gastrointestinaltrakt

In der Humanernährung zielt die Verabreichung von Präbiotika vornehmlich auf die selektive Stimulation der Bifidobakterien und Laktobazillen im Verdauungstrakt. Bifidobakterien sind u.a. in der Lage, sich an die Oberfläche der Enterozyten anzuheften und somit die Adhäsion von Enteropathogenen zu inhibieren (*Bernet et al., 1993; Kleesen et al., 2001; Harmsen et al.*, *2002)*. Bei anderen Spezies steht die Förderung der Bifidobakterien meist weniger im Fokus, da diese nur in geringer Anzahl im Verdauungstrakt resident sind. In der Ernährung von Schweinen als Vertreter der Omnivora ist man sich über eine stimulierende Wirkung von Präbiotika hinsichtlich der o.g. Vertreter der intestinalen Mikroflora uneinig. *Janczyk et al. (2010)* zeigten in ihrer Studie mit Ferkeln (28 d) bei Supplementation von 1,5 % Inulin eine erhöhte mikrobielle Diversität sowie eine höhere Artenvielfalt im Dünndarmbereich, wohingegen dies auf das Colon nur tendenziell zutraf. Da Bifidobakterien im porcinen Gastrointestinaltrakt eine untergeordnete Rolle spielen (*Loh et al., 2006)*, lag der Fokus dieser Studie auf der Identifizierung der gesamten Laktobazillen. Dabei zeigte sich kein signifikanter Effekt des zugeführten Präbiotikums auf die Anzahl der Bakterien. Ähnliche Ergebnisse resultieren auch aus der Studie von *Böhmer et al. (2005)* mit adulten, männlich kastrierten Schweinen bei Supplementation von 2 % Inulin (PG 10 - 12). Andere Untersuchungen hingegen zeigten einen stimulierenden Einfluss von Inulin auf die porcine Mikroflora hinsichtlich der Anzahl an Bifidobakterien und Laktobazillen *(Patterson et al., 2010)*.

Untersuchungen zur Wirkung von Präbiotika auf den Gastrointestinaltrakt herbivorer, wiederkäuender Spezies sind nur in sehr begrenztem Umfang vorhanden. Zumeist ist ein positiver Effekt hinsichtlich der Etablierung einer Mikroflora lediglich bei Kälbern beschrieben, da bei adulten Tieren das zugesetzte Präbiotikum bereits im Pansen fermentiert wird und demnach keine adäquaten Mengen den Dickdarm erreichen *(Uyeno et al., 2015)*.

Aufgrund der forcierten Bildung von SCFA kann der luminale pH-Wert absinken, wobei die Anzahl der Laktobazillen sowie Bifidobakterien erhöht wird, da diese optimal an ein saures Milieu angepasst sind (*Blaut, 2002*). Laktobazillen produzieren als Fermentationsprodukt primär D-Laktat, welches durch andere kommensale Bakterien im Verdauungstrakt in SCFA umgesetzt wird (*Reischer, 2012*). Meist unter Beteiligung von Laktat kann der pH-Wert im Darmlumen so weit absinken, dass die Lebensfähigkeit einiger Pathogene (v.a. bestimmte Clostridien) herabgesetzt wird. D-Laktat kann auch durch ein spezifsches Enzym (D-2-Hydroxysäuren-Dehydrogenase) in Pyruvat umgesetzt werden. Allerdings ist der Umfang bei Säugetieren, mit Ausnahme der Wiederkäuer, gering (*Tubbs, 1965; nach Reischer, 2012*).

3.2 Intermediärstoffwechsel

Beim mikrobiellen Abbau von Präbiotika im Gastrointestinaltrakt werden vor allem kurzkettige Fettsäuren (SCFA; primär Acetat, Propionat und Butyrat) gebildet, wobei Butyrat quantitativ am geringsten vertreten, für den Stoffwechsel des Darmepithels jedoch besonders bedeutsam ist *(Plöger et al., 2012*; Abb. 3). So werden SCFA zum Teil bereits im Epithelium metabolisiert *(Herrmann et al., 2011; Bach Knudsen, 2012)*

bzw. über die Zirkulation im Organismus verteilt und in den Stoffwechsel von Geweben (z.B. Fettgewebe, Muskelzellen) und Organen (z.B. der Leber) integriert *(Bergmann, 1990)*. Butyrat ist die Hauptenergiequelle der Colonozyten *(Scheppach, 1994)*, Acetat und Propionat werden u. a. über die Portalvene zur Leber transportiert und sind dort an Gluconeogenese, Lipogenese und Fettsäurenoxidation beteiligt *(Wähler, 2015)*.

Die Supplementierung von präbiotischen Wirksubstanzen zielt zumeist auf eine erhöhte mikrobielle Produktion von SCFA, vor allem Butyrat. In diversen Studien u. a. mit Schweinen (*Loh et al., 2006; Vhile et al., 2012)* bzw. gnotobiotisch aufgezogenen Ratten, welche mit humaner Faecalflora inkubiert wurden *(Kleesen et al., 2001)*, war die Produktion von SCFA im Gastrointestinaltrakt infolge der Fütterung von Inulin in unterschiedlichen Konzentrationen im Alleinfutter (30 - 121 bzw. 50 g/kg) signifikant gesteigert. Untersuchungen zum systemischen Einfluss einer erhöhten SCFA Konzentration nach Präbiotika-Fütterung sind rar. Eine verringerte Konzentration an Triglyceriden im Serum nach Präbiotika-Gabe wurde bei Indischen Leoparden *(Panthera pardus fusca; Pradhan et al., 2015)*, bei Broiler-Küken (*Velasco et al., 2010)* sowie Ratten (*Fiordaliso et al., 1995)* gemessen. In Studien zum Einfluss von Präbiotika generell bzw. im Besonderen auch von Fruktanen auf den humanen Lipidmetabolismus wurden eine verminderte Genexpression lipogener Enzyme *(Delzenne und Kok, 2001)* sowie ein reduzierter Triglycerid-Gehalt im Serum in Abhängigkeit von der Ausprägung der Lipämie (*Delzenne und Williams, 2002*) beschrieben. Weiterhin wird angegeben, dass die beim Abbau von Präbiotika gebildeten SCFA die Produktion von Peptiden (u. a. Glukagon-like Peptid 1, GLP-1; Ghrelin) in den endokrinen Zellen beeinflussen können *(Delzenne et al., 2005)*. Eine erhöhte Konzentration an GLP-1 sowie Ghrelin nach der Fütterung lag bei Ratten vor *(Cani et al., 2004; Delzenne et al., 2007)*, aber auch in Humanstudien *(Tarini und Wolever, 2010)*. Eine nutrigenomische Studie von *Sevane et al. (2014)* mit Broiler-Küken zeigte nach Fütterung von 5 g Inulin eine Modifikation der Expression verschiedener Gene in beide Richtungen.

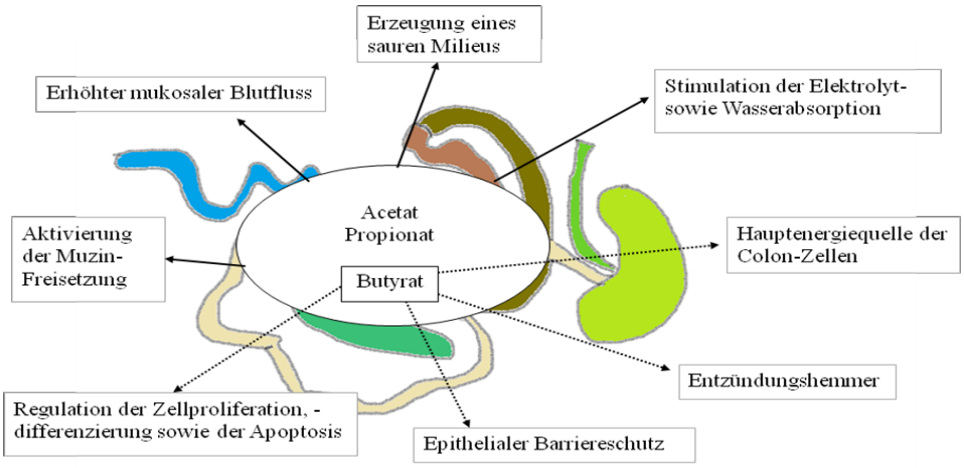

Abb. 3: Übersicht über den Einfluss von Butyrat als Fermentationsprodukt des mikrobiellen Stoffwechsels auf den Organismus (nach *Plöger et al., 2012*)

3.3 Immunstatus

Präbiotika können direkt und/oder indirekt (über ihre Fermentationsprodukte) auf das Immunsystem einwirken. In einer Studie von *Roller et al.* (*2004*) mit Ratten führte die Fütterung von Inulin und Oligofruktose (100 g/kg) zu einer erhöhten Bildung von Interleukin-10 sowie zu einer vermehrten Produktion von sekretorischen Immunglobulinen der Klasse A. Die gestiegene Cytokin-Produktion deutet darauf hin, dass das Präbiotikum selbst und/oder dessen Metabolite regulatorisch auf diesen Prozess einwirken. Die kurzkettigen Fettsäuren (SCFA) als Fermentationsendprodukte können dabei auf die Immunzellen im GALT („gut-associated lymphoid tissue“) wirken (*Seifert und Watzl, 2007*). Es ist allerdings noch nicht bekannt, über welchen Signalweg intralumiale SCFA von den Leukozyten detektiert werden. Weiterhin wird postuliert, dass spezifische Kohlenhydrate (z.B. Fruktose, Mannose) über spezifische Kohlenhydrat-Rezeptoren der Immunzellen mit Ihnen interagieren. Für Mannose wurde bereits ein spezifischer Rezeptor identifiziert *(Brown und Gordon, 2001)*. Phagozytotische Zellen sowie u.a. kleine Untergruppen von T- und B - Lymphozyten enthalten spezifische Rezeptoren, welche eine Vielzahl von β-1,3- sowie β-1,6- verknüpften Glukanen aus Pilzen und Pflanzen erkennen. Weiterhin wird auch beschrieben, dass sich der Immunstatus der Nachkommen über die maternale Supplementation mit präbiotischen Substanzen positiv beeinflussen lässt. In der Studie von *Le Bourgot et al.* (*2014*) wurde nach Fütterung von scFOS (10 g/d) im peripartalen Zeitraum an Sauen eine erhöhte kolostrale Immunität sowie ein stimulierender Effekt auf die Entwicklung und Reifung des mukosalen Immunsystems der Ferkel beschrieben.

3.4 Resorptionsprozesse

Ein weiterer positiver Aspekt der Präbiotika-Fütterung ist der stimulierende Einfluss auf Resorptionsprozesse im Gastrointestinaltrakt. Butyrat, als Fermentationsprodukt des mikrobiellen Abbaus der Präbiotika, stimuliert die Synthese von Mukus *(Finnie et al., 1995)* sowie forciert die Bildung von „tight junctions“ *in vitro (Peng et al., 2009)* einhergehend mit einer verringerten parazellulären Durchlässigkeit der Darmbarriere. Pathogene Bakterien sowie deren Toxine können somit in geringerem Umfang über das Epithel in den Blutkreislauf aufgenommen werden. Des Weiteren können präbiotische Supplemente die Nährstoffresorption beeinflussen. Einerseits kann dies über eine Veränderung der Zottenlängen in den verschiedenen Darmabschnitten erfolgen. *Pierce et al. (2006)* zeigten in einer Untersuchung mit Ferkeln (21. Lebenstag), dass sich die Länge der Villi im Jejunum signifikant erhöhte bei der Fütterung von 150 g Laktose und 15 g Inulin pro Kilogramm Alleinfutter. Vergleichbare Ergebnisse erbrachte eine Studie mit Kälbern *(Masanetz et al., 2010)*. Hierbei führte die Zugabe von 2 % Laktose zum Milchaustauscher zu einer tendenziellen Erhöhung der Zottenlängen im Jejunum sowie Ileum, die Zugabe von 2 % Inulin zum Milchaustauscher zeigte allerdings eine tendenzielle Abnahme in beiden Darmabschnitten. In einer *in vitro* Studie mit Zellkulturen führte die Zugabe von Inulin (1 mg/ml) zu einer vermehrten Resorption von Glukose durch einen AMP-aktivierten Protein-Kinase- sowie über einen Phosphatidylinositol-3-Kinase-Weg *(Yun et al., 2009)*. Durch die Fütterung von Präbiotika zeigt sich, wie oben beschrieben,

auch auf systemischer Ebene ein Effekt auf die Nährstoffresorption. In einer Untersuchung von *Tako et al. (2008)* führte die Supplementation von Inulin (4 % im Alleinfutter) an Schweine zu einer erhöhten Expression von Genen, welche diverse Eisen-Transporter in den Enterocyten kodieren. In einer Studie mit Legehennen führte die Supplementation von Inulin (~ 0,94 g/d) zu einer verbesserten Resorption von Ca, P und Zn *(Swiatkiewicz et al., 2010)*. Der zugrundeliegende Mechanismus einer verbesserten Verfügbarkeit von Mengen- und Spurenelementen ist sehr komplex und resultiert vermutlich aus einer verbesserten Löslichkeit der Mineralstoffe aufgrund der pH-Wert Senkung als Folge der mikrobiellen Bildung von SCFA aus den Präbiotika *(Samanta et al., 2013)*.

4. Anwendung in der Ernährung von Pferden

Präbiotika können, wie z. B. auch Zellulose, Hemizellulose oder Pektin, im equinen Verdauungstrakt nicht durch körpereigene Enzyme hydrolysiert werden und gelangen somit nahezu unverdaut in den Dickdarmbereich. Dort können sie als Substrat den Stoffwechsel der autochthonen Mikrobiota stimulieren. Bei der Verwendung von ITF wird eine Dosierung von 0,2 g/kg LM * d^{-1} (~ 0,1 kg/d bei einem 500 kg schweren Pferd) empfohlen *(Julliand und Zeyner, 2013)*. Infolge substanziell höherer Fruktan-Dosierungen (~ 10 kg/d bei einem 500 kg schweren Pferd) kann Hufrehe ausgelöst werden *(Elliot und Bailey, 2006; Longland und Byrd, 2006; van Eps und Pollit, 2006, 2009; Milinovich et al., 2010; Onishi et al., 2012)*. Pathophysiologisch kommen dafür sowohl eine massive Störung der Dickdarmfermentation (caecale Acidose) als auch eine postprandiale Hyperinsulinämie bei metabolisch disponierten Tieren (Equines Metabolisches Syndrom) in Betracht. Experimentell wurde Hufrehe bereits bei einer Dosierung von 7,5 g Oligofruktose pro kg LM (verabreicht über eine Nasenschlundsonde) ausgelöst *(Eps und Pollit*, *2006)*.

4.1 Gastrointestinaltrakt

In der Humanernährung soll durch präbiotische Gaben an ITF die Laktobazillen und Bifidobakterien stimuliert werden. Beim Pferd ist die Existenz von Bifidobakterien im Verdauungstrakt nach Kenntnis der Autoren bisher nicht sicher bewiesen. Der Gastrointestinaltrakt von Pferden beherbergt eine Vielzahl von Mikroorganismen (Tab. 2), von denen u. a. *Streptococcus bovis* dazu fähig ist, Inulin zu fermentieren (*Harlow et al., 2014*). In einer *in vitro* Studie wurde gezeigt, dass Fruktane (Phlein-Typ-Fruktane aus Gräsern) im vorderen Verdauungstrakt nicht abgebaut werden, sondern zu einem erheblichen Anteil in den Dickdarm gelangen (*Ince et al., 2014*). Ein kleiner Anteil der Fruktane wird vermutlich bereits durch saure Hydrolyse (im Magen) gespalten (*Wichert et al., 2015*) bzw. praecaecal fermentiert (*Coenen et al., 2006*). Dies ist per se eher kritisch zu sehen. Hinzu kommt, dass im vorderen Verdauungstrakt abgebaute Fruktane im Dickdarm nicht mehr wirksam werden können. Der Einfluss von präbiotischen Zulagen auf die gastrointestinale Mikroflora

erbrachte *in vivo* unterschiedliche Ergebnisse. In einer Studie von *Berg et al.* (*2005*) wurden Jährlinge mit Fruktooligosacchariden (FOS) in unterschiedlichen Konzentrationen (8 bzw. 24 FOS/d) supplementiert. Die Anzahl an *Laktobazillen* blieb durch die Zugabe von FOS unverändert, allerdings war bei Fütterung von 8 g FOS/d die Anzahl der *Escherichia coli*-Kolonien im Kot vermindert. Kurzkettige Fruktooligosaccharide (scFOS) können z. B. bei einer abrupten Futterumstellung einer Veränderung der mikrobiellen Zusammensetzung entgegenwirken. So wiesen *Respondek et al.* (*2008*) nach, dass die Fütterung von scFOS eine Erhöhung der Anzahl an *Laktobazillen* und *Streptokokken* nach Futterumstellung im Vergleich zur Kontrollgruppe verhinderte. Dieser Effekt ist besonders interessant, da an sich beim Pferd eine Stimulation von Laktobazillen im Dickdarm, wie sie als präbiotischer Effekt bei anderen monogastrischen Spezies begrüßt wird, eher kritisch zu sehen ist.
Präbiotika sollen neben der Keimzahl auch die Stoffwechselaktivität „erwünschter" Mikroorganismen im Darm erhöhen und damit die Bildung von SCFA begünstigen. Wie eingangs beschrieben, wird vor allem n-Butyrat eine Schlüsselrolle im Hinblick auf die Barrierefunktion des Dickdarms zugesprochen. In der o. g. Studie von *Berg et al.* (*2005*) wurden mit zunehmender Dosis an FOS auch steigende Konzentrationen an SCFA (u. a. auch von Butyrat) im Kot gemessen. *Respondek et al.* (*2008*) stellten keine solche Zunahme von SCFA im Caecum und Colon von Pferden nach Supplementation von scFOS fest. Bei *Berg et al. (2005)* war der Anstieg der Konzentration an SCFA im Kot von einem entsprechenden pH-Wert-Abfall begleitet. Die Fütterung von 3 g Inulin/kg LM*d, (dies entspricht dem 15-fachen der als präbiotisch empfohlenen Dosierung) reduzierte den pH-Wert der Faeces adulter Ponys ebenfalls signifikant (*Crawford et al., 2007)*, was jedoch in der hier beschriebenen Größenordnung als kritisches Ereignis denn als präbiotischer Effekt zu werten ist (Abb. 4).

Tab. 3: Übersicht über die dominanten Bakterien des Verdauungstraktes (Ausschnitt)

Abschnitt	Mikroorganismen	Keimdichte (KBE)
Magen	*Laktobacillus spp.*	3,78 – 7,09[a]
	Streptococcus spp.	7,3 – 7,5[b]
	Cellulolyten	2,48[c]
Dünndarm	*Laktobacillus spp.*	6,12 – 7,2[b]
	Enterococcus spp.	5,3 – 7,9[d]
	Proteolyten	6,46 – 7,19[e]
Caecum	*Laktobacillus spp.*	5,62 – 6,87[f]
	Streptococcus spp.	5,9 – 7,2[g]
	Laktat-Verwerter	5,39 – 6,18[f]
	Cellulolyten	5,0 – 6,0[b]
Colon	*Lactobacillus spp.*	6,63 – 8,58[f]
	Streptococcus spp.	6,1 – 7,5[g]
	Laktat-Verwerter	6,49 – 7,33[f]
	Cellulolyten	5,5 – 5,8[g]

[a]Varloud et al. (2007); [b]De Fombelle et al. (2003); [c]Kern et al. (1974); [d]Kollarczik et al. (1995); [e]Mackie und Wilkins (1988); [f]Julliand et al. (2001); [g]De Fombelle et al. (2001)

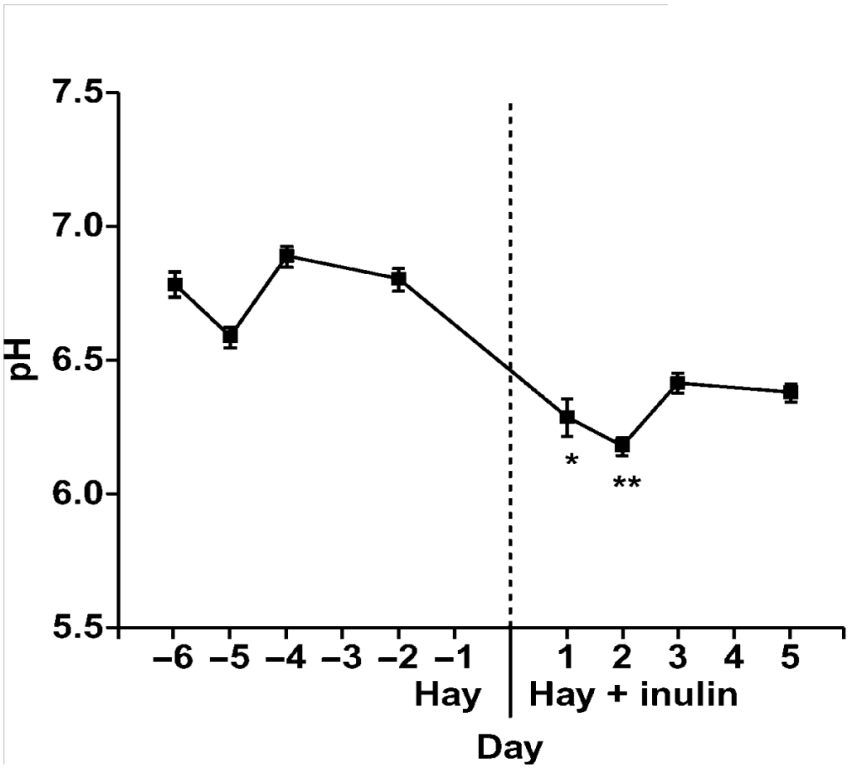

Abb. 4: Einfluss von Inulin (3 g/kg LM * d^{-1}) auf den pH-Wert (MW ± SEM) in dem Faeces von 11 Ponys; * P < 0,05, ** P < 0,001 (*Crawford et al., 2007*)

4.2 Intermediärstoffwechsel

Auf der systemischen Wirkebene zielt die Fütterung von Präbiotika u. a. auf eine Verbesserung des Glukose- und Insulinstoffwechsels. Ergebnisse aus Studien mit Pferden zeigen allerdings primär einen eher konträren Einfluss auf die Insulinsekretion. In einer Studie von *Bailey et al. (2007)* mit Ponys führte die Aufnahme von Inulin (3 g/kg LM * d^{-1}) zu einem tendenziellen Anstieg der Insulinkonzentration im Serum (24,6 – 106,2 mU/L; vgl. Heufütterung: 10,7 – 79,1 mU/L), aber zu keiner Veränderung der Glukosekonzentration im Plasma (4,8 – 5,2 mmol/L; vgl. Heufütterung: 5,1 – 5,4 mmol/L). Ein anderes Resultat wurde bei der Supplementation von 45 g scFOS pro Pferd und Tag an übergewichtige Araber (BCS 8,1/9) erzielt (Abb. 5; *Respondek et al., 2011*). Hierbei kam es zu einer moderaten Absenkung der Konzentration an Insulin aber nicht an Glukose im Blutplasma.

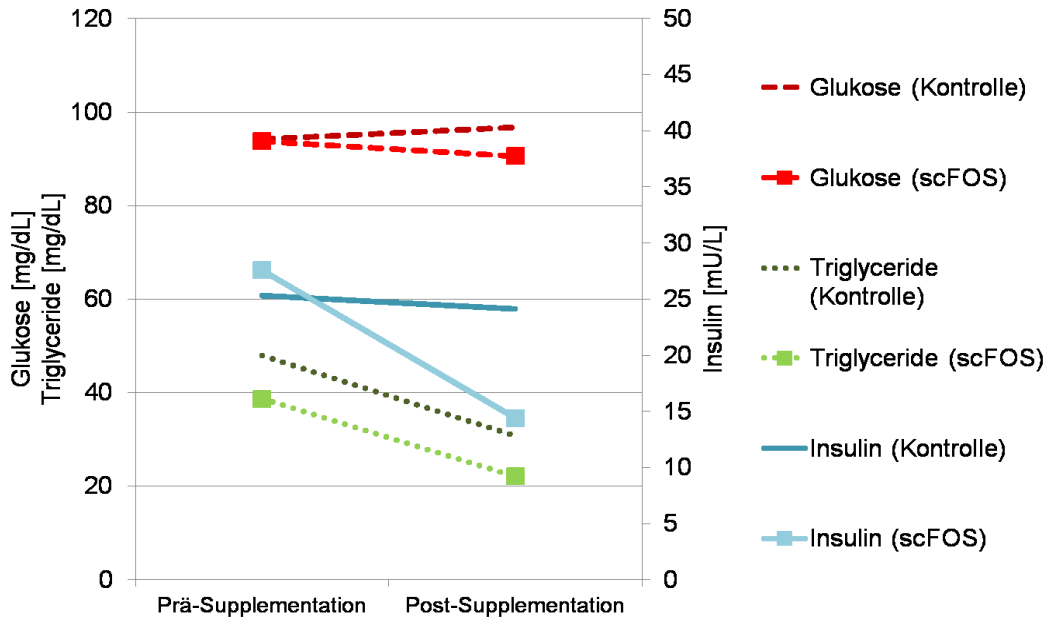

Abb. **5**: Basalwerte von Insulin (in mU/L), Glukose und Triglyceriden (in mg/dL) im Plasma von übergewichtigen Arabern vor (Prä-Supplementation; t = 0 Wochen) und nach (Post-Supplementation; t = 6 Wochen) Fütterung von kurzkettigen Fruktooligosacchariden (scFOS; 45 g/d) und Maltodextrin (Kontrolle) (nach *Respondek et al., 2011*)

Bei der Fermentation von Fruktanen im Verdauungstrakt wird diese durch mikrobielle Enzyme (Fruktanasen) in Fruktose gespalten, welche weiter zu organischen Säuren umgesetzt werden kann. Sollte direkt Fruktose absorbiert werden so führt diese im Vergleich zu Glukose zu einer geringeren Insulinreaktion (*Borer et al., 2012*). Ein teil der Fruktose wird in der Leber rasch in Fett umgewandelt und im Körper deponiert (*Johnson et al.,* 2013). Dies wiederum kann mit einer verminderten Ansprechbarkeit der Gewebe auf Leptin verbunden sein und zu Adipositas beitragen. Dies beeinflusst die Insulinsensitivität ungünstig. Allerdings spielt die Absorption von Fruktose aus Fruktanen im Vergleich zur mikrobiellen Fermentation wohl nur eine sehr untergeordnete Rolle. Eine Schlüsselrolle hinsichtlich endokrin vermittelter postprandialer Effekte spielt das gastrointestinale Hormon GLP-1 (siehe *Delzenne et al., 2007*). Bisher sind nur wenige Studien zum Metabolismus des GLP-1 bei Pferden durchgeführt wurden. *Bamford et al.* (*2015*) zeigten durch Fütterung von mikronisiertem Mais, dass die Konzentration von GLP-1 zwischen verschiedenen Rassen (Ponys, Standardtypen, Andalusier) variierte und hochgradig mit der Insulin-Konzentration korreliert war. Im Zusammenhang mit der Supplementierung von Präbiotika wurden nach bisherigem Kenntnisstand noch keine Untersuchungen an Pferden durchgeführt.

4.3 Immunstatus

Präbiotika wirken nicht nur auf die Mikroflora im Verdauungstrakt, sondern können sich auch positiv auf den Immunstatus auswirken (siehe oben). Die dem zugrunde liegenden Mechanismen sind noch nicht vollständig bekannt. Eine stimulierende Wirkung von FOS, Mannanoligosacchariden oder Galaktooligosacchariden auf

diverse Immunparameter beim Pferd (sowie Fohlen) konnte bisher nicht belegt werden (*Gürbüz et al., 2010; Vendrig et al., 2014*).

4.4 Resorptionsprozesse

Bisher gibt es nur wenig experimentell basierte Evidenz über Transportmechanismen im equinen Verdauungstrakt. *Dyer et al. (2002)* zeigten *in vitro*, dass Glukose und Galaktose im Dünndarm über SGLT1 (Natrium-abhängiger Glukose-Co-Transporter 1) absorbiert werden. Dies wurde auch von *Cehak et al. (2009)* unter Verwendung von Gewebe aus dem Jejunum bestätigt. Im Jejunum wurden weiterhin sekundär aktivierte, H^+-abhängige Co-Transporte sowie H^+- und Na^+-unabhängige Transportmechanismen für Peptide verifiziert *(Cehak et al., 2013)*. Eine umfangreiche *in vitro* Untersuchung zum Calcium- sowie Phosphat-Transport in verschiedenen Abschnitten des Verdauungstraktes zeigte die signifikant höchste Absorption von Calcium aus dem Duodenum, für Phosphat im Jejunum *(Cehak et al., 2012)*. Die Absorption von Butyrat im Dickdarm von Pferden erfolgt über Natrium-unabhängige Membrantransporter, welche über einen pH-Wert Gradienten angetrieben werden (*Nedjadi et al., 2014*). Nach bisherigem Kenntnistand liegen keine Studien zum Einfluss von Präbiotika auf Transportprozesse im equinen Verdauungstrakt vor.

5. Ausblick

Derzeit sind einige Präparate auf dem Markt, welche als präbiotisch für Pferde beworben werden. Wissenschaftlich fundierte Ergebnisse sind allerdings nur in sehr begrenztem Umfang vorhanden. Dennoch konnten *Julliand und Zeyner (2013)* vorläufige Empfehlungen zur präbiotischen Dosierung von Inulin-Typ-Fruktanen beim Pferd ableiten. In der Potenz höhere Mengen an Fruktanen können dagegen, unabhängig vom Typ, Hufrehe auslösen. Neben der Dosierung hat auch die Zusammensetzung des Präbiotikums (Polymerisationsgrad, begleitende mono- und dimere Kohlenhydrate) einen erheblichen Einfluss auf seine Wirksamkeit. Das Präbiotikum sollte den Magen möglichst unbeschadet passieren und auch vor dem Abbau durch körpereigene Enzyme im Dünndarm geschützt sein. Wahrscheinlich werden jedoch schon im vorderen Verdauungstrakt erhebliche Mengen an Fruktanen abgebaut (*Coenen et al., 2006; Ince et al., 2014*), sodass weit weniger Wirksubstanz den Dickdarm erreicht als angenommen. Dies könnte durch saure Hydrolyse im Magen oder bereits im terminalen Verdauungstrakt durch mikrobiellen Abbau geschehen. Je höher der Polymerisationsgrad, desto wahrscheinlicher erreicht das Molekül nahezu unverdaut den Dickdarm und kann am ursprünglich dafür avisierten Ort fermentiert werden. Eine gesteigerte mikrobielle Fermentation im Magen-Dünndarm-Bereich ist jedoch unerwünscht. Präbiotika mit geringem Polymerisationsgrad (z.B. scFOS) könnten evtl. durch eine entsprechende galenische Behandlung so geschützt werden, dass sie weitgehend unbeschadet den Dickdarm erreichen. Bei der Verwendung von Präbiotika pflanzlichen Ursprungs (z. B. Inulin aus Topinambur) empfiehlt es sich, auf die jahreszeitlichen, natürlichen Schwankungen der Fruktangehalte sowie deren Polymerisationsgrade zu achten.

Mitunter sinkt der Polymerisationsgrad bei veränderten Temperaturen (*Gupta und Kaur, 2000*). In der Praxis sollte demnach zum Zeitpunkt der Ernte der Gehalt an Wirksubstanz bestimmt werden, sodass die gewünschte Dosierung bei der Fütterung erreicht wird. Die Ergebnisse aus der Literatur zur Anwendung von Präbiotika bei Pferden sind kritisch zu hinterfragen, da zumeist eine detaillierte Analyse des eingesetzten Präbiotikums fehlt. Der Fokus sollte neben der Wirkung der eingesetzten Substanz vorab zunächst auf einer Charakterisierung des Präbiotikums liegen. Neben physikochemischen Charakteristika der mit präbiotischen Intentionen eingesetzten Fruktane (meist ITF) ist die spezifische gastrointestinale und metabolische Situation des jeweiligen Pferdes mit entscheidend für den Erfolg einer präbiotischen Anwendungen und damit immer auch eine Einzelfallentscheidung.

Literaturverzeichnis

Azorín-Ortuño, M.; Urbán, C.; Cerón, J. J.; Tecles, F.; Allende, A.; Tomás-Barberán, F. A.; Espín, J. C. (2009): Effect of low inulin doses with different polymerisation degree on lipid metabolism, mineral absorption, and intestinal microbiota in rats with fat-supplemented diet, Food Chemistry 113 4, 1058–1065.

Bach Knudsen, K. E. (2012): Prebiotic with special emphasis on butyrogenic carbohydrates and the effects on gut function. In: 24. Hülsenberger Gespräche (H. Wilhelm Schaumann Stiftung, Hrsg.), 84–90.

Bacon, J. S. D.; Loxley, R. (1952): Seasonal changes in the carbohydrates of the Jerusalem Artichoke tuber, Biochemical Journal 51 (2), 208–213.

Bailey, S. R.; Menzies-Gow, N.; Harris, P. A.; Habershon-Butcher, J. L.; Crawford, C.; Berhane, Y.; Boston, R. C.; Elliot, J. (2007): Effect of dietary fructans and dexamethasone administration on the insulin response of ponies predisposed to laminitis, JAVMA 231 (9), 1365–1373.

Bamford, N. J.; Baskerville, C. L.; Harris, P. A.; Bailey, S. R. (2015): Postprandial glucose, insulin, and glucagon-like peptide-1 responses of different equine breeds adapted to meals containing micronized maize, Journal of Animal Science 93 (7), 3377–3383.

Berg, E. L.; Fu, C. J.; Porter, J. H.; Kerley, M. S. (2005): Fructooligosaccharide supplementation in the yearling horse: Effects on fecal pH, microbial content and volatile fatty acid concentration, Journal of Animal Science 83, 1549–1553.

Bergmann, E. N. (1990): Energy Contributions of Volatile Fatty Acids From the Gastrointestinal Tract in Various Species, Physiological Reviews 70 (2), 567–590.

Bernet, M.-F.; Brassart, D.; Neeser, J.-R.; Servin, A. L. (1993): Adhesion of humans bifidobacterial strains to cultural human intestinal epithelial cells and inhibition of enteropathogen-cell interaction, Applied and Environmental Microbiology 59 (12), 4121–4128.

Blaut, M. (2002): Relationship of prebiotics and food to intestinal microflora, European journal of nutrition 41 Suppl 1, I11-6.

Böhmer, B. M.; Branner, G. R.; Roth-Maier, D. A. (2005): Precaecal and faecal digestibility of inulin (DP 10-12) or an inulin/Enterococcus faecium mix and effects on nutrient digestibility and microbial gut flora, J Anim Physiol Anim Nutr 89 (11-12), 388–396.

Bonnett, G. D.; Sims, I. M.; Simpson, R. J.; Cairns, A. J. (1997): Structural diversity of fructans in relation to the taxonomy of teh Poaceae, The New Phytologist 136 (1), 11–17.

Borer, K. E.; Bailey, S. R.; Menzies-Gow, N. J.; Harris, P. A.; Elliott, J. (2012): Effect of feeding glucose, fructose, and inulin on blood glucose and insulin concentrations in normal ponies and those predisposed to laminitis, Journal of Animal Science 90 (9), 3003–3011.

Brown, G. D.; Gordon, S. (2001): A new receptor for beta-glucans, Nature 413, 36–37.

Cani, P. D.; Dewever, C.; Delzenne, N. M. (2004): Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats, British Journal of Nutrition 92 (3), 521–526.

Cehak, A.; Burmester, M.; Geburek, F.; Feige, K.; Breves, G. (2009): Electrophysiological characterization of electrolyte and nutrient transport across the small intestine in horses, Journal of Animal Physiology and Animal Nutrition 93 3, 287–294.

Cehak, A.; Schröder, B.; Feige, K.; Breves, G. (2013): In vitro studies on intestinal peptide transport in horses, Journal of Animal Science 91 11, 5220–5228.

Cehak, A.; Wilkens, M. R.; Guschlbauer, M.; Mrochen, N.; Schröder, B.; Feige, K.; Breves, G. (2012): In vitro studies on intestinal calcium and phosphate transport in horses, Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 161 2, 259–264.

Coenen, M.; Mößeler, A.; Vervuert, I. (2006): Fermentative gases in breath indictae the Inulin and starch start to be degrated by microbial fermentation in the stomach and small intestine of the horse in contrast to Pectin and Cellulose, The Journal of Nutritition 136, 2108S-2110S.

Crawford, C.; Sepulveda, M. F.; Elliott, J.; Harris, P. A.; Bailey, S. R. (2007): Dietary fructan carbohydrate increases amine production in the equine large intestine: implications for pasture-associated laminitis, Journal of Animal Science 85 (11), 2949–2958.

Dahlhoff, S. (2002): Fruktangehalt im Gras von Pferdeweiden während der Weidesaison, Dissertation, Tierärztliche Hochschule Hannover

Delzenne, N. M.; Cani, P. D.; Daubioul, C.; Neyrinck, A. M. (2005): Impact of inulin and oligofructose on gastrointestinal peptides, British Journal of Nutrition 93 (S1), S157.

Delzenne, N. M.; Cani, P. D.; Neyrinck, A. M. (2007): Modulation of Glucagon-like peptide 1 and energy metabolism by inulin and oligofructose: experimental data, Journal of Nutrition 137, 2547S-2551S.

Delzenne, N. M.; Kok, N. (2001): Effects of fructans-type prebiotics on lipid metabolism, The American Journal of Clinical Nutrition 73(suppl.), 456S-458S.

Delzenne, N. M.; Williams, C. M. (2002): Prebiotics and lipid metabolism, Current Opinion in Lipidology 13 (1), 61–67.

Dyer, J.; Fernandez-Castano Merediz, E.; Salmon, K. S.; Proudman, C. J.; Edwards, G. B.; Shirazi-Beechey, S. P. (2002): Molecular characterisation of carbohydrate digestion and absorption in equine small intestine, Equine Veterinary Journal 34, 349–358.

Elliott, J.; Bailey, S. R. (2006): Gastrointestinal derived factors are potential triggers for the development of acute equine laminitis, Journal of Nutrition 136 (7), 2103S-2017S.

Eps, A. W. van; Pollit, C. C. (2006): Equine laminitis induced with oligofructose, Equine Veterinary Journal 38 (3), 203–208.

Eps, A. W. van; Pollit, C. C. (2009): Equine laminitis model: Lamellar histopathology seven days after induction with oligofructose, Equine Veterinary Journal 41(8), 735–740.

Finnie, I. A.; Dwarakanath, A. D.; Taylor, B. A.; Rhodes J.M. (1995): Colonic mucin synthesis is increased by sodium butyrate, Gut 36, 93–99.

Fiordaliso, M.; Kok, N.; Desager, J.-P.; Goethals, F.; Deboyser, D.; Roberfroid, M.; Delzenne, N. (1995): Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats, Lipids 30 (2), 163–167.

Fombelle, A. de; Julliand, V.; Drogoul, C.; Jacotot, E. (2001): Feeding and microbial disorders in horses: 1-effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities, Journal of Equine Veterinary Science 21 9, 439–445.

Fombelle, A. de; Varloud, M.; Goachet, A. G.; Jacotot, E.; Philippeau, C.; Drogoul, C.; Julliand, V. (2003): Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets, Animal Science 77, 293–304.

Gupta, A. K.; Kaur, N.: Fructan metabolism in jerusalem artichoke and chicory. In: Gupta, Kaur (Hrsg.) 2000 – Carbohydrate Reserves in Plants- Synthesis, Bd. 26, 223–248.

Gürbüz, E.; Inal, F.; Ata, S. Ü.; Citil, Ö. B.; Kav, K.; Kücükkaya, F. (2010): Effects of supplemental fructo-oligosaccharide and mannan-oligosaccharide on nutrient digestibilities, volatile fatty acid concentrations and immune function in horses, Turkish Journal of Veterinary & Animal Science 34 (1), 39–44.

Harlow, B. E.; Lawrence, L. M.; Kagan, I. A.; Flythe, M. D. (2014): Inhibition of fructan-fermenting equine faecal bacteria and Streptococcus bovis by hops (Humulus lupulus L.) β-acid, Journal of applied microbiology 117 (2), 329–339.

Harmsen, H.; Raangs, G. C.; Franks, A. H.; Wildeboer-Veloo, A.; Welling, G. W. (2002): The effect of the prebiotic inulin and probiotic Bifidobacterium longum on the fecal microflora of healthy volunteers measured by FISH and DGGE, Microbial Ecology in Health and Disease 14, 211–219.

Herrmann, J.; Hermes, R.; Breves, G. (2011): Transepithelial transport and intraepithelial metabolism of short-chain fatty acids (SCFA) in the porcine proximal colon are influenced by SCFA concentration and luminal pH, Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 158 1, 169–176.

Ince, J. C.; Longland, A. C.; Moore-Colyer, M. J. S.; Harris, P. A. (2014): In vitro degradation of grass fructan by equid gastrointestinal digesta, Grass Forage Sci 69 (3), 514–523.

Janczyk, P.; Pieper, R.; Smidt, H.; Souffrant, W. B. (2010): Effect of alginate and inulin on intestinal microbial ecology of weanling pigs reared under different husbandry conditions, FEMS Microbiology Ecology 72 1, 132–142.

Johnson, R. J.; Rivard, C.; Lanaspa, M. A.; Otabachian-Smith, S.; Ishimoto, T.; Cicerchi, C.; Cheeke, P. R.; Macintosh, B.; Hess, T. (2013): Fructokinase, Fructans, Intestinal Permeability, and Metabolic Syndrome: An Equine Connection?, Journal of Equine Veterinary Science 33 (2), 120–126.

Julliand, V.; Fombelle, A. de; Drogoul, C.; Jacotot, E. (2001): Feeding and microbial disorders in horses: Part 3—Effects of three hay:grain ratios on microbial profile and activities, Journal of Equine Veterinary Science 21 11, 543–546.

Julliand, V.; Zeyner, A. (2013): Dietary strategies for optimizing gastro intestinal health: an update on pre- and probiotics. In: Horse Health Nutrition, European Equine Health & Nutrition Congress, 75–84.

Kern, D. L.; Slyter, L. L.; Leffel, E. C.; Weaver, J. M.; Oltjen, R. R. (1974): Ponies vs. steers: Microbial and Chemical Characteristics of intestinal ingesta, Journal of Animal Science 38, 559–564.

Kleessen, B.; Hartmann, L.; Blaut, M. (2001): Oligofructose and long-chain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora, British Journal of Nutrition 86 (2), 291.

Kollarczik, B.; Flothow, C.; Radicke, S.; Kaaden, O.-R. (1995): Einfluß von Fetten auf das Keimspektrum des Jejunums von Pferden, Journal of Animal Physiology and Animal Nutrition 73, 225–232.

Le Bourgot, C.; Ferret-Bernard, S.; Le Normand, L.; Savary, G.; Menendez-Aparicio, E.; Blat, S.; Appert-Bossard, E.; Respondek, F.; Le Huërou-Luron, I. (2014): Maternal short-chain fructooligosaccharide supplementation influences intestinal immune system maturation in piglets, PLoS ONE 9, e107508.

Li, W.; Zhang, J.; Yu, C.; Li, Q.; Dong, F.; Wang, G.; Gu, G.; Guo, Z. (2015): Extraction, degree of polymerization determination and prebiotic effect evaluation of inulin from Jerusalem artichoke, Carbohydrate polymers 121, 315–319.

Loh, G.; Eberhard, M.; Brunner, R. M.; Henning, U.; Kuhla, S.; Kleessen, B.; Metges, C. C. (2006): Inulin alters the intestinal microbiota and short-chain fatty acid concentrations in growing pigs regardless of their basal diet, The Journal of Nutritition 136, 1198–1202.

Longland, A. C.; Byrd, B. M. (2006): Pasture nonstructural carbohydrates and equine laminitis, Journal of Nutrition 136 (7), 2099S–2102.

Mackie, R. I.; Wilkins, C. A. (1988): Enumeration of Anaerobic Bacterial Microflora of the Equine Gastrointestinal Tract, Applied and Environmental Microbiology 54, 2155–2160.

Masanetz, S.; Wimmer, N.; Plitzner, C.; Limbeck, E.; Preißinger, W.; Pfaffl, M. W. (2010): Effects of inulin and lactulose on the intestinal morphology of calves, Animal 4 05, 739.

Milinovich, G. J.; Klieve, A. V.; Pollitt, C. C.; Trott, D. J. (2010): Microbial Events in the Hindgut During Carbohydrate-induced Equine Laminitis, Veterinary Clinics of North America: Equine Practice 26 (1), 79–94.

Nedjadi, T.; Moran, A. W.; Al-Rammahi, M. A.; Shirazi-Beechey, S. P. (2014): Characterization of butyrate transport across the luminal membranes of equine large intestine, Experimental Physiology 99 (10), 1335–1347.

Onishi, J. C.; Park, J.-W.; Prado, J.; Eades, S. C.; Mirza, M. H.; Fugaro, M. N.; Häggblom, M. M.; Reinemeyer, C. R. (2012): Intestinal bacterial overgrowth includes potential pathogens in the carbohydrate overload models of equine acute laminitis, Veterinary Microbiology 159 (3-4), 354–363.

Patterson, J. K.; Yasuda, K.; Welch, R. M.; Miller, D. D.; Lei, X. G. (2010): Supplemental Dietary Inulin of Variable Chain Lengths Alters Intestinal Bacterial Populations in Young Pigs, Journal of Nutrition 140 12, 2158–2161.

Peng, L.; Li, Z.-R.; Green, R. S.; Holzman, I. R.; Lin, J. (2009): Butyrate Enhances the Intestinal Barrier by Facilitating Tight Junction Assembly via Activation of AMP-Activated Protein Kinase in Caco-2 Cell Monolayers, Journal of Nutrition 139 (9), 1619–1625.

Pierce, K. M.; Sweeney, T.; Brophy, P. O.; Callan, J. J.; Fitzpatrick, E.; McCarthy, P.; O'Doherty, J. V. (2006): The effect of lactose and inulin on intestinal morphology, selected microbial populations and volatile fatty acid concentrations in the gastro-intestinal tract of the weanling pig, ASC 82 03.

Plöger, S.; Stumpff, F.; Penner, G. B.; Schulzke, J.-D.; Gäbel, G.; Martens, H.; Shen, Z.; Günzel, D.; Aschenbach, J. R. (2012): Microbial butyrate and its role for barrier function in the gastrointestinal tract, Annals of the New York Academy of Sciences 1258, 52–59.

Pradhan, S. K.; Das, A.; Kullu, S. S.; Saini, M.; Pattanaik, A. K.; Dutta, N.; Sharma, A. K. (2015): Effect of feeding Jerusalem artichoke (Helianthus tuberosus) root as prebiotic on nutrient utilization, fecal characteristics and serum metabolite profile of captive Indian leopard (Panthera pardus fusca) fed a meat-on-bone diet, Zoo biology 34 (2), 153–162.

Reischer, N.(2012) : Dynamik der Serumkonzentration und Ausscheidung von D-Laktat bei jungen Kälbern mit Durchfall, Dissertation, Ludwig-Maximilians-Universität München.

Respondek, F.; Goachet, A. G.; Julliand, V. (2008): Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet, Journal of Animal Science 86 (2), 316–323.

Respondek, F.; Myers, K.; Smith, T. L.; Wagner, A.; Geor, R. J. (2011): Dietary supplementation with short-chain fructo-oligosaccharides improves insulin sensitivity in obese horses, Journal of Animal Science 89 (1), 77–83.

Roberfroid, M. (2007): Prebiotics: The concept revisited, Journal of Nutrition 137, 830S-837S.

Roberfroid, M.; Gibson, G. R.; Hoyles, L.; McCartney, A. L.; Rastall, R.; Rowland, I.; Wolvers, D.; Watzl, B.; Szajewska, H.; Stahl, B.; Guarner, F.; Respondek, F.; Whelan, K.; Coxam, V.; Davicco, M.-J.; Léotoing, L.; Wittrant, Y.; Delzenne, N. M.; Cani, P. D.; Neyrinck, A. M.; Meheust, A. (2010): Prebiotic effects: metabolic and health benefits, British Journal of Nutrition 104 (S2), S1-S62.

Roller, M.; Rechkemmer G.; Watzl, B. (2004): Prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis modulates intestinal immune functions in rats, Journal of Nutrition 134 (1), 153–156.

Samanta, A. K.; Jayapal, N.; Jayaram, C.; Roy, S.; Kolte, A. P.; Senani, S.; Sridhar, M. (2015): Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications, Bioactive Carbohydrates and Dietary Fibre 5 (1), 62–71.

Samanta, A. K.; Jayapal, N.; Senani, S.; Kolte, A. P.; Sridhar, M. (2013): Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora, Sociedade Brasileira de Microbiologia 44 (1), 1–14.

Scheppach, W. (1994): Effects of short chain fatty acids on gut morphology and function, Gut Supplement 1, S35-S38.

Seifert, S.; Watzl, B. (2007): Inulin and oligofructose: Review of experimental data on immune modulation, The Journal of Nutritition 137, 2563S-2567S.

Sevane, N.; Bialade, F.; Velasco, S.; Rebolé, A.; Rodríguez, M. L.; Ortiz, L. T.; Cañón, J.; Dunner, S.; te Pas, Marinus FW. (2014): Dietary Inulin Supplementation Modifies Significantly the Liver Transcriptomic Profile of Broiler Chickens, PLoS ONE 9 6, e98942.

Świątkiewicz, S.; Koreleski, J.; Arczewska-Włosek, A. (2010): Effect of prebiotic fructans and organic acids on mineral retention in laying hens, Acta Agriculturae Scandinavica, Section A - Animal Science 60 (2), 125–128.

Tako, E.; Glahn, R. P.; Welch, R. M.; Lei, X.; Yasuda, K.; Miller, D. D. (2008): Dietary inulin affects the expression of intestinal enterocyte iron transporters, receptors and storage protein and alters the microbiota in the pig intestine, The British journal of nutrition 99 3, 472–480.

Tarini, J.; Wolever, T. M. S. (2010): The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects, Applied physiology, nutrition, and metabolism = Physiologie appliquée, nutrition et métabolisme 35 1, 9–16.

Tubbs, P. K. (1965): The metabolism of D-alpha-hydroxy acids in animal tissues, Annals of the New York Academy of Sciences 119 (3), 920–926.

Uyeno, Y.; Shigemori, S.; Shimosato, T. (2015): Effect of Probiotics/Prebiotics on Cattle Health and Productivity, Microbes and environments / JSME 30 2, 126–132.

van de Wiele, T.; Boon, N.; Possemiers, S.; Jacobs, H.; Verstraete, W. (2007): Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects, Journal of applied microbiology 102 2, 452–460.

Varloud, M.; Fonty, G.; Roussel, A.; Guyonvarch, A.; Julliand, V. (2007): Postprandial kinetics of some biotic and abiotic characteristics of the gastric ecosystem of horses fed a pelleted concentrate meal, Journal of Animal Science 85 10, 2508–2516.

Velasco, S.; Ortiz, L. T.; Alzueta, C.; Rebolé, A.; Treviño, J.; Rodríguez, M. L. (2010): Effect of inulin supplementation and dietary fat source on performance, blood serum metabolites, liver lipids, abdominal fat deposition, and tissue fatty acid composition in broiler chickens, Poultry science 89 8, 1651–1662.

Vendrig, J. C.; Coffeng, L. E.; Fink-Gremmels, J. (2014): Effects of orally administered galacto-oligosaccharides on immunological parameters in foals: a pilot study, BMC Veterinary Research 10 (1), 367.

Vhile, S. G.; Kjos, N. P.; Sørum, H.; Overland, M. (2012): Feeding Jerusalem artichoke reduced skatole level and changed intestinal microbiota in the gut of entire male pigs, Animal 6 (5), 807–814.

Wähler, R. (2015): Präbiotika und die Behandlung des metabolischen Syndroms, OM & Ernährung 152 (Sonderdruck), 2–8.

Wichert, B.; Strauch, S.; Zeyner, A.; Suter, D.; Liesegang, A. (2015): Pre-digestion of feedstuff for further use in in vitro hindgut digestion trials. In: Proceedings of the 19th congress of the European Society of Veterinary and Comparative Nutrition. Toulouse, Frankreich, 35.

Yun, H.; Lee, J. H.; Park, C. E.; Kim, M.-J.; Min, B.-I.; Bae, H.; Choe, W.; Kang, I.; Kim, S.-S.; Ha, J. (2009): Inulin increases glucose transport in C2C12 myotubes and HepG2 cells via activation of AMP-activated protein kinase and phosphatidylinositol 3-kinase pathways, Journal of medicinal food 12 5, 1023–1028.

Zentek, J.; Marquart, B.; Pietrzak, T. (2002): Intestinal effects of mannanoligosaccharides, transoligosaccharides, lactose and lactulose in dogs, Journal of Nutrition 132, 1682S-1684S.

Zeyner, A.; Schrödel, W.; Sahm, U.; Lengwenat, O.; Krüger, M. (2003): Effects of lactulose as feed additive on microbiological and immunological parameters in faeces and blood of healthy horses. Proc. 18th Equine Nutr. Physiol. Symp.. East Lansing (Michigan, USA): June 04-07 2003, 283-284.

Zeyner, A.; Schrödl, W.; Sahm, U.; Lengwenat, O.; Coenen, M.; Krüger, M. (2003): Investigations on the safety of lactulose as feed additive for adult healthy horses. In: Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier (Hrsg.: Schubert, R.; Flachowsky, G.; Jahreis, G.; Bitsch, R.), 196-201.

Supplementary material

Table S1: Distribution of microorganisms in the equine GIT (according to OZEKI et al., 1973; CANN et al., 2005; KULIKOV et al., 2007; LIGGENSTOFFER et al., 2010; SADET-BOURGETAU and JULLIAND, 2010; SHEPHERD et al., 2012; DICKS et al., 2014; FERNANDES et al., 2014; LWIN and MATSUI, 2014; COSTA et al., 2015; JULLIAND and GRIMM, 2016; JULLIAND and GRIMM, 2017)*

Microorganism	**part of the GIT**					
	STO	SI	CAE	CV	CD	fae.
protozoa						
Allantosoma	-	-	x	x	x	x
Alloizona	-	-	x	x	x	x
Ampullacula	-	-	-	x	x	x
Blepharoconus	-	-	x	x	x	x
Blepharocorys	-	-	x	x	x	x
Blepharoprosthium	-	-	x	x	x	x
Blepharosphaera	-	-	x	x	x	x
Blepharozoum	-	-	x	x	-	-
Bundleia	-	-	x	x	x	x
Buetschlia	-	-	x	-	x	-
Callimastix[1]	-	-	x	-	x	-
Charonnautes	-	-	-	x	x	x
Charonina	-	-	-	-	-	x
Chlamydobundleia	-	-	-	-	-	x
Circodium	-	-	-	-	-	x
Cochliatoxum	-	-	-	x	x	x
Cycloposthium	-	-	x	x	x	x
Didesmis	-	-	x	x	x	x
Ditoxum	-	-	-	x	x	x
Endamoeba	-	-	x	-	x	-
Hemiporodon	-	-	-	-	x	x
Holophryoides	-	-	x	x	x	x
Ochoterenaia	-	-	-	-	-	x
Oikomonas	-	-	x	-	-	-
Paraisotrichopsis	-	-	-	-	-	x
Polymorpha	-	-	x	x	x	x
Prorodonopsis	-	-	-	x	x	x
Spirodinium	-	-	-	x	x	x
Tetratoxum	-	-	-	x	x	x
Triadinium	-	-	x	x	x	x
Trichomonas	-	-	x	-	x	-
Tripalmaria	-	-	-	x	x	x
Walskana	-	-	-	-	-	x

Microorganism		part of the GIT					
		STO	SI	CAE	CV	CD	fae.
fungi							
Anaeromyces		-	-	-	-	-	x
Caecomyces		-	-	-	-	-	x
Candida		-	-	x (not specified)			
Geotrichum		-	-	x (not specified)			
Neocallimastix		-	-	-	-	-	x
Piromyces		-	-	x	-	-	x
Torulopsis		-	-	x (not specified)			
bacteriophages[2]							
Microviridae		-	-	-	-	-	x
Myoviridae		-	-	-	-	-	x
Orthopoviridae		-	-	-	-	-	x
Podoviridae		-	-	-	-	-	x
Siphoviridae		-	-	-	-	-	x
archaea							
Methanobrevibacter spp.		-	-	-	-	-	x
Methanocorpusculum spp.		-	-	-	-	-	x
Methanomicrococcus spp.		-	-	-	-	-	x
Methanosphaera		-	-	-	-	-	x
bacteria							
family	genus						
Acidaminococcaceae		-	-	x	x	x	-
Acidaminococcaceae	*Acidaminococcus*	-	-	-	-	-	x
Anaeroplasmataceae		-	-	-	-	-	x
Bacteroidaceae	*Bacteroides*	-	-	x	-	-	-
Campylobacteraceae	*Campylobacter*	-	-	-	x	x	-
Clostridiaceae	*Acetivibrio*	-	-	-	-	-	x
Clostridiaceae	*Clostridium*	x	x	x	x	x	-
Clostridiaceae	*Sarcina*	x	-	-	-	-	-
Clostridiales Family XIII incertae sedis	*Mogibacterium*	-	-	-	-	-	x
Coriobacteriaceae		-	-	-	-	x	x
Coriobacteriaceae	*Asaccharobacter*	-	-	-	-	-	x
Coriobacteriaceae	*Denitrobacterium*	-	-	-	-	-	x
Enterobacteriaceae	*Enterobacter*	-	x	-	-	-	-
Enterobacteriaceae	*Proteus*	-	x	-	-	-	-
Enterococcaceae	*Enterococcus*	-	x	-	-	-	x
Erysipelotrichaceae		-	-	x	x	x	x
Eubacteriaceae	*Eubacterium*	-	-	x	x	x	x
Fibrobacteraceae	*Fibrobacter*	-	-	x	x	x	x
Heliobacteraceae	*Heliobacter*	x	-	-	-	-	-
Lachnospiraceae	*Anaerosporobacter*	-	-	-	-	-	x
Lachnospiraceae	*Blautia*	-	-	-	-	-	x
Lachnospiraceae	*Butyrivibrio*	-	-	x	x	x	x
Lachnospiraceae	*Coprococcus*	-	-	-	-	-	x
Lachnospiraceae	*Pseudobutyrivibrio*	-	-	-	-	-	x
Lachnospiraceae	*Roseburia*	-	-	-	-	-	x
Lactobacillaceae	*Lactobacillus*	x	x	x	x	x	x
Moraxellaceae	*Moraxella*	x	-	-	-	-	-

Microorganism		part of the GIT					
		STO	SI	CAE	CV	CD	fae.
Mycoplasmateles incertae sedis		-	-	-	-	-	x
Oscillospiraceae	*Oscillobacter*	-	-	-	-	-	x
Pasteurellaceae		-	x	-	-	-	-
Pasteurellaceae	*Actinobacillus*	x	x	-	-	-	x
Porphyromonadaceae		-	-	x	-	-	-
Porphyromonadaceae	*Porphyromonas*	x	-	-	-	-	-
Prevotellaceae	*Prevotella*	x	-	x	x	x	x
Pseudomonaceae	*Pseudomonas*	x	x	-	-	-	-
Ruminococcaceae	*Faecalibacterium*	-	-	-	-	-	x
Ruminococcaceae	*Papillibacter*	-	-	-	-	-	x
Ruminococcaceae	*Ruminococcus*	-	-	x	x	x	x
Ruminococcaceae	*Sporobacter*	-	-	x	x	x	-
Spirochaeteceae	*Treponema*	-	-	-	x	-	x
Staphylococcaceae	*Staphylococcus*	-	x	x	-	-	-
Streptococcaceae	*Streptococcus*	x	x	x	-	-	x
Succinivibrionaceae	*Succinivibrio*	-	-	-	-	-	x
Veillonellaceae	*Megaspaera*	-	-	x	x	x	-
Veillonellaceae	*Veillonella*	-	-	x	x	x	-

CD = colon dorsale; CV = colon ventrale; fae. = faeces; GIT = gastrointestinal tract; SI = small intestine; STO = stomach; - = no reference; [1] = classified now as *Neocallimastix* (fungi); [2] = only classified ones are listed; * = The list raise no claim to be completeness. Unknown or unclassified members are excluded.

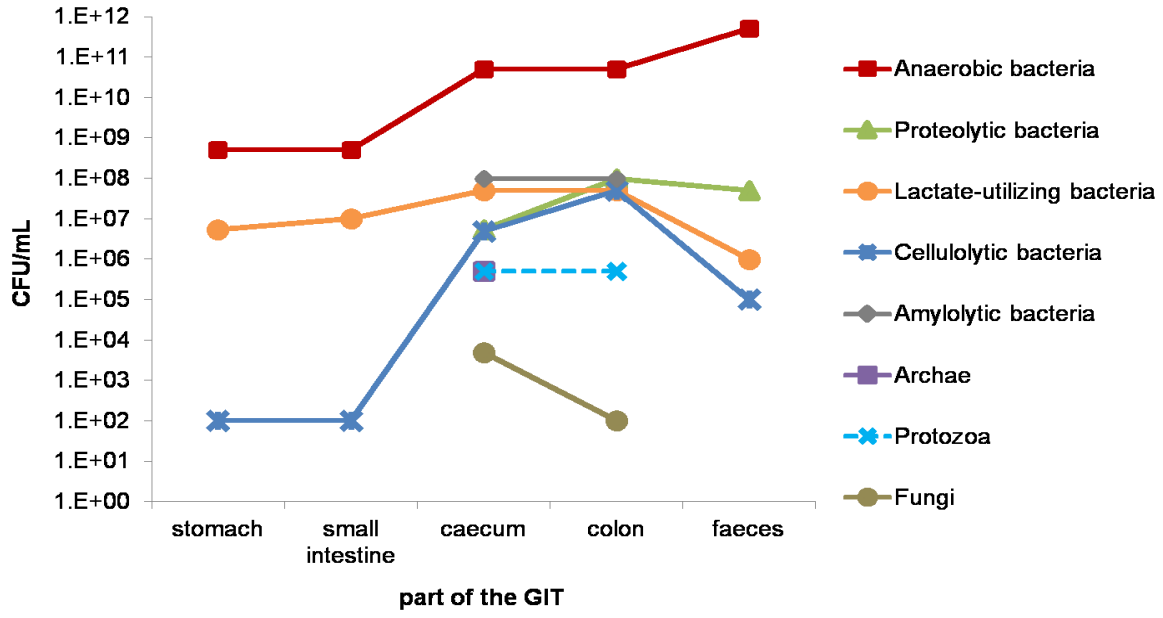

Fig. S1: Concentration (in CFU/mL) of different microbial groups along the equine gastrointestinal tract (according to MACKIE and WILKINS, 1988; JULLIAND, 1992; DE FOMBELLE et al., 2003; SADET-BOURGETAU and JULLIAND, 2010; DICKS et al., 2014; JULLIAND and GRIMM, 2016)

Table S2: Core microbial community at the family respectively genus level[1] in the equine gastrointestinal tract after feeding of JAM in comparison to CON

[%]	part of GIT													
	PN	PG	SI	CAE	CV	CD	CT	PN	PG	SI	CAE	CV	CD	CT
	CON							JAM						
proportion of not shared sequences	90.5	66.7	88.9	63.4	78.3	82.4	82.6	88.9	96.4	89.7	63.4	81.6	75.5	83.0
proportion of core microbiota	**9.5**	**33.3**	**11.1**	**36.6**	**21.7**	**17.6**	**17.4**	**11.1**	**3.6**	**10.3**	**36.6**	**18.4**	**24.5**	**17.0**
⇨ core microbiota consisting of:														
Bacteroidales[#] - BS11-gut group				0.4										
Bacteroidales[#] - S24-7				3.4	2.3	1.5	2.1				4.0	1.7	2.6	1.9
Christenenellaceae-g				0.8	0.8	0.4						1.0		0.9
Clostridiaceae - Clostridium				0.4		0.4				2.2	1.0		0.5	
Clostridiaceae - Sarcina		2.1	0.9	0.5	0.6	0.6	0.4			0.6	0.5	0.3	0.6	0.2
Clostridales[#]-g												0.4	0.4	
[Cyanobacteria] - 4C0d-2*											0.7			
Desulfovibrionaceae - Desulfovibrio											0.6			
Erysipelotrichaceae-g				1.5	0.5		0.5				2.9			
Lachnospiraceae - Blautia				0.3										
Lachnospiraceae - Coprococcus											0.5			
Lachnospiraceae-g	0.5	0.9	2.3	14.3	9.4	9.6	6.6				5.1	6.5	6.6	4.5
Lachnospiraceae-incertae sedis						0.2								
Lachnospiraceae - Johnsonella													0.3	
Lachnospiraceae - Marvinbryantia				0.4	0.3									
Lactobacillaceae - Lactobacillus	9.0	27.0	7.7	6.1	3.1	2.6	2.3	10.7	3.6	7.6	7.9	4.5	6.1	3.2
Mollicutes[§] - RF16													0.4	
Pasteurellaceae - Actinobacillus		0.9												
Porphyromonadaceae - Paludibacter													0.4	
Prevotellaceae - Prevotella				1.0							1.3			
Prevotellaceae - Xylanibacter		0.7	0.2	1.9	1.2	0.8					3.9	1.7	1.9	

[%]	part of GIT													
	PN	PG	SI	CAE	CV	CD	CT	PN	PG	SI	CAE	CV	CD	CT
	CON							JAM						
Rikenellaceae - RC9-gut group											0.7		2.0	1.9
Ruminococccaceae-g				2.0	2.4	1.4	3.9				2.5	1.7	2.0	2.7
Ruminococcaceae-incertae sedis				1.0			0.5							
Ruminococcaceae - Ruminococcus				2.7							3.6	0.6	0.8	0.5
Spirochaetaceae - Treponema					1.1		1.1				1.4			1.2
Streptococcaceae - Streptococcus		1.6						0.4						

Phylogenetic classification at the above-mentioned level, where possible. Variations are marked with the signs see below.
CAE = caecum; CD = colon dorsale; CON = placebo group; CT = colon transversum; CV = colon ventrale; g = uncultured; GIT = gastrointestinal tract; JAM = prebiotic feeding group (Jerusalem artichoke meal); PG = *pars glandularis*; PN = *pars nonglandularis*; SI = small intestine
* = phylum, # = order, § = class

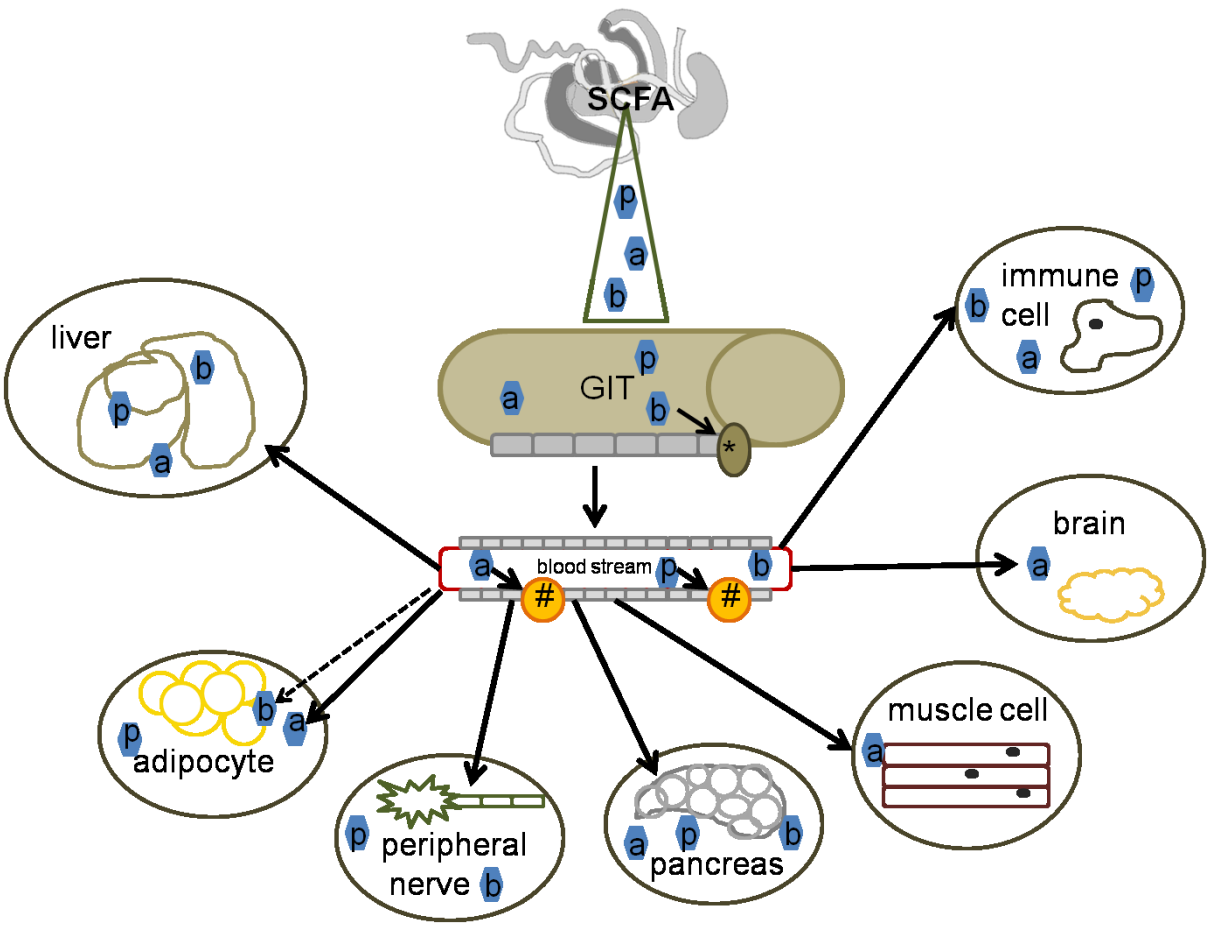

Fig. S2: Systemic direct (⟶) and indirect (⋯⋯▸) effects of SCFA on the organism (according to GUILLOTEAU et al., 2010; KASABUCHI et al., 2015; WÄHLER, 2015; MORRISON and PRESTON, 2016; Mc NABNEY and HENAGAN, 2017) [a = acetic acid; b = butyric acid; GIT = gastrointestinal tract; p = propionic acid; SCFA = short chain fatty acid; * = enterocyte; # = blood vessel]

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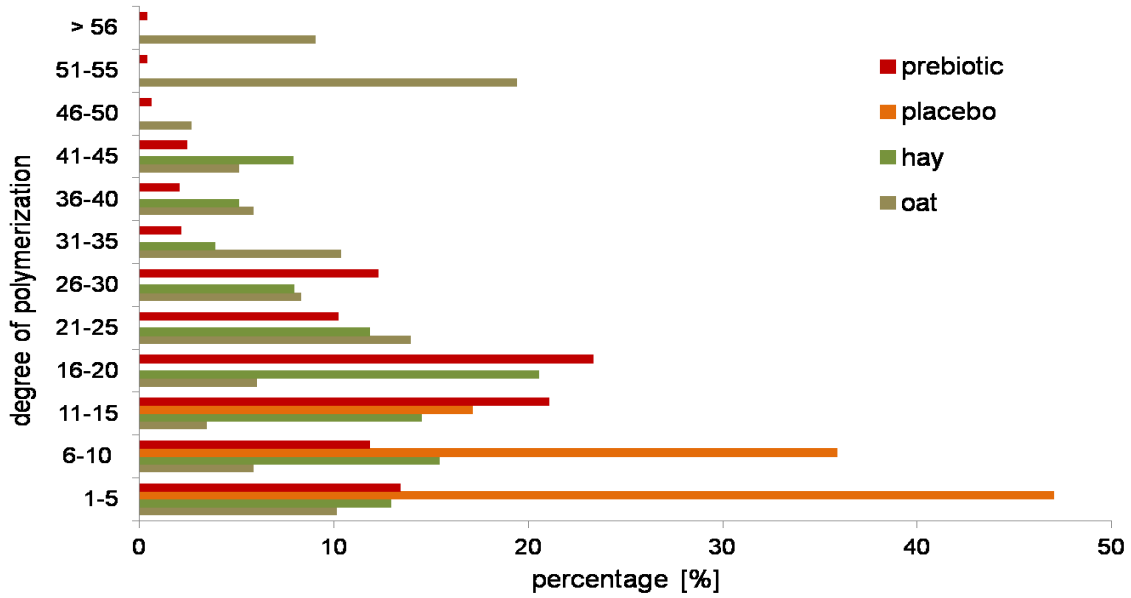

Fig. S1: Percentage (%) of the degree of polymerization in the dietary components.

Table S1: Water-soluble carbohydrate and starch content (each in g/kg DM) in the chyme of the different parts in the gastrointestinal tract (GIT)

Feeding group	Part of the GIT	water soluble carbohydrate				starch
		glucose	fructose	sucrose	fructan	
CON	PN	16.7	14.8	8.1	47.4	177.0
	PG	12.8	10.6	4.2	23.3	125.8
	CAE	0.0	0.0	0.0	0.0	8.3
	CV	0.0	0.0	0.0	0.0	3.4
	CD	0.0	0.0	0.0	0.0	4.6
	CT	0.0	0.0	0.0	0.0	2.4
JAM	PN	13.8	15.4	7.1	61.8	177.7
	PG	9.2	12.1	9.9	43.0	136.6
	CAE	0.0	0.0	0.0	0.0	1.2
	CV	0.0	0.0	0.0	0.0	5.4
	CD	0.0	0.0	0.0	0.0	3.3
	CT	0.0	0.0	0.0	0.0	3.6

CAE = caecum; CD = colon dorsale; CON = placebo group; CT = colon transversum; CV = colon ventrale; GIT = gastrointestinal tract; JAM = prebiotic feeding group (Jerusalem artichoke meal); PG = *pars glandularis*; PN = *pars nonglandularis*

Table S2: Library composition including individual primers for each sample and two mock communities

sample ID	barcode sequence	UniTag 1-27-DegS	UniTag2-338R-I	UniTag2-338R-II	digesta source	diet suppl.	library
1	CTGGATAA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	JAM	b11747
2	ATAAGGTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	JAM	b11747
3	AATAAGGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	JAM	b11747
4	TACTTATC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	JAM	b11747
5	ATCTCAGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	JAM	b11747
6	GTCAACGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	JAM	b11747
7	ATGTTCCA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	JAM	b11747
8	CGTCTGAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	CON	b11747
9	CCAAGTCA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	CON	b11747
10	GGAGTATG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	CON	b11747
11	TGGTTGAC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	CON	b11747
12	GCCTCATC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	CON	b11747
13	TTCTGAAC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	CON	b11747
16	GAGTTCAT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	JAM	b11747
17	CTTCATGG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	JAM	b11747

18	TCAGGCGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	JAM	b11747
19	AGAGATAA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	JAM	b11747
20	GGTCAGAT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	JAM	b11747
21	CCGTCTGC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	JAM	b11747
22	GGAGCGCA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	JAM	b11747
57	CGACCGCG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	CON	b11747
24	CGTCCTCC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	JAM	b11747
25	AGTACTGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	JAM	b11747
26	CGCGCCAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	JAM	b11747
27	TAATACGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	JAM	b11747
58	TCGTCGCC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	CON	b11747
31	GGCCAGTA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	JAM	b11747
32	CTGAGTTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	CON	b11747
33	TCTCTACC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	CON	b11747
34	CCGAGGCG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	CON	b11747
35	GAAGCTCG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	CON	b11747
36	AAGGTAAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	CON	b11747

37	ATGCGATT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	CON	b11747
38	AAGATCGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	CON	b11747
39	ATATAGGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	CON	b11747
41	TATATTGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	CON	b11747
42	TCCGACCT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	CON	b11747
43	TCCAGCTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	CON	b11747
44	GCTTGATG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	CON	b11747
47	GTAAGAAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	JAM	b11747
49	GAACGCTG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	JAM	b11747
50	CTGACCGG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	JAM	b11747
52	CATCAGTT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	JAM	b11747
53	ACCGGAAT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	JAM	b11747
55	TACCTACT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	CON	b11747
56	TGCCTCTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	JAM	b11747
Mock. I.lib.1	GATGATAA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	mock	NA	b11747
Mock. III.lib.1	ACTGCTCT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	mock	NA	b11747
59	CTGGATAA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	CON	b11748

60	ATAAGGTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	CON	b11748
61	AATAAGGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	CON	b11748
62	TACTTATC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	CON	b11748
64	ATCTCAGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	CON	b11748
65	GTCAACGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	JAM	b11748
71	ATGTTCCA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	JAM	b11748
66	CGTCTGAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	JAM	b11748
67	CCAAGTCA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	JAM	b11748
68	GGAGTATG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	JAM	b11748
69	TGGTTGAC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	JAM	b11748
30	GCCTCATC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	JAM	b11748
72	TTCTGAAC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	JAM	b11748
73	GAGTTCAT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	JAM	b11748
75	CTTCATGG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	JAM	b11748
76	TCAGGCGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	JAM	b11748
77	AGAGATAA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	JAM	b11748
80	GGTCAGAT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	JAM	b11748

87	CCGTCTGC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	CON	b11748
82	GGAGCGCA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	CON	b11748
83	CGACCGCG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	CON	b11748
84	CGTCCTCC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	CON	b11748
85	AGTACTGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	CON	b11748
86	CGCGCCAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	CON	b11748
88	TAATACGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	CON	b11748
23	TCGTCGCC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	CON	b11748
89	GGCCAGTA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	CON	b11748
90	CTGAGTTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	gaster mix	CON	b11748
91	TCTCTACC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CAE	CON	b11748
92	CCGAGGCG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	CON	b11748
94	GAAGCTCG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	CON	b11748
95	AAGGTAAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	CON	b11748
96	ATGCGATT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	CON	b11748
97	AAGATCGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	JAM	b11748
51	ATATAGGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CV	JAM	b11748

54	TATATTGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	SI	CON	b11748
40	TCCGACCT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	CON	b11748
28	TCCAGCTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	JAM	b11748
74	GCTTGATG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	JAM	b11748
81	GTAAGAAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PN	CON	b11748
93	GAACGCTG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	CON	b11748
70	CTGACCGG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	JAM	b11748
14	CATCAGTT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	CON	b11748
15	ACCGGAAT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	CON	b11748
29	TACCTACT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	JAM	b11748
45	TGCCTCTC	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	CON	b11748
Mock. I.lib.1	GATGATAA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	mock	NA	b11748
Mock. III.lib.1	ACTGCTCT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	mock	NA	b11748
46	AAGGTAAG	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	CON	b11746
48	ATGCGATT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	PG	JAM	b11746
63	AAGATCGT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	CON	b11746
78	ATATAGGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CD	JAM	b11746

79	TATATTGA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	CT	JAM	b11746
Mock. I.lib.1	GATGATAA	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	mock	NA	b11746
Mock. III.lib.1	ACTGCTCT	GAGCCGTAGCCAGTCTGCGTTYG ATYMTGGCTCAG	GCCGTGACCGTGACATCGGC WGCCTCCCGTAGGAGT	GCCGTGACCGTGACACGGCWGC CACCCGTAGGTGT	mock	NA	b11746

CAE = caecum; CD = colon dorsale; CON = placebo group; CT = colon transversum; CV = colon ventrale; JAM = prebiotic feeding group (Jerusalem artichoke meal); NA = not available (no supplementation); PG = *pars glandularis*; PN = *pars nonglandularis*; SI = small intestine

Table S3: Base pairs per sample

sample ID	digesta source	diet supplementation	library	base pairs
1	CAE	JAM	b11747	1151
2	gaster mix	JAM	b11747	9283
3	PN	JAM	b11747	33,712
4	PG	JAM	b11747	19,675
5	CV	JAM	b11747	14,583
6	CD	JAM	b11747	15,242
7	CT	JAM	b11747	5735
8	SI	CON	b11747	25,586
9	PN	CON	b11747	11,506
10	PG	CON	b11747	21,365
11	gaster mix	CON	b11747	5637
12	CAE	CON	b11747	6809
13	CV	CON	b11747	3189
16	PN	JAM	b11747	34,526
17	PG	JAM	b11747	32,085
18	gaster mix	JAM	b11747	4348
19	CAE	JAM	b11747	11,442
20	CV	JAM	b11747	9544
21	CD	JAM	b11747	6271
22	CT	JAM	b11747	13,362
57	CT	CON	b11747	49,300
24	PG	JAM	b11747	47,453
25	gaster mix	JAM	b11747	35,054
26	CAE	JAM	b11747	2179
27	CV	JAM	b11747	5705
58	PG	CON	b11747	1493
31	SI	JAM	b11747	38,249
32	SI	CON	b11747	51,404
33	PN	CON	b11747	4914
34	PG	CON	b11747	7592
35	gaster mix	CON	b11747	18,044
36	CAE	CON	b11747	8044
37	CV	CON	b11747	9663
38	CD	CON	b11747	13,830
39	CT	CON	b11747	6705
41	PG	CON	b11747	10,248
42	gaster mix	CON	b11747	3908
43	CAE	CON	b11747	2450
44	CV	CON	b11747	10,798
47	PN	JAM	b11747	35,530
49	gaster mix	JAM	b11747	70,641
50	CAE	JAM	b11747	7042
52	CD	JAM	b11747	5890
53	CT	JAM	b11747	11,566
55	SI	CON	b11747	16,255
56	SI	JAM	b11747	14,830

Mock.I.lib.1	mock	NA	b11747	40,909
Mock.III.lib.1	mock	NA	b11747	42,724
59	gaster mix	CON	b11748	22,944
60	CAE	CON	b11748	23,585
61	CV	CON	b11748	13,086
62	CD	CON	b11748	3748
64	SI	CON	b11748	121,560
65	PN	JAM	b11748	70,291
71	CT	JAM	b11748	19,925
66	PG	JAM	b11748	39,801
67	gaster mix	JAM	b11748	42,011
68	CAE	JAM	b11748	11,308
69	CV	JAM	b11748	2904
30	SI	JAM	b11748	249
72	SI	JAM	b11748	14,782
73	PN	JAM	b11748	20,459
75	gaster mix	JAM	b11748	35,418
76	CAE	JAM	b11748	963
77	CV	JAM	b11748	16,842
80	SI	JAM	b11748	33,588
87	CT	CON	b11748	3938
82	PG	CON	b11748	30,277
83	gaster mix	CON	b11748	21,820
84	CAE	CON	b11748	9442
85	CV	CON	b11748	12,195
86	CD	CON	b11748	8796
88	PN	CON	b11748	12,024
23	PN	CON	b11748	2031
89	PG	CON	b11748	28,926
90	gaster mix	CON	b11748	39,657
91	CAE	CON	b11748	2897
92	CV	CON	b11748	6269
94	CT	CON	b11748	7849
95	SI	CON	b11748	171,624
96	SI	CON	b11748	204,888
97	SI	JAM	b11748	75,324
51	CV	JAM	b11748	6129
54	SI	CON	b11748	8385
40	PN	CON	b11748	2391
28	CD	JAM	b11748	3336
74	PG	JAM	b11748	90,878
81	PN	CON	b11748	72,616
93	CD	CON	b11748	38,208
70	CD	JAM	b11748	22,195
14	CD	CON	b11748	18,234
15	CT	CON	b11748	24,352
29	CT	JAM	b11748	5134
45	CD	CON	b11748	4582
Mock.I.lib.1	mock	NA	b11748	80,605

Mock.III.lib.1	mock	NA	b11748	60,075
46	CT	CON	b11746	19,802
48	PG	JAM	b11746	102,754
63	CT	CON	b11746	24,430
78	CD	JAM	b11746	12,146
79	CT	JAM	b11746	3710
Mock.I.lib.1	mock	NA	b11746	68,179
Mock.III.lib.1	mock	NA	b11746	54,609

CAE = caecum; CD = colon dorsale; CON = placebo group; CT = colon transversum; CV = colon ventrale; JAM = prebiotic feeding group (Jerusalem artichoke meal); NA = not available (no supplementation); PG = *pars glandularis*; PN = *pars nonglandularis*; SI = small intestine

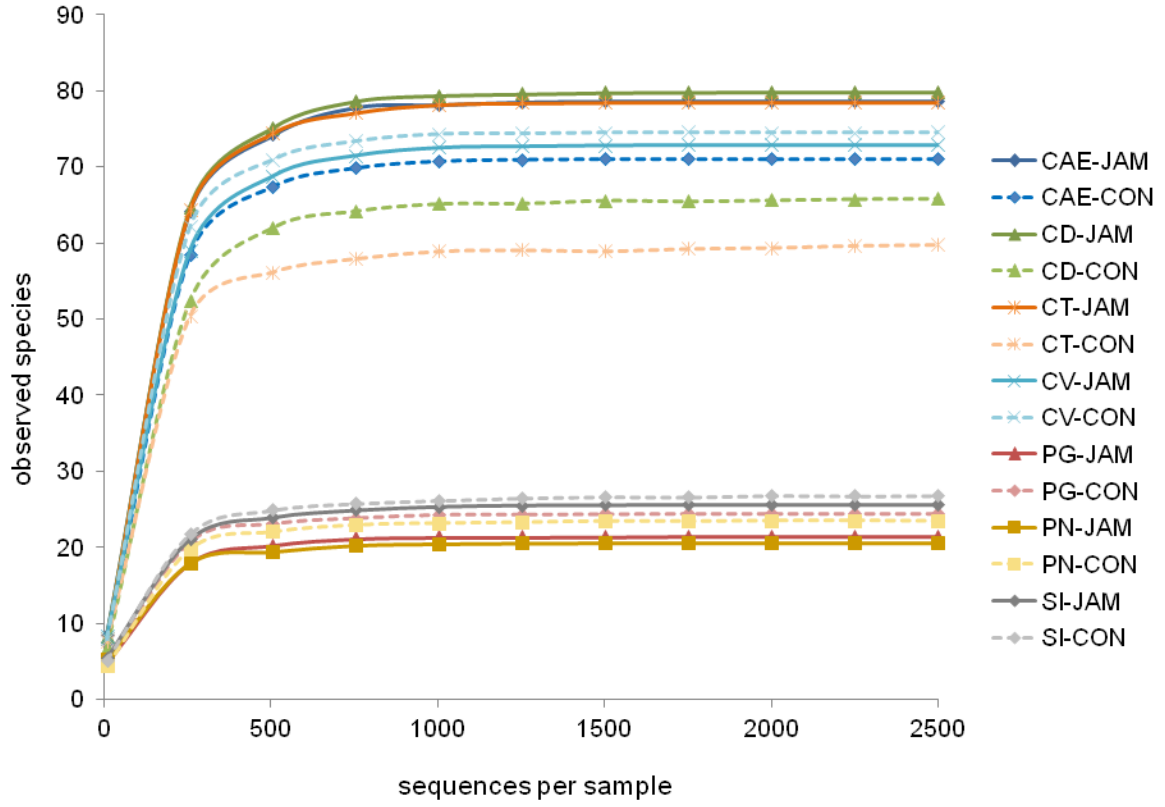

Fig. S2: Rarefraction curve represents adequate number of species after sub sampling of 2,500 reads per sample

The dashed line indicate the CON (placebo) feeding group and the continuous line indicate the JAM (prebiotic) feeding group separated for the different parts of the GIT: CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG =(*pars glandularis*), PN (*pars nonglandularis*) and SI (small intestine).

Table S4: Beta diversity in the different regions of the digestive tract and in relation to the feeding groups

	No	12	3	6	8	9	7	4	2	10	11	12	3	1	6	8	9	4	2	5	10	11
No	part	PN-JAM	PN-JAM	PN-JAM	PN-JAM	PN-JAM	PN-CON	PN-CON	PN-CON	PN-CON	PN-CON	PG-JAM	PG-JAM	PG-JAM	PG-JAM	PG-JAM	PG-JAM	PG-CON	PG-CON	PG-CON	PG-CON	PG-CON
12	PN-JAM	0.000	0.545	0.556	0.417	0.524	0.588	0.529	0.455	0.520	0.615	0.333	0.684	0.692	0.619	0.524	0.600	0.455	0.455	0.760	0.545	0.391
3	PN-JAM	0.545	0.000	0.375	0.364	0.474	0.625	0.467	0.300	0.478	0.500	0.520	0.294	0.417	0.474	0.579	0.444	0.200	0.200	0.478	0.300	0.143
6	PN-JAM	0.556	0.375	0.000	0.444	0.467	0.714	0.091	0.375	0.579	0.600	0.619	0.538	0.500	0.467	0.733	0.429	0.375	0.375	0.579	0.500	0.412
8	PN-JAM	0.417	0.364	0.444	0.000	0.143	0.588	0.412	0.273	0.280	0.308	0.407	0.263	0.538	0.524	0.238	0.200	0.364	0.273	0.360	0.182	0.304
9	PN-JAM	0.524	0.474	0.467	0.143	0.000	0.677	0.429	0.368	0.273	0.391	0.500	0.375	0.565	0.556	0.333	0.176	0.474	0.368	0.364	0.158	0.400
7	PN-CON	0.588	0.625	0.714	0.588	0.677	0.000	0.704	0.625	0.543	0.611	0.568	0.724	0.667	0.548	0.677	0.600	0.625	0.563	0.714	0.625	0.515
4	PN-CON	0.529	0.467	0.091	0.412	0.429	0.704	0.000	0.333	0.556	0.579	0.600	0.500	0.579	0.571	0.714	0.385	0.333	0.333	0.556	0.467	0.375
2	PN-CON	0.455	0.300	0.375	0.273	0.368	0.625	0.333	0.000	0.304	0.417	0.440	0.294	0.500	0.579	0.579	0.333	0.300	0.100	0.391	0.300	0.143
10	PN-CON	0.520	0.478	0.579	0.280	0.273	0.543	0.556	0.304	0.000	0.333	0.571	0.400	0.630	0.545	0.455	0.333	0.478	0.304	0.385	0.217	0.333
11	PN-CON	0.615	0.500	0.600	0.308	0.391	0.611	0.579	0.417	0.333	0.000	0.586	0.333	0.571	0.391	0.478	0.364	0.500	0.417	0.481	0.333	0.440
12	PG-JAM	0.333	0.520	0.619	0.407	0.500	0.568	0.600	0.440	0.571	0.586	0.000	0.545	0.655	0.500	0.583	0.565	0.440	0.440	0.643	0.520	0.385
3	PG-JAM	0.684	0.294	0.538	0.263	0.375	0.724	0.500	0.294	0.400	0.333	0.545	0.000	0.524	0.500	0.500	0.333	0.412	0.294	0.400	0.294	0.333
1	PG-JAM	0.692	0.417	0.500	0.538	0.565	0.667	0.579	0.500	0.630	0.571	0.655	0.524	0.000	0.478	0.826	0.545	0.583	0.583	0.630	0.500	0.520
6	PG-JAM	0.619	0.474	0.467	0.524	0.556	0.548	0.571	0.579	0.545	0.391	0.500	0.500	0.478	0.000	0.667	0.412	0.474	0.579	0.636	0.474	0.500
8	PG-JAM	0.524	0.579	0.733	0.238	0.333	0.677	0.714	0.579	0.455	0.478	0.583	0.500	0.826	0.667	0.000	0.412	0.579	0.474	0.455	0.368	0.500
9	PG-JAM	0.600	0.444	0.429	0.200	0.176	0.600	0.385	0.333	0.333	0.364	0.565	0.333	0.545	0.412	0.412	0.000	0.444	0.333	0.429	0.222	0.368
4	PG-CON	0.455	0.200	0.375	0.364	0.474	0.625	0.333	0.300	0.478	0.500	0.440	0.412	0.583	0.474	0.579	0.444	0.000	0.200	0.391	0.300	0.143
2	PG-CON	0.455	0.200	0.375	0.273	0.368	0.563	0.333	0.100	0.304	0.417	0.440	0.294	0.583	0.579	0.474	0.333	0.200	0.000	0.391	0.300	0.048
5	PG-CON	0.760	0.478	0.579	0.360	0.364	0.714	0.556	0.391	0.385	0.481	0.643	0.400	0.630	0.636	0.455	0.429	0.391	0.391	0.000	0.304	0.417
10	PG-CON	0.545	0.300	0.500	0.182	0.158	0.625	0.467	0.300	0.217	0.333	0.520	0.294	0.500	0.474	0.368	0.222	0.300	0.300	0.304	0.000	0.238
11	PG-CON	0.391	0.143	0.412	0.304	0.400	0.515	0.375	0.143	0.333	0.440	0.385	0.333	0.520	0.500	0.500	0.368	0.143	0.048	0.417	0.238	0.000
3	SI-JAM	0.417	0.273	0.556	0.250	0.238	0.588	0.529	0.182	0.280	0.462	0.333	0.368	0.538	0.524	0.429	0.400	0.273	0.182	0.360	0.182	0.130

6	SI-JAM	0.538	0.417	0.400	0.462	0.478	0.611	0.474	0.417	0.481	0.571	0.448	0.524	0.500	0.391	0.739	0.455	0.417	0.417	0.556	0.417	0.360
8	SI-JAM	0.500	0.615	0.636	0.500	0.440	0.579	0.619	0.538	0.379	0.600	0.548	0.652	0.533	0.600	0.680	0.500	0.538	0.538	0.586	0.385	0.481
9	SI-JAM	0.524	0.474	0.600	0.143	0.111	0.742	0.571	0.368	0.273	0.304	0.500	0.250	0.652	0.556	0.222	0.294	0.474	0.368	0.364	0.158	0.400
12	SI-JAM	0.652	0.524	0.529	0.391	0.500	0.697	0.500	0.429	0.500	0.360	0.538	0.333	0.520	0.400	0.600	0.474	0.429	0.429	0.500	0.429	0.455
10	SI-CON	0.630	0.520	0.619	0.407	0.417	0.568	0.600	0.360	0.357	0.448	0.600	0.455	0.448	0.583	0.583	0.478	0.520	0.360	0.429	0.360	0.385
4	SI-CON	0.385	0.417	0.500	0.385	0.478	0.500	0.474	0.417	0.407	0.571	0.448	0.524	0.571	0.565	0.565	0.545	0.333	0.333	0.556	0.417	0.280
2	SI-CON	0.742	0.517	0.600	0.548	0.643	0.756	0.667	0.586	0.625	0.576	0.647	0.538	0.697	0.643	0.643	0.630	0.586	0.517	0.625	0.586	0.533
5	SI-CON	0.576	0.484	0.704	0.515	0.533	0.581	0.692	0.419	0.412	0.600	0.500	0.571	0.543	0.667	0.667	0.586	0.419	0.419	0.471	0.419	0.375
7	SI-CON	0.636	0.400	0.500	0.273	0.368	0.500	0.467	0.200	0.304	0.333	0.520	0.294	0.500	0.368	0.474	0.222	0.400	0.200	0.391	0.300	0.238
11	SI-CON	0.478	0.524	0.529	0.304	0.400	0.697	0.500	0.429	0.500	0.440	0.462	0.333	0.600	0.500	0.500	0.474	0.429	0.429	0.500	0.429	0.455
11	SI-CON	0.545	0.500	0.500	0.273	0.368	0.750	0.467	0.400	0.478	0.417	0.440	0.294	0.583	0.474	0.474	0.444	0.400	0.400	0.478	0.400	0.429
3	CAE-JAM	0.814	0.756	0.838	0.628	0.700	0.509	0.889	0.756	0.682	0.689	0.696	0.737	0.822	0.700	0.650	0.744	0.805	0.756	0.727	0.707	0.762
6	CAE-JAM	0.805	0.641	0.714	0.561	0.632	0.529	0.765	0.641	0.571	0.628	0.682	0.667	0.721	0.684	0.684	0.676	0.692	0.641	0.619	0.590	0.650
8	CAE-JAM	0.730	0.771	0.806	0.514	0.588	0.574	0.800	0.714	0.579	0.641	0.650	0.750	0.795	0.706	0.588	0.636	0.714	0.714	0.684	0.600	0.722
4	CAE-CON	0.773	0.667	0.737	0.545	0.610	0.444	0.784	0.667	0.600	0.652	0.660	0.692	0.739	0.707	0.659	0.700	0.714	0.667	0.644	0.619	0.674
2	CAE-CON	0.870	0.727	0.800	0.652	0.721	0.464	0.846	0.727	0.660	0.708	0.755	0.756	0.792	0.721	0.721	0.714	0.773	0.727	0.702	0.682	0.733
7	CAE-CON	0.810	0.700	0.778	0.619	0.692	0.462	0.829	0.700	0.628	0.636	0.689	0.676	0.773	0.692	0.692	0.737	0.750	0.700	0.674	0.650	0.707
10	CAE-CON	0.707	0.692	0.771	0.512	0.579	0.451	0.824	0.692	0.571	0.535	0.682	0.667	0.721	0.632	0.579	0.676	0.744	0.692	0.667	0.590	0.700
11	CAE-CON	0.750	0.667	0.692	0.563	0.655	0.476	0.760	0.667	0.576	0.588	0.657	0.630	0.765	0.586	0.655	0.714	0.667	0.667	0.636	0.600	0.677
12	CV-JAM	0.682	0.667	0.737	0.500	0.610	0.407	0.784	0.667	0.600	0.609	0.660	0.692	0.696	0.659	0.610	0.600	0.714	0.667	0.689	0.619	0.674
3	CV-JAM	0.667	0.529	0.600	0.389	0.515	0.391	0.655	0.529	0.514	0.526	0.538	0.548	0.632	0.576	0.576	0.563	0.588	0.529	0.568	0.529	0.543
1	CV-JAM	0.784	0.600	0.677	0.514	0.588	0.447	0.733	0.600	0.526	0.590	0.700	0.625	0.692	0.647	0.647	0.636	0.657	0.600	0.579	0.543	0.611
6	CV-JAM	0.822	0.674	0.744	0.600	0.714	0.455	0.789	0.674	0.652	0.660	0.708	0.700	0.745	0.667	0.714	0.659	0.721	0.674	0.696	0.674	0.682
8	CV-JAM	0.700	0.789	0.824	0.600	0.676	0.480	0.879	0.789	0.659	0.667	0.674	0.771	0.810	0.676	0.622	0.722	0.789	0.789	0.756	0.684	0.795
9	CV-JAM	0.636	0.613	0.704	0.455	0.533	0.488	0.769	0.677	0.529	0.543	0.611	0.643	0.771	0.667	0.467	0.655	0.677	0.613	0.647	0.548	0.625

4	CV-CON	0.722	0.588	0.667	0.500	0.576	0.391	0.724	0.647	0.568	0.632	0.641	0.677	0.737	0.697	0.576	0.688	0.647	0.588	0.622	0.588	0.600
2	CV-CON	0.864	0.714	0.789	0.636	0.707	0.481	0.838	0.714	0.644	0.696	0.745	0.744	0.783	0.707	0.707	0.700	0.762	0.714	0.689	0.667	0.721
5	CV-CON	0.805	0.641	0.714	0.561	0.632	0.412	0.765	0.641	0.571	0.628	0.682	0.667	0.721	0.684	0.684	0.676	0.692	0.641	0.619	0.590	0.650
7	CV-CON	0.730	0.657	0.742	0.568	0.647	0.362	0.800	0.714	0.632	0.641	0.650	0.688	0.795	0.706	0.588	0.758	0.714	0.657	0.684	0.657	0.667
10	CV-CON	0.850	0.737	0.824	0.650	0.730	0.440	0.879	0.737	0.659	0.619	0.721	0.714	0.762	0.622	0.676	0.722	0.789	0.737	0.707	0.684	0.744
11	CV-CON	0.771	0.697	0.724	0.600	0.688	0.422	0.786	0.697	0.611	0.622	0.632	0.667	0.784	0.625	0.688	0.742	0.697	0.697	0.667	0.636	0.706
12	CD-JAM	0.810	0.700	0.778	0.571	0.641	0.462	0.829	0.700	0.628	0.682	0.689	0.730	0.773	0.692	0.641	0.684	0.750	0.700	0.674	0.650	0.707
3	CD-JAM	0.778	0.674	0.744	0.556	0.619	0.491	0.789	0.674	0.609	0.660	0.667	0.700	0.745	0.714	0.667	0.707	0.721	0.674	0.652	0.628	0.682
1	CD-JAM	0.850	0.737	0.824	0.650	0.730	0.520	0.879	0.737	0.659	0.667	0.767	0.714	0.810	0.676	0.676	0.722	0.789	0.737	0.707	0.684	0.744
6	CD-JAM	0.830	0.733	0.805	0.617	0.682	0.544	0.800	0.689	0.625	0.673	0.720	0.714	0.796	0.773	0.727	0.721	0.733	0.689	0.667	0.644	0.696
8	CD-JAM	0.684	0.667	0.750	0.474	0.543	0.500	0.806	0.667	0.538	0.550	0.610	0.636	0.750	0.657	0.543	0.647	0.722	0.667	0.641	0.556	0.676
9	CD-JAM	0.714	0.750	0.778	0.571	0.641	0.538	0.829	0.750	0.628	0.591	0.644	0.730	0.773	0.641	0.641	0.737	0.750	0.750	0.721	0.650	0.756
4	CD-CON	0.805	0.692	0.771	0.610	0.684	0.490	0.824	0.692	0.619	0.581	0.682	0.667	0.721	0.632	0.684	0.730	0.744	0.692	0.667	0.641	0.700
2	CD-CON	0.826	0.682	0.750	0.609	0.674	0.464	0.795	0.682	0.617	0.667	0.714	0.707	0.750	0.721	0.721	0.714	0.727	0.682	0.660	0.636	0.689
5	CD-CON	0.857	0.750	0.833	0.667	0.744	0.577	0.886	0.750	0.674	0.682	0.733	0.730	0.818	0.692	0.692	0.737	0.800	0.750	0.721	0.700	0.756
7	CD-CON	0.800	0.684	0.765	0.600	0.676	0.400	0.818	0.684	0.610	0.619	0.674	0.657	0.762	0.676	0.676	0.722	0.737	0.684	0.659	0.632	0.692
10	CD-CON	0.600	0.571	0.667	0.333	0.407	0.450	0.739	0.571	0.419	0.375	0.576	0.520	0.625	0.481	0.407	0.538	0.643	0.571	0.548	0.429	0.586
11	CD-CON	0.771	0.636	0.724	0.543	0.625	0.556	0.786	0.636	0.556	0.514	0.632	0.600	0.676	0.563	0.625	0.677	0.697	0.636	0.611	0.576	0.647
12	CT-JAM	0.800	0.684	0.765	0.600	0.676	0.480	0.818	0.684	0.610	0.619	0.674	0.657	0.762	0.676	0.676	0.722	0.737	0.684	0.659	0.632	0.692
3	CT-JAM	0.758	0.677	0.704	0.576	0.667	0.488	0.769	0.742	0.647	0.714	0.667	0.786	0.829	0.667	0.600	0.724	0.677	0.677	0.706	0.677	0.688
1	CT-JAM	0.850	0.789	0.824	0.700	0.784	0.520	0.879	0.789	0.707	0.714	0.721	0.771	0.810	0.676	0.730	0.778	0.789	0.789	0.756	0.737	0.795
6	CT-JAM	0.867	0.721	0.795	0.644	0.714	0.491	0.842	0.721	0.652	0.702	0.750	0.750	0.787	0.714	0.714	0.707	0.767	0.721	0.696	0.674	0.727
8	CT-JAM	0.737	0.722	0.813	0.579	0.657	0.500	0.871	0.778	0.641	0.650	0.707	0.758	0.850	0.714	0.543	0.706	0.778	0.722	0.744	0.667	0.730
9	CT-JAM	0.714	0.750	0.778	0.571	0.641	0.500	0.829	0.750	0.628	0.591	0.644	0.730	0.773	0.641	0.641	0.737	0.750	0.750	0.721	0.650	0.756
4	CT-CON	0.730	0.657	0.742	0.514	0.588	0.447	0.800	0.657	0.579	0.538	0.600	0.625	0.692	0.588	0.588	0.697	0.714	0.657	0.632	0.600	0.667
2	CT-CON	0.842	0.667	0.750	0.632	0.714	0.375	0.806	0.722	0.641	0.700	0.756	0.758	0.800	0.714	0.657	0.706	0.722	0.667	0.692	0.667	0.676

5	CT-CON	0.857	0.800	0.833	0.714	0.744	0.500	0.886	0.800	0.721	0.682	0.733	0.784	0.727	0.641	0.744	0.737	0.850	0.800	0.767	0.750	0.805
7	CT-CON	0.765	0.750	0.786	0.647	0.742	0.227	0.778	0.750	0.714	0.611	0.676	0.724	0.778	0.613	0.677	0.667	0.750	0.688	0.771	0.750	0.697
11	CT-CON	0.850	0.737	0.824	0.650	0.730	0.440	0.879	0.737	0.659	0.667	0.721	0.714	0.810	0.676	0.676	0.722	0.789	0.737	0.707	0.684	0.744
10	CT-CON	0.860	0.756	0.838	0.674	0.750	0.396	0.889	0.756	0.682	0.644	0.739	0.737	0.733	0.650	0.700	0.744	0.805	0.756	0.727	0.707	0.762
	No	3	6	8	9	12	10	4	2	5	7	11	11	3	6	8	4	2	7	10	11	3
No	part	SI-JAM	SI-JAM	SI-JAM	SI-JAM	SI-JAM	SI-CON	SI-CON	SI-CON	SI-CON	SI-CON	SI-CON	SI-CON	CAE-JAM	CAE-JAM	CAE-JAM	CAE-CON	CAE-CON	CAE-CON	CAE-CON	CAE-CON	SI-JAM
12	PN-JAM	0.417	0.538	0.500	0.524	0.652	0.630	0.385	0.742	0.576	0.636	0.478	0.545	0.814	0.805	0.730	0.773	0.870	0.810	0.707	0.750	0.417
3	PN-JAM	0.273	0.417	0.615	0.474	0.524	0.520	0.417	0.517	0.484	0.400	0.524	0.500	0.756	0.641	0.771	0.667	0.727	0.700	0.692	0.667	0.273
6	PN-JAM	0.556	0.400	0.636	0.600	0.529	0.619	0.500	0.600	0.704	0.500	0.529	0.500	0.838	0.714	0.806	0.737	0.800	0.778	0.771	0.692	0.556
8	PN-JAM	0.250	0.462	0.500	0.143	0.391	0.407	0.385	0.548	0.515	0.273	0.304	0.273	0.628	0.561	0.514	0.545	0.652	0.619	0.512	0.563	0.250
9	PN-JAM	0.238	0.478	0.440	0.111	0.500	0.417	0.478	0.643	0.533	0.368	0.400	0.368	0.700	0.632	0.588	0.610	0.721	0.692	0.579	0.655	0.238
7	PN-CON	0.588	0.611	0.579	0.742	0.697	0.568	0.500	0.756	0.581	0.500	0.697	0.750	0.509	0.529	0.574	0.444	0.464	0.462	0.451	0.476	0.588
4	PN-CON	0.529	0.474	0.619	0.571	0.500	0.600	0.474	0.667	0.692	0.467	0.500	0.467	0.889	0.765	0.800	0.784	0.846	0.829	0.824	0.760	0.529
2	PN-CON	0.182	0.417	0.538	0.368	0.429	0.360	0.417	0.586	0.419	0.200	0.429	0.400	0.756	0.641	0.714	0.667	0.727	0.700	0.692	0.667	0.182
10	PN-CON	0.280	0.481	0.379	0.273	0.500	0.357	0.407	0.625	0.412	0.304	0.500	0.478	0.682	0.571	0.579	0.600	0.660	0.628	0.571	0.576	0.280
11	PN-CON	0.462	0.571	0.600	0.304	0.360	0.448	0.571	0.576	0.600	0.333	0.440	0.417	0.689	0.628	0.641	0.652	0.708	0.636	0.535	0.588	0.462
12	PG-JAM	0.333	0.448	0.548	0.500	0.538	0.600	0.448	0.647	0.500	0.520	0.462	0.440	0.696	0.682	0.650	0.660	0.755	0.689	0.682	0.657	0.333
3	PG-JAM	0.368	0.524	0.652	0.250	0.333	0.455	0.524	0.538	0.571	0.294	0.333	0.294	0.737	0.667	0.750	0.692	0.756	0.676	0.667	0.630	0.368
1	PG-JAM	0.538	0.500	0.533	0.652	0.520	0.448	0.571	0.697	0.543	0.500	0.600	0.583	0.822	0.721	0.795	0.739	0.792	0.773	0.721	0.765	0.538
6	PG-JAM	0.524	0.391	0.600	0.556	0.400	0.583	0.565	0.643	0.667	0.368	0.500	0.474	0.700	0.684	0.706	0.707	0.721	0.692	0.632	0.586	0.524
8	PG-JAM	0.429	0.739	0.680	0.222	0.600	0.583	0.565	0.643	0.667	0.474	0.500	0.474	0.650	0.684	0.588	0.659	0.721	0.692	0.579	0.655	0.429
9	PG-JAM	0.400	0.455	0.500	0.294	0.474	0.478	0.545	0.630	0.586	0.222	0.474	0.444	0.744	0.676	0.636	0.700	0.714	0.737	0.676	0.714	0.400
4	PG-CON	0.273	0.417	0.538	0.474	0.429	0.520	0.333	0.586	0.419	0.400	0.429	0.400	0.805	0.692	0.714	0.714	0.773	0.750	0.744	0.667	0.273
2	PG-CON	0.182	0.417	0.538	0.368	0.429	0.360	0.333	0.517	0.419	0.200	0.429	0.400	0.756	0.641	0.714	0.667	0.727	0.700	0.692	0.667	0.182
5	PG-CON	0.360	0.556	0.586	0.364	0.500	0.429	0.556	0.625	0.471	0.391	0.500	0.478	0.727	0.619	0.684	0.644	0.702	0.674	0.667	0.636	0.360
10	PG-CON	0.182	0.417	0.385	0.158	0.429	0.360	0.417	0.586	0.419	0.300	0.429	0.400	0.707	0.590	0.600	0.619	0.682	0.650	0.590	0.600	0.182
11	PG-CON	0.130	0.360	0.481	0.400	0.455	0.385	0.280	0.533	0.375	0.238	0.455	0.429	0.762	0.650	0.722	0.674	0.733	0.707	0.700	0.677	0.130
3	SI-JAM	0.000	0.385	0.429	0.238	0.478	0.333	0.308	0.613	0.333	0.273	0.391	0.364	0.674	0.610	0.622	0.591	0.696	0.667	0.610	0.625	0.000
6	SI-JAM	0.385	0.000	0.467	0.565	0.520	0.517	0.429	0.455	0.486	0.417	0.520	0.500	0.778	0.674	0.744	0.696	0.750	0.727	0.721	0.647	0.385
8	SI-JAM	0.429	0.467	0.000	0.520	0.556	0.290	0.267	0.714	0.297	0.538	0.407	0.462	0.830	0.733	0.610	0.750	0.800	0.783	0.733	0.722	0.429

9	SI-JAM	0.238	0.565	0.520	0.000	0.400	0.417	0.478	0.571	0.533	0.368	0.300	0.263	0.650	0.632	0.588	0.610	0.721	0.641	0.526	0.586	0.238
12	SI-JAM	0.478	0.520	0.556	0.400	0.000	0.308	0.440	0.600	0.563	0.333	0.273	0.238	0.762	0.700	0.667	0.721	0.778	0.707	0.650	0.613	0.478
10	SI-CON	0.333	0.517	0.290	0.417	0.308	0.000	0.310	0.647	0.278	0.280	0.308	0.360	0.739	0.636	0.600	0.660	0.714	0.689	0.636	0.657	0.333
4	SI-CON	0.308	0.429	0.267	0.478	0.440	0.310	0.000	0.636	0.314	0.417	0.200	0.250	0.778	0.721	0.590	0.696	0.792	0.773	0.721	0.706	0.308
2	SI-CON	0.613	0.455	0.714	0.571	0.600	0.647	0.636	0.000	0.650	0.586	0.600	0.586	0.680	0.667	0.818	0.647	0.698	0.633	0.667	0.590	0.613
5	SI-CON	0.333	0.486	0.297	0.533	0.563	0.278	0.314	0.650	0.000	0.484	0.438	0.484	0.769	0.680	0.652	0.698	0.745	0.725	0.720	0.707	0.333
7	SI-CON	0.273	0.417	0.538	0.368	0.333	0.280	0.417	0.586	0.484	0.000	0.429	0.400	0.707	0.641	0.657	0.667	0.682	0.700	0.641	0.667	0.273
11	SI-CON	0.391	0.520	0.407	0.300	0.273	0.308	0.200	0.600	0.438	0.429	0.000	0.048	0.714	0.700	0.556	0.674	0.778	0.707	0.650	0.613	0.391
11	SI-CON	0.364	0.500	0.462	0.263	0.238	0.360	0.250	0.586	0.484	0.400	0.048	0.000	0.707	0.692	0.543	0.667	0.773	0.700	0.641	0.600	0.364
3	CAE-JAM	0.674	0.778	0.830	0.650	0.762	0.739	0.778	0.680	0.769	0.707	0.714	0.707	0.000	0.200	0.357	0.111	0.169	0.180	0.233	0.294	0.674
6	CAE-JAM	0.610	0.674	0.733	0.632	0.700	0.636	0.721	0.667	0.680	0.641	0.700	0.692	0.200	0.000	0.370	0.180	0.238	0.220	0.276	0.347	0.610
8	CAE-JAM	0.622	0.744	0.610	0.588	0.667	0.600	0.590	0.818	0.652	0.657	0.556	0.543	0.357	0.370	0.000	0.333	0.356	0.273	0.370	0.467	0.622
4	CAE-CON	0.591	0.696	0.750	0.610	0.721	0.660	0.696	0.647	0.698	0.667	0.674	0.667	0.111	0.180	0.333	0.000	0.182	0.129	0.148	0.231	0.591
2	CAE-CON	0.696	0.750	0.800	0.721	0.778	0.714	0.792	0.698	0.745	0.682	0.778	0.773	0.169	0.238	0.356	0.182	0.000	0.156	0.270	0.370	0.696
7	CAE-CON	0.667	0.727	0.783	0.641	0.707	0.689	0.773	0.633	0.725	0.700	0.707	0.700	0.180	0.220	0.273	0.129	0.156	0.000	0.186	0.240	0.667
10	CAE-CON	0.610	0.721	0.733	0.526	0.650	0.636	0.721	0.667	0.720	0.641	0.650	0.641	0.233	0.276	0.370	0.148	0.270	0.186	0.000	0.306	0.610
11	CAE-CON	0.625	0.647	0.722	0.586	0.613	0.657	0.706	0.590	0.707	0.667	0.613	0.600	0.294	0.347	0.467	0.231	0.370	0.240	0.306	0.000	0.625
12	CV-JAM	0.636	0.696	0.625	0.610	0.674	0.574	0.565	0.686	0.623	0.619	0.535	0.571	0.302	0.377	0.263	0.250	0.333	0.290	0.311	0.385	0.636
3	CV-JAM	0.500	0.579	0.700	0.515	0.600	0.590	0.579	0.535	0.644	0.529	0.543	0.529	0.345	0.358	0.469	0.250	0.414	0.333	0.358	0.227	0.500
1	CV-JAM	0.568	0.641	0.707	0.588	0.667	0.600	0.692	0.636	0.652	0.600	0.667	0.657	0.321	0.296	0.440	0.263	0.288	0.273	0.333	0.244	0.568
6	CV-JAM	0.689	0.702	0.796	0.714	0.727	0.708	0.745	0.654	0.741	0.628	0.727	0.721	0.125	0.161	0.345	0.108	0.164	0.175	0.258	0.321	0.689
8	CV-JAM	0.700	0.762	0.636	0.622	0.692	0.628	0.619	0.787	0.673	0.737	0.538	0.579	0.356	0.368	0.208	0.300	0.387	0.310	0.298	0.375	0.700
9	CV-JAM	0.576	0.714	0.730	0.467	0.688	0.667	0.657	0.650	0.714	0.677	0.625	0.613	0.385	0.440	0.391	0.321	0.455	0.333	0.360	0.366	0.576
4	CV-CON	0.556	0.684	0.750	0.576	0.714	0.641	0.632	0.628	0.689	0.647	0.657	0.647	0.200	0.321	0.429	0.143	0.310	0.296	0.245	0.318	0.556
2	CV-CON	0.682	0.739	0.792	0.707	0.767	0.702	0.783	0.686	0.736	0.667	0.767	0.762	0.206	0.246	0.368	0.188	0.091	0.194	0.279	0.385	0.682
5	CV-CON	0.610	0.674	0.733	0.632	0.700	0.636	0.721	0.708	0.680	0.641	0.700	0.692	0.267	0.241	0.370	0.213	0.270	0.220	0.310	0.347	0.610
7	CV-CON	0.622	0.744	0.805	0.588	0.722	0.700	0.692	0.682	0.739	0.714	0.667	0.657	0.286	0.296	0.320	0.193	0.322	0.164	0.222	0.244	0.622
10	CV-CON	0.700	0.762	0.818	0.676	0.692	0.674	0.810	0.745	0.755	0.632	0.744	0.737	0.356	0.404	0.547	0.367	0.355	0.379	0.368	0.458	0.700

11	CV-CON	0.657	0.676	0.744	0.625	0.647	0.684	0.730	0.714	0.727	0.697	0.647	0.636	0.333	0.269	0.333	0.236	0.298	0.170	0.231	0.209	0.657
12	CD-JAM	0.619	0.727	0.783	0.641	0.756	0.689	0.727	0.673	0.725	0.650	0.707	0.700	0.180	0.288	0.273	0.129	0.250	0.167	0.288	0.280	0.619
3	CD-JAM	0.600	0.702	0.755	0.619	0.727	0.667	0.702	0.654	0.704	0.674	0.682	0.674	0.125	0.226	0.345	0.108	0.194	0.175	0.258	0.283	0.600
1	CD-JAM	0.700	0.762	0.818	0.676	0.744	0.721	0.810	0.702	0.755	0.684	0.744	0.737	0.186	0.333	0.434	0.200	0.258	0.241	0.263	0.333	0.700
6	CD-JAM	0.660	0.755	0.765	0.682	0.739	0.680	0.755	0.741	0.714	0.689	0.739	0.733	0.333	0.281	0.400	0.284	0.275	0.292	0.406	0.491	0.660
8	CD-JAM	0.579	0.700	0.667	0.486	0.676	0.610	0.650	0.689	0.660	0.667	0.568	0.556	0.333	0.345	0.294	0.276	0.400	0.286	0.309	0.391	0.579
9	CD-JAM	0.667	0.727	0.739	0.590	0.659	0.689	0.727	0.755	0.765	0.700	0.659	0.650	0.410	0.458	0.382	0.355	0.406	0.333	0.390	0.440	0.667
4	CD-CON	0.659	0.721	0.778	0.632	0.650	0.636	0.767	0.708	0.720	0.641	0.700	0.692	0.300	0.345	0.370	0.279	0.333	0.254	0.345	0.347	0.659
2	CD-CON	0.652	0.708	0.760	0.674	0.733	0.673	0.750	0.660	0.709	0.682	0.733	0.727	0.138	0.206	0.322	0.121	0.088	0.125	0.238	0.296	0.652
5	CD-CON	0.714	0.773	0.826	0.692	0.756	0.733	0.818	0.755	0.765	0.700	0.756	0.750	0.410	0.424	0.491	0.387	0.344	0.367	0.458	0.520	0.714
7	CD-CON	0.650	0.714	0.773	0.622	0.692	0.674	0.762	0.660	0.714	0.684	0.692	0.684	0.288	0.298	0.358	0.200	0.290	0.138	0.263	0.208	0.650
10	CD-CON	0.467	0.625	0.647	0.333	0.517	0.515	0.625	0.622	0.641	0.500	0.517	0.500	0.429	0.404	0.395	0.400	0.500	0.375	0.234	0.316	0.467
11	CD-CON	0.600	0.676	0.744	0.563	0.588	0.579	0.730	0.571	0.682	0.576	0.647	0.636	0.407	0.385	0.542	0.382	0.439	0.358	0.385	0.349	0.600
12	CT-JAM	0.650	0.714	0.773	0.622	0.692	0.674	0.762	0.702	0.714	0.684	0.692	0.684	0.356	0.368	0.472	0.333	0.387	0.310	0.404	0.292	0.650
3	CT-JAM	0.636	0.714	0.784	0.667	0.750	0.722	0.657	0.750	0.762	0.677	0.688	0.677	0.346	0.400	0.348	0.321	0.418	0.412	0.440	0.415	0.636
1	CT-JAM	0.750	0.762	0.773	0.730	0.692	0.721	0.762	0.787	0.755	0.737	0.692	0.684	0.390	0.439	0.396	0.433	0.290	0.345	0.439	0.458	0.750
6	CT-JAM	0.689	0.745	0.796	0.714	0.773	0.708	0.787	0.769	0.741	0.674	0.773	0.767	0.313	0.258	0.414	0.262	0.224	0.270	0.355	0.434	0.689
8	CT-JAM	0.684	0.800	0.810	0.600	0.784	0.756	0.750	0.733	0.787	0.722	0.730	0.722	0.368	0.418	0.333	0.345	0.433	0.357	0.345	0.391	0.684
9	CT-JAM	0.667	0.727	0.739	0.590	0.659	0.689	0.727	0.755	0.765	0.700	0.659	0.650	0.410	0.458	0.418	0.355	0.406	0.367	0.322	0.440	0.667
4	CT-CON	0.568	0.692	0.756	0.529	0.611	0.600	0.692	0.682	0.696	0.600	0.611	0.600	0.321	0.370	0.440	0.298	0.390	0.345	0.333	0.289	0.568
2	CT-CON	0.684	0.750	0.810	0.714	0.784	0.707	0.750	0.733	0.745	0.667	0.784	0.778	0.333	0.382	0.333	0.276	0.267	0.250	0.345	0.348	0.684
5	CT-CON	0.762	0.773	0.739	0.744	0.707	0.644	0.773	0.796	0.725	0.700	0.707	0.700	0.443	0.458	0.527	0.419	0.406	0.433	0.458	0.520	0.762
7	CT-CON	0.765	0.778	0.842	0.742	0.697	0.730	0.778	0.756	0.814	0.625	0.758	0.750	0.434	0.451	0.489	0.370	0.429	0.346	0.333	0.429	0.765
11	CT-CON	0.700	0.762	0.818	0.676	0.744	0.721	0.810	0.745	0.755	0.684	0.744	0.737	0.322	0.368	0.472	0.367	0.290	0.345	0.404	0.375	0.700
10	CT-CON	0.721	0.778	0.745	0.700	0.667	0.609	0.733	0.760	0.692	0.659	0.667	0.659	0.387	0.400	0.429	0.333	0.385	0.344	0.333	0.373	0.721
	No	12	3	1	6	8	9	4	2	5	7	10	11	12	3	1	6	8	9	4	2	5
No	part	CV-JAM	CV-JAM	CV-JAM	CV-JAM	CV-JAM	CV-JAM	CV-CON	CV-CON	CV-CON	CV-CON	CV-CON	CV-CON	CD-JAM	CD-JAM	CD-JAM	CD-JAM	CD-JAM	CD-JAM	CD-CON	CD-CON	CD-CON
12	PN-JAM	0.682	0.667	0.784	0.822	0.700	0.636	0.722	0.864	0.805	0.730	0.850	0.771	0.810	0.778	0.850	0.830	0.684	0.714	0.805	0.826	0.857
3	PN-JAM	0.667	0.529	0.600	0.674	0.789	0.613	0.588	0.714	0.641	0.657	0.737	0.697	0.700	0.674	0.737	0.733	0.667	0.750	0.692	0.682	0.750
6	PN-JAM	0.737	0.600	0.677	0.744	0.824	0.704	0.667	0.789	0.714	0.742	0.824	0.724	0.778	0.744	0.824	0.805	0.750	0.778	0.771	0.750	0.833
8	PN-JAM	0.500	0.389	0.514	0.600	0.600	0.455	0.500	0.636	0.561	0.568	0.650	0.600	0.571	0.556	0.650	0.617	0.474	0.571	0.610	0.609	0.667

9	PN-JAM	0.610	0.515	0.588	0.714	0.676	0.533	0.576	0.707	0.632	0.647	0.730	0.688	0.641	0.619	0.730	0.682	0.543	0.641	0.684	0.674	0.744
7	PN-CON	0.407	0.391	0.447	0.455	0.480	0.488	0.391	0.481	0.412	0.362	0.440	0.422	0.462	0.491	0.520	0.544	0.500	0.538	0.490	0.464	0.577
4	PN-CON	0.784	0.655	0.733	0.789	0.879	0.769	0.724	0.838	0.765	0.800	0.879	0.786	0.829	0.789	0.879	0.800	0.806	0.829	0.824	0.795	0.886
2	PN-CON	0.667	0.529	0.600	0.674	0.789	0.677	0.647	0.714	0.641	0.714	0.737	0.697	0.700	0.674	0.737	0.689	0.667	0.750	0.692	0.682	0.750
10	PN-CON	0.600	0.514	0.526	0.652	0.659	0.529	0.568	0.644	0.571	0.632	0.659	0.611	0.628	0.609	0.659	0.625	0.538	0.628	0.619	0.617	0.674
11	PN-CON	0.609	0.526	0.590	0.660	0.667	0.543	0.632	0.696	0.628	0.641	0.619	0.622	0.682	0.660	0.667	0.673	0.550	0.591	0.581	0.667	0.682
12	PG-JAM	0.660	0.538	0.700	0.708	0.674	0.611	0.641	0.745	0.682	0.650	0.721	0.632	0.689	0.667	0.767	0.720	0.610	0.644	0.682	0.714	0.733
3	PG-JAM	0.692	0.548	0.625	0.700	0.771	0.643	0.677	0.744	0.667	0.688	0.714	0.667	0.730	0.700	0.714	0.714	0.636	0.730	0.667	0.707	0.730
1	PG-JAM	0.696	0.632	0.692	0.745	0.810	0.771	0.737	0.783	0.721	0.795	0.762	0.784	0.773	0.745	0.810	0.796	0.750	0.773	0.721	0.750	0.818
6	PG-JAM	0.659	0.576	0.647	0.667	0.676	0.667	0.697	0.707	0.684	0.706	0.622	0.625	0.692	0.714	0.676	0.773	0.657	0.641	0.632	0.721	0.692
8	PG-JAM	0.610	0.576	0.647	0.714	0.622	0.467	0.576	0.707	0.684	0.588	0.676	0.688	0.641	0.667	0.676	0.727	0.543	0.641	0.684	0.721	0.692
9	PG-JAM	0.600	0.563	0.636	0.659	0.722	0.655	0.688	0.700	0.676	0.758	0.722	0.742	0.684	0.707	0.722	0.721	0.647	0.737	0.730	0.714	0.737
4	PG-CON	0.714	0.588	0.657	0.721	0.789	0.677	0.647	0.762	0.692	0.714	0.789	0.697	0.750	0.721	0.789	0.733	0.722	0.750	0.744	0.727	0.800
2	PG-CON	0.667	0.529	0.600	0.674	0.789	0.613	0.588	0.714	0.641	0.657	0.737	0.697	0.700	0.674	0.737	0.689	0.667	0.750	0.692	0.682	0.750
5	PG-CON	0.689	0.568	0.579	0.696	0.756	0.647	0.622	0.689	0.619	0.684	0.707	0.667	0.674	0.652	0.707	0.667	0.641	0.721	0.667	0.660	0.721
10	PG-CON	0.619	0.529	0.543	0.674	0.684	0.548	0.588	0.667	0.590	0.657	0.684	0.636	0.650	0.628	0.684	0.644	0.556	0.650	0.641	0.636	0.700
11	PG-CON	0.674	0.543	0.611	0.682	0.795	0.625	0.600	0.721	0.650	0.667	0.744	0.706	0.707	0.682	0.744	0.696	0.676	0.756	0.700	0.689	0.756
3	SI-JAM	0.636	0.500	0.568	0.689	0.700	0.576	0.556	0.682	0.610	0.622	0.700	0.657	0.619	0.600	0.700	0.660	0.579	0.667	0.659	0.652	0.714
6	SI-JAM	0.696	0.579	0.641	0.702	0.762	0.714	0.684	0.739	0.674	0.744	0.762	0.676	0.727	0.702	0.762	0.755	0.700	0.727	0.721	0.708	0.773
8	SI-JAM	0.625	0.700	0.707	0.796	0.636	0.730	0.750	0.792	0.733	0.805	0.818	0.744	0.783	0.755	0.818	0.765	0.667	0.739	0.778	0.760	0.826
9	SI-JAM	0.610	0.515	0.588	0.714	0.622	0.467	0.576	0.707	0.632	0.588	0.676	0.625	0.641	0.619	0.676	0.682	0.486	0.590	0.632	0.674	0.692
12	SI-JAM	0.674	0.600	0.667	0.727	0.692	0.688	0.714	0.767	0.700	0.722	0.692	0.647	0.756	0.727	0.744	0.739	0.676	0.659	0.650	0.733	0.756
10	SI-CON	0.574	0.590	0.600	0.708	0.628	0.667	0.641	0.702	0.636	0.700	0.674	0.684	0.689	0.667	0.721	0.680	0.610	0.689	0.636	0.673	0.733
4	SI-CON	0.565	0.579	0.692	0.745	0.619	0.657	0.632	0.783	0.721	0.692	0.810	0.730	0.727	0.702	0.810	0.755	0.650	0.727	0.767	0.750	0.818
2	SI-CON	0.686	0.535	0.636	0.654	0.787	0.650	0.628	0.686	0.708	0.682	0.745	0.714	0.673	0.654	0.702	0.741	0.689	0.755	0.708	0.660	0.755
5	SI-CON	0.623	0.644	0.652	0.741	0.673	0.714	0.689	0.736	0.680	0.739	0.755	0.727	0.725	0.704	0.755	0.714	0.660	0.765	0.720	0.709	0.765
7	SI-CON	0.619	0.529	0.600	0.628	0.737	0.677	0.647	0.667	0.641	0.714	0.632	0.697	0.650	0.674	0.684	0.689	0.667	0.700	0.641	0.682	0.700
11	SI-CON	0.535	0.543	0.667	0.727	0.538	0.625	0.657	0.767	0.700	0.667	0.744	0.647	0.707	0.682	0.744	0.739	0.568	0.659	0.700	0.733	0.756
11	SI-CON	0.571	0.529	0.657	0.721	0.579	0.613	0.647	0.762	0.692	0.657	0.737	0.636	0.700	0.674	0.737	0.733	0.556	0.650	0.692	0.727	0.750
3	CAE-JAM	0.302	0.345	0.321	0.125	0.356	0.385	0.200	0.206	0.267	0.286	0.356	0.333	0.180	0.125	0.186	0.333	0.333	0.410	0.300	0.138	0.410
6	CAE-JAM	0.377	0.358	0.296	0.161	0.368	0.440	0.321	0.246	0.241	0.296	0.404	0.269	0.288	0.226	0.333	0.281	0.345	0.458	0.345	0.206	0.424

8	CAE-JAM	0.263	0.469	0.440	0.345	0.208	0.391	0.429	0.368	0.370	0.320	0.547	0.333	0.273	0.345	0.434	0.400	0.294	0.382	0.370	0.322	0.491
4	CAE-CON	0.250	0.250	0.263	0.108	0.300	0.321	0.143	0.188	0.213	0.193	0.367	0.236	0.129	0.108	0.200	0.284	0.276	0.355	0.279	0.121	0.387
2	CAE-CON	0.333	0.414	0.288	0.164	0.387	0.455	0.310	0.091	0.270	0.322	0.355	0.298	0.250	0.194	0.258	0.275	0.400	0.406	0.333	0.088	0.344
7	CAE-CON	0.290	0.333	0.273	0.175	0.310	0.333	0.296	0.194	0.220	0.164	0.379	0.170	0.167	0.175	0.241	0.292	0.286	0.333	0.254	0.125	0.367
10	CAE-CON	0.311	0.358	0.333	0.258	0.298	0.360	0.245	0.279	0.310	0.222	0.368	0.231	0.288	0.258	0.263	0.406	0.309	0.390	0.345	0.238	0.458
11	CAE-CON	0.385	0.227	0.244	0.321	0.375	0.366	0.318	0.385	0.347	0.244	0.458	0.209	0.280	0.283	0.333	0.491	0.391	0.440	0.347	0.296	0.520
12	CV-JAM	0.000	0.357	0.439	0.292	0.200	0.396	0.357	0.375	0.311	0.263	0.433	0.345	0.129	0.262	0.367	0.343	0.345	0.387	0.410	0.333	0.452
3	CV-JAM	0.357	0.000	0.306	0.333	0.423	0.289	0.250	0.357	0.396	0.306	0.462	0.362	0.296	0.263	0.423	0.458	0.360	0.407	0.396	0.310	0.519
1	CV-JAM	0.439	0.306	0.000	0.276	0.434	0.391	0.306	0.263	0.296	0.280	0.434	0.292	0.345	0.241	0.283	0.433	0.333	0.491	0.333	0.254	0.527
6	CV-JAM	0.292	0.333	0.276	0.000	0.377	0.444	0.228	0.169	0.258	0.310	0.410	0.321	0.206	0.182	0.213	0.265	0.356	0.429	0.290	0.164	0.397
8	CV-JAM	0.200	0.423	0.434	0.377	0.000	0.347	0.423	0.367	0.404	0.208	0.393	0.216	0.276	0.377	0.393	0.429	0.185	0.345	0.368	0.323	0.448
9	CV-JAM	0.396	0.289	0.391	0.444	0.347	0.000	0.289	0.396	0.400	0.217	0.510	0.318	0.333	0.370	0.429	0.464	0.191	0.294	0.320	0.345	0.529
4	CV-CON	0.357	0.250	0.306	0.228	0.423	0.289	0.000	0.286	0.283	0.265	0.423	0.319	0.259	0.193	0.231	0.390	0.280	0.407	0.283	0.241	0.444
2	CV-CON	0.375	0.357	0.263	0.169	0.367	0.396	0.286	0.000	0.311	0.333	0.333	0.309	0.258	0.200	0.233	0.254	0.345	0.419	0.344	0.091	0.419
5	CV-CON	0.311	0.396	0.296	0.258	0.404	0.400	0.283	0.311	0.000	0.259	0.263	0.269	0.254	0.194	0.333	0.250	0.309	0.322	0.276	0.238	0.356
7	CV-CON	0.263	0.306	0.280	0.310	0.208	0.217	0.265	0.333	0.259	0.000	0.396	0.125	0.200	0.241	0.321	0.400	0.216	0.345	0.296	0.254	0.455
10	CV-CON	0.433	0.462	0.434	0.410	0.393	0.510	0.423	0.333	0.263	0.396	0.000	0.373	0.414	0.377	0.357	0.302	0.370	0.276	0.298	0.323	0.310
11	CV-CON	0.345	0.362	0.292	0.321	0.216	0.318	0.319	0.309	0.269	0.125	0.373	0.000	0.283	0.321	0.333	0.414	0.224	0.358	0.269	0.263	0.396
12	CD-JAM	0.129	0.296	0.345	0.206	0.276	0.333	0.259	0.258	0.254	0.200	0.414	0.283	0.000	0.143	0.276	0.292	0.321	0.367	0.322	0.219	0.400
3	CD-JAM	0.262	0.263	0.241	0.182	0.377	0.370	0.193	0.200	0.194	0.241	0.377	0.321	0.143	0.000	0.213	0.294	0.322	0.333	0.290	0.134	0.429
1	CD-JAM	0.367	0.423	0.283	0.213	0.393	0.429	0.231	0.233	0.333	0.321	0.357	0.333	0.276	0.213	0.000	0.333	0.333	0.414	0.263	0.226	0.414
6	CD-JAM	0.343	0.458	0.433	0.265	0.429	0.464	0.390	0.254	0.250	0.400	0.302	0.414	0.292	0.294	0.333	0.000	0.410	0.323	0.313	0.246	0.354
8	CD-JAM	0.345	0.360	0.333	0.356	0.185	0.191	0.280	0.345	0.309	0.216	0.370	0.224	0.321	0.322	0.333	0.410	0.000	0.250	0.236	0.300	0.429
9	CD-JAM	0.387	0.407	0.491	0.429	0.345	0.294	0.407	0.419	0.322	0.345	0.276	0.358	0.367	0.333	0.414	0.323	0.250	0.000	0.220	0.344	0.300
4	CD-CON	0.410	0.396	0.333	0.290	0.368	0.320	0.283	0.344	0.276	0.296	0.298	0.269	0.322	0.290	0.263	0.313	0.236	0.220	0.000	0.270	0.288
2	CD-CON	0.333	0.310	0.254	0.164	0.323	0.345	0.241	0.091	0.238	0.254	0.323	0.263	0.219	0.134	0.226	0.246	0.300	0.344	0.270	0.000	0.406
5	CD-CON	0.452	0.519	0.527	0.397	0.448	0.529	0.444	0.419	0.356	0.455	0.310	0.396	0.400	0.429	0.414	0.354	0.429	0.300	0.288	0.406	0.000
7	CD-CON	0.300	0.308	0.245	0.279	0.286	0.306	0.308	0.300	0.193	0.132	0.321	0.137	0.207	0.246	0.321	0.365	0.259	0.310	0.263	0.226	0.379
10	CD-CON	0.480	0.286	0.302	0.490	0.391	0.282	0.333	0.440	0.404	0.302	0.435	0.317	0.458	0.412	0.435	0.585	0.273	0.417	0.362	0.423	0.583

11	CD-CON	0.527	0.277	0.375	0.429	0.451	0.364	0.362	0.382	0.500	0.417	0.451	0.348	0.434	0.357	0.412	0.517	0.306	0.396	0.269	0.368	0.509
12	CT-JAM	0.300	0.385	0.321	0.377	0.357	0.388	0.385	0.367	0.263	0.245	0.321	0.255	0.241	0.279	0.357	0.302	0.370	0.345	0.298	0.323	0.414
3	CT-JAM	0.358	0.333	0.435	0.370	0.306	0.429	0.289	0.358	0.360	0.304	0.388	0.409	0.294	0.259	0.388	0.429	0.362	0.333	0.360	0.345	0.412
1	CT-JAM	0.433	0.538	0.358	0.377	0.357	0.551	0.423	0.333	0.368	0.396	0.321	0.294	0.448	0.410	0.393	0.429	0.370	0.345	0.263	0.355	0.310
6	CT-JAM	0.323	0.474	0.379	0.242	0.410	0.444	0.368	0.231	0.161	0.345	0.246	0.286	0.270	0.273	0.311	0.147	0.390	0.333	0.290	0.284	0.302
8	CT-JAM	0.345	0.440	0.373	0.390	0.222	0.319	0.360	0.448	0.382	0.216	0.370	0.265	0.321	0.390	0.370	0.443	0.231	0.393	0.273	0.400	0.393
9	CT-JAM	0.387	0.481	0.382	0.429	0.345	0.373	0.370	0.419	0.288	0.309	0.241	0.321	0.400	0.333	0.379	0.385	0.250	0.133	0.254	0.375	0.400
4	CT-CON	0.404	0.306	0.280	0.379	0.321	0.304	0.265	0.368	0.333	0.240	0.283	0.250	0.345	0.276	0.283	0.400	0.216	0.273	0.111	0.322	0.382
2	CT-CON	0.276	0.400	0.333	0.322	0.259	0.404	0.320	0.276	0.273	0.176	0.296	0.265	0.214	0.254	0.296	0.344	0.346	0.357	0.273	0.233	0.357
5	CT-CON	0.387	0.556	0.527	0.460	0.414	0.529	0.481	0.452	0.390	0.455	0.276	0.396	0.400	0.460	0.448	0.446	0.393	0.367	0.322	0.469	0.233
7	CT-CON	0.407	0.391	0.404	0.382	0.360	0.349	0.348	0.407	0.412	0.234	0.360	0.244	0.385	0.455	0.400	0.474	0.333	0.423	0.333	0.393	0.462
11	CT-CON	0.367	0.423	0.396	0.377	0.321	0.510	0.423	0.400	0.263	0.321	0.214	0.294	0.345	0.344	0.429	0.333	0.370	0.345	0.333	0.323	0.276
10	CT-CON	0.365	0.418	0.393	0.375	0.288	0.423	0.382	0.365	0.300	0.286	0.186	0.259	0.377	0.406	0.356	0.364	0.298	0.311	0.267	0.354	0.344
	No	12	3	1	6	8	9	4	2	5	7	11	10	-	-	-	-	-	-	-	-	-
No	part	CT-JAM	CT-JAM	CT-JAM	CT-JAM	CT-JAM	CT-JAM	CT-CON	CT-CON	CT-CON	CT-CON	CT-CON	CT-CON	-	-	-	-	-	-	-	-	-
12	PN-JAM	0.800	0.758	0.850	0.867	0.737	0.714	0.730	0.842	0.857	0.765	0.850	0.860	-	-	-	-	-	-	-	-	-
3	PN-JAM	0.684	0.677	0.789	0.721	0.722	0.750	0.657	0.667	0.800	0.750	0.737	0.756	-	-	-	-	-	-	-	-	-
6	PN-JAM	0.765	0.704	0.824	0.795	0.813	0.778	0.742	0.750	0.833	0.786	0.824	0.838	-	-	-	-	-	-	-	-	-
8	PN-JAM	0.600	0.576	0.700	0.644	0.579	0.571	0.514	0.632	0.714	0.647	0.650	0.674	-	-	-	-	-	-	-	-	-
9	PN-JAM	0.676	0.667	0.784	0.714	0.657	0.641	0.588	0.714	0.744	0.742	0.730	0.750	-	-	-	-	-	-	-	-	-
7	PN-CON	0.480	0.488	0.520	0.491	0.500	0.500	0.447	0.375	0.500	0.227	0.440	0.396	-	-	-	-	-	-	-	-	-
4	PN-CON	0.818	0.769	0.879	0.842	0.871	0.829	0.800	0.806	0.886	0.778	0.879	0.889	-	-	-	-	-	-	-	-	-
2	PN-CON	0.684	0.742	0.789	0.721	0.778	0.750	0.657	0.722	0.800	0.750	0.737	0.756	-	-	-	-	-	-	-	-	-
10	PN-CON	0.610	0.647	0.707	0.652	0.641	0.628	0.579	0.641	0.721	0.714	0.659	0.682	-	-	-	-	-	-	-	-	-
11	PN-CON	0.619	0.714	0.714	0.702	0.650	0.591	0.538	0.700	0.682	0.611	0.667	0.644	-	-	-	-	-	-	-	-	-
12	PG-JAM	0.674	0.667	0.721	0.750	0.707	0.644	0.600	0.756	0.733	0.676	0.721	0.739	-	-	-	-	-	-	-	-	-
3	PG-JAM	0.657	0.786	0.771	0.750	0.758	0.730	0.625	0.758	0.784	0.724	0.714	0.737	-	-	-	-	-	-	-	-	-
1	PG-JAM	0.762	0.829	0.810	0.787	0.850	0.773	0.692	0.800	0.727	0.778	0.810	0.733	-	-	-	-	-	-	-	-	-
6	PG-JAM	0.676	0.667	0.676	0.714	0.714	0.641	0.588	0.714	0.641	0.613	0.676	0.650	-	-	-	-	-	-	-	-	-
8	PG-JAM	0.676	0.600	0.730	0.714	0.543	0.641	0.588	0.657	0.744	0.677	0.676	0.700	-	-	-	-	-	-	-	-	-
9	PG-JAM	0.722	0.724	0.778	0.707	0.706	0.737	0.697	0.706	0.737	0.667	0.722	0.744	-	-	-	-	-	-	-	-	-

4	PG-CON	0.737	0.677	0.789	0.767	0.778	0.750	0.714	0.722	0.850	0.750	0.789	0.805	-	-	-	-	-	-	-	-	-
2	PG-CON	0.684	0.677	0.789	0.721	0.722	0.750	0.657	0.667	0.800	0.688	0.737	0.756	-	-	-	-	-	-	-	-	-
5	PG-CON	0.659	0.706	0.756	0.696	0.744	0.721	0.632	0.692	0.767	0.771	0.707	0.727	-	-	-	-	-	-	-	-	-
10	PG-CON	0.632	0.677	0.737	0.674	0.667	0.650	0.600	0.667	0.750	0.750	0.684	0.707	-	-	-	-	-	-	-	-	-
11	PG-CON	0.692	0.688	0.795	0.727	0.730	0.756	0.667	0.676	0.805	0.697	0.744	0.762	-	-	-	-	-	-	-	-	-
3	SI-JAM	0.650	0.636	0.750	0.689	0.684	0.667	0.568	0.684	0.762	0.765	0.700	0.721	-	-	-	-	-	-	-	-	-
6	SI-JAM	0.714	0.714	0.762	0.745	0.800	0.727	0.692	0.750	0.773	0.778	0.762	0.778	-	-	-	-	-	-	-	-	-
8	SI-JAM	0.773	0.784	0.773	0.796	0.810	0.739	0.756	0.810	0.739	0.842	0.818	0.745	-	-	-	-	-	-	-	-	-
9	SI-JAM	0.622	0.667	0.730	0.714	0.600	0.590	0.529	0.714	0.744	0.742	0.676	0.700	-	-	-	-	-	-	-	-	-
12	SI-JAM	0.692	0.750	0.692	0.773	0.784	0.659	0.611	0.784	0.707	0.697	0.744	0.667	-	-	-	-	-	-	-	-	-
10	SI-CON	0.674	0.722	0.721	0.708	0.756	0.689	0.600	0.707	0.644	0.730	0.721	0.609	-	-	-	-	-	-	-	-	-
4	SI-CON	0.762	0.657	0.762	0.787	0.750	0.727	0.692	0.750	0.773	0.778	0.810	0.733	-	-	-	-	-	-	-	-	-
2	SI-CON	0.702	0.750	0.787	0.769	0.733	0.755	0.682	0.733	0.796	0.756	0.745	0.760	-	-	-	-	-	-	-	-	-
5	SI-CON	0.714	0.762	0.755	0.741	0.787	0.765	0.696	0.745	0.725	0.814	0.755	0.692	-	-	-	-	-	-	-	-	-
7	SI-CON	0.684	0.677	0.737	0.674	0.722	0.700	0.600	0.667	0.700	0.625	0.684	0.659	-	-	-	-	-	-	-	-	-
11	SI-CON	0.692	0.688	0.692	0.773	0.730	0.659	0.611	0.784	0.707	0.758	0.744	0.667	-	-	-	-	-	-	-	-	-
11	SI-CON	0.684	0.677	0.684	0.767	0.722	0.650	0.600	0.778	0.700	0.750	0.737	0.659	-	-	-	-	-	-	-	-	-
3	CAE-JAM	0.356	0.346	0.390	0.313	0.368	0.410	0.321	0.333	0.443	0.434	0.322	0.387	-	-	-	-	-	-	-	-	-
6	CAE-JAM	0.368	0.400	0.439	0.258	0.418	0.458	0.370	0.382	0.458	0.451	0.368	0.400	-	-	-	-	-	-	-	-	-
8	CAE-JAM	0.472	0.348	0.396	0.414	0.333	0.418	0.440	0.333	0.527	0.489	0.472	0.429	-	-	-	-	-	-	-	-	-
4	CAE-CON	0.333	0.321	0.433	0.262	0.345	0.355	0.298	0.276	0.419	0.370	0.367	0.333	-	-	-	-	-	-	-	-	-
2	CAE-CON	0.387	0.418	0.290	0.224	0.433	0.406	0.390	0.267	0.406	0.429	0.290	0.385	-	-	-	-	-	-	-	-	-
7	CAE-CON	0.310	0.412	0.345	0.270	0.357	0.367	0.345	0.250	0.433	0.346	0.345	0.344	-	-	-	-	-	-	-	-	-
10	CAE-CON	0.404	0.440	0.439	0.355	0.345	0.322	0.333	0.345	0.458	0.333	0.404	0.333	-	-	-	-	-	-	-	-	-
11	CAE-CON	0.292	0.415	0.458	0.434	0.391	0.440	0.289	0.348	0.520	0.429	0.375	0.373	-	-	-	-	-	-	-	-	-
12	CV-JAM	0.300	0.358	0.433	0.323	0.345	0.387	0.404	0.276	0.387	0.407	0.367	0.365	-	-	-	-	-	-	-	-	-
3	CV-JAM	0.385	0.333	0.538	0.474	0.440	0.481	0.306	0.400	0.556	0.391	0.423	0.418	-	-	-	-	-	-	-	-	-
1	CV-JAM	0.321	0.435	0.358	0.379	0.373	0.382	0.280	0.333	0.527	0.404	0.396	0.393	-	-	-	-	-	-	-	-	-

6	CV-JAM	0.377	0.370	0.377	0.242	0.390	0.429	0.379	0.322	0.460	0.382	0.377	0.375	-	-	-	-	-	-	-	-	-
8	CV-JAM	0.357	0.306	0.357	0.410	0.222	0.345	0.321	0.259	0.414	0.360	0.321	0.288	-	-	-	-	-	-	-	-	-
9	CV-JAM	0.388	0.429	0.551	0.444	0.319	0.373	0.304	0.404	0.529	0.349	0.510	0.423	-	-	-	-	-	-	-	-	-
4	CV-CON	0.385	0.289	0.423	0.368	0.360	0.370	0.265	0.320	0.481	0.348	0.423	0.382	-	-	-	-	-	-	-	-	-
2	CV-CON	0.367	0.358	0.333	0.231	0.448	0.419	0.368	0.276	0.452	0.407	0.400	0.365	-	-	-	-	-	-	-	-	-
5	CV-CON	0.263	0.360	0.368	0.161	0.382	0.288	0.333	0.273	0.390	0.412	0.263	0.300	-	-	-	-	-	-	-	-	-
7	CV-CON	0.245	0.304	0.396	0.345	0.216	0.309	0.240	0.176	0.455	0.234	0.321	0.286	-	-	-	-	-	-	-	-	-
10	CV-CON	0.321	0.388	0.321	0.246	0.370	0.241	0.283	0.296	0.276	0.360	0.214	0.186	-	-	-	-	-	-	-	-	-
11	CV-CON	0.255	0.409	0.294	0.286	0.265	0.321	0.250	0.265	0.396	0.244	0.294	0.259	-	-	-	-	-	-	-	-	-
12	CD-JAM	0.241	0.294	0.448	0.270	0.321	0.400	0.345	0.214	0.400	0.385	0.345	0.377	-	-	-	-	-	-	-	-	-
3	CD-JAM	0.279	0.259	0.410	0.273	0.390	0.333	0.276	0.254	0.460	0.455	0.344	0.406	-	-	-	-	-	-	-	-	-
1	CD-JAM	0.357	0.388	0.393	0.311	0.370	0.379	0.283	0.296	0.448	0.400	0.429	0.356	-	-	-	-	-	-	-	-	-
6	CD-JAM	0.302	0.429	0.429	0.147	0.443	0.385	0.400	0.344	0.446	0.474	0.333	0.364	-	-	-	-	-	-	-	-	-
8	CD-JAM	0.370	0.362	0.370	0.390	0.231	0.250	0.216	0.346	0.393	0.333	0.370	0.298	-	-	-	-	-	-	-	-	-
9	CD-JAM	0.345	0.333	0.345	0.333	0.393	0.133	0.273	0.357	0.367	0.423	0.345	0.311	-	-	-	-	-	-	-	-	-
4	CD-CON	0.298	0.360	0.263	0.290	0.273	0.254	0.111	0.273	0.322	0.333	0.333	0.267	-	-	-	-	-	-	-	-	-
2	CD-CON	0.323	0.345	0.355	0.284	0.400	0.375	0.322	0.233	0.469	0.393	0.323	0.354	-	-	-	-	-	-	-	-	-
5	CD-CON	0.414	0.412	0.310	0.302	0.393	0.400	0.382	0.357	0.233	0.462	0.276	0.344	-	-	-	-	-	-	-	-	-
7	CD-CON	0.214	0.388	0.357	0.279	0.259	0.276	0.283	0.222	0.448	0.280	0.286	0.254	-	-	-	-	-	-	-	-	-
10	CD-CON	0.435	0.436	0.478	0.529	0.364	0.375	0.256	0.455	0.542	0.400	0.478	0.388	-	-	-	-	-	-	-	-	-
11	CD-CON	0.451	0.455	0.412	0.500	0.429	0.434	0.250	0.469	0.509	0.422	0.451	0.444	-	-	-	-	-	-	-	-	-
12	CT-JAM	0.000	0.388	0.393	0.246	0.333	0.345	0.245	0.222	0.379	0.400	0.250	0.288	-	-	-	-	-	-	-	-	-
3	CT-JAM	0.388	0.000	0.388	0.444	0.362	0.373	0.304	0.191	0.529	0.488	0.347	0.423	-	-	-	-	-	-	-	-	-
1	CT-JAM	0.393	0.388	0.000	0.344	0.407	0.310	0.321	0.333	0.310	0.400	0.286	0.288	-	-	-	-	-	-	-	-	-
6	CT-JAM	0.246	0.444	0.344	0.000	0.356	0.333	0.345	0.288	0.333	0.418	0.279	0.281	-	-	-	-	-	-	-	-	-
8	CT-JAM	0.333	0.362	0.407	0.356	0.000	0.286	0.216	0.231	0.464	0.333	0.296	0.298	-	-	-	-	-	-	-	-	-
9	CT-JAM	0.345	0.373	0.310	0.333	0.286	0.000	0.236	0.321	0.400	0.385	0.345	0.246	-	-	-	-	-	-	-	-	-
4	CT-CON	0.245	0.304	0.321	0.345	0.216	0.236	0.000	0.255	0.345	0.319	0.321	0.250	-	-	-	-	-	-	-	-	-
2	CT-CON	0.222	0.191	0.333	0.288	0.231	0.321	0.255	0.000	0.429	0.333	0.259	0.298	-	-	-	-	-	-	-	-	-
5	CT-CON	0.379	0.529	0.310	0.333	0.464	0.400	0.345	0.429	0.000	0.385	0.345	0.213	-	-	-	-	-	-	-	-	-
7	CT-CON	0.400	0.488	0.400	0.418	0.333	0.385	0.319	0.333	0.385	0.000	0.400	0.245	-	-	-	-	-	-	-	-	-

11	CT-CON	0.250	0.347	0.286	0.279	0.296	0.345	0.321	0.259	0.345	0.400	0.000	0.254	-	-	-	-	-	-	-	-	-
10	CT-CON	0.288	0.423	0.288	0.281	0.298	0.246	0.250	0.298	0.213	0.245	0.254	0.000	-	-	-	-	-	-	-	-	-

No = number of the animal in the trial
CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG =(*pars glandularis*), PN (*pars nonglandularis*) and SI (small intestine) for the CON feeding group (placebo) and the JAM feeding group (Jerusalem artichoke meal).

Table S5: Relative abundance (in %) at the genus level for the individual horses in the JAM and CON groups

part	taxa	CON						JAM					
		animal											
		2	4	5	7	10	11	1	3	6	8	9	12
PN	*Acinetobacter*	1.3	0.9	-	1.7	0.0	0.5	-	0.4	1.7	0.4	0.0	2.9
	Actinobacillus	10.7	2.2	-	5.0	1.9	0.0	-	0.3	11.5	0.7	1.2	0.0
	Asaia	0.0	0.0	-	2.8	0.2	0.0	-	0.0	0.0	0.0	0.0	0.0
	Bacillus	0.0	0.0	-	0.0	0.0	1.0	-	0.0	0.0	0.0	0.0	0.0
	Chloroplast	0.0	0.0	-	3.7	0.0	0.0	-	1.8	0.0	0.0	0.0	2.2
	Clostridium	0.3	0.0	-	0.0	0.8	1.1	-	0.0	0.0	3.3	0.3	0.0
	Enterobacter	0.0	0.0	-	0.7	0.0	0.0	-	0.0	0.0	0.0	0.0	4.2
	Halomonas	0.0	0.0	-	0.0	0.0	0.8	-	0.0	0.0	0.0	0.0	0.0
	IS(Lachnospiraceae)	0.4	0.0	-	2.6	0.9	0.5	-	12.9	0.0	1.2	0.4	0.0
	Lactobacillus	69.7	69.0	-	77.6	70.8	76.9	-	80.8	73.1	78.5	76.0	83.2
	Lactococcus	0.0	0.0	-	0.0	1.4	0.3	-	0.0	0.0	6.7	15.9	0.5
	Neisseria	0.7	0.0	-	0.0	0.0	0.0	-	0.9	0.0	0.0	0.0	1.1
	Pasteurella	3.2	0.0	-	0.6	1.0	0.0	-	0.0	0.0	0.0	0.0	0.5
	Rothia	0.0	0.0	-	0.0	0.2	0.0	-	0.0	0.0	0.0	0.0	0.3
	Sarcina	0.0	0.0	-	0.4	1.0	1.0	-	0.3	0.0	4.7	0.4	0.0
	Streptococcus	12.2	22.1	-	0.0	12.5	1.9	-	2.0	8.8	1.6	0.4	0.6
	Un(Acidobacteria)	0.0	0.0	-	0.0	0.0	0.4	-	0.0	0.0	0.0	0.0	0.0
	Un (Christensenellaceae)	0.0	0.0	-	0.0	0.0	0.0	-	0.4	4.5	0.0	0.0	0.0
	Un(Lachnospiraceae)	0.6	5.8	-	4.5	6.5	4.6	-	0.0	0.4	0.5	4.2	1.4
	Un (Peptostreptococcaceae)	0.0	0.0	-	0.0	0.0	1.4	-	0.0	0.0	0.0	0.0	0.0
	Un(Ruminococcaceae)	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	0.7	0.0	0.7
	Un(S24-7)	0.0	0.0	-	0.0	0.3	0.7	-	0.0	0.0	0.0	0.0	0.0
	Weissella	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	1.1	1.2	2.4
	Xylanibacter	0.8	0.0	-	0.0	2.4	8.9	-	0.3	0.0	0.7	0.0	0.0
PG	*Acinetobacter*	1.6	0.5	0.0	-	0.0	1.2	0.5	0.6	1.1	0.0	0.9	1.8
	Actinobacillus	4.6	5.7	1.1	-	0.5	1.2	4.7	0.0	0.0	0.0	0.7	0.0
	Chloroplast	0.0	0.4	0.0	-	1.3	0.3	0.7	0.0	0.7	0.0	0.0	1.1
	Clostridium	0.0	0.0	0.3	-	12.7	0.0	0.6	0.8	23.0	0.0	1.5	2.5
	Dialister	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Enterococcus	0.0	0.0	0.0	-	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
	Escherichia Shigella	0.0	0.0	0.0	-	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
	Gardnerella	0.0	0.3	2.7	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS(Lachnospiraceae)	0.5	0.0	10.0	-	0.4	0.9	2.0	1.0	0.0	2.3	2.5	0.0
	Lactobacillus	82.4	82.9	75.9	-	47.8	82.6	83.1	95.1	36.3	85.5	88.1	81.2
	Lactococcus	0.0	0.0	0.0	-	1.2	0.0	0.0	0.0	0.0	2.9	2.3	0.0
	Moraxella	0.0	0.0	0.4	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Neisseria	0.3	0.5	0.0	-	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.7
	Paenibacillus	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
	Pasteurella	2.3	0.0	0.0	-	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.4
	Phyllobacterium	0.0	0.0	0.0	-	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
	Pseudomonas	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4

	Sarcina	0.6	0.6	0.4	-	27.5	0.3	0.0	1.2	36.6	0.3	2.4	2.6
	Sharpea	0.0	0.0	0.8	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Streptococcus	6.1	3.1	4.4	-	2.1	7.1	0.3	0.3	0.0	0.0	0.0	1.2
	Treponema	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
	Un(Anaerolineaceae)	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
	Un(ARKICE-90)	0.0	0.0	0.0	-	0.0	0.0	5.1	0.0	0.0	0.0	0.0	0.0
	Un (Christensenellaceae)	0.0	0.0	0.0	-	0.0	0.0	0.6	0.0	0.5	0.0	0.0	0.0
	Un(Lachnospiraceae)	0.8	4.0	1.5	-	3.8	2.4	0.0	0.0	0.3	0.6	1.6	3.4
	Un (Peptostreptococcaceae)	0.0	0.0	0.0	-	0.0	0.0	0.3	0.0	0.9	0.0	0.0	0.0
	Un(Ruminococcaceae)	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.3
	Un(S24-7)	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
	Variovorax	0.0	0.0	0.0	-	0.0	0.0	0.8	1.0	0.0	0.0	0.0	0.0
	Veillonella	0.0	0.0	1.4	-	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0
	Weissella	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0	3.0	0.0	2.8
	Xylanibacter	0.7	2.1	0.8	-	2.7	3.2	0.0	0.0	0.0	0.3	0.0	0.0
SI	*Acinetobacter*	33.0	0.6	0.0	0.5	0.0	0.5	-	0.0	0.5	0.0	0.4	0.5
	Actinobacillus	0.0	48.9	49.7	4.9	1.0	0.0	-	2.0	40.7	16.8	0.0	0.0
	Asaia	0.0	0.3	5.7	0.0	0.0	0.0	-	0.0	0.0	0.3	0.0	0.0
	Blautia	0.4	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Cedecea	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.3	0.0	0.0
	Cellusilyticum	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.3	0.0	0.0	0.0
	Chloroplast	0.0	0.3	0.8	0.0	0.0	0.0	-	0.6	2.1	2.5	0.0	0.0
	Clostridium	0.0	0.0	3.5	2.1	16.4	5.4	-	3.6	10.3	9.0	21.0	4.0
	Curtobacterium	0.3	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Devosia	0.6	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Enterobacter	0.0	1.6	2.2	0.0	0.6	1.8	-	0.0	0.0	1.0	0.0	0.0
	Escherichia Shigella	0.0	3.2	5.6	0.0	9.7	3.1	-	0.0	0.0	10.8	0.0	0.7
	Gardnerella	0.0	0.0	0.4	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	IS(Lachnospiraceae)	0.3	0.0	0.4	3.1	0.6	0.0	-	1.3	0.0	0.0	72.3	0.0
	IS (Peptostreptococcaceae)	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.3
	Lactobacillus	53.2	7.0	8.0	76.1	16.5	63.1	-	85.1	0.7	32.7	1.1	47.9
	Lactococcus	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	3.7	0.7	0.0
	Neisseria	0.0	0.0	0.5	0.0	0.0	0.0	-	0.4	0.0	0.0	0.0	0.0
	Ochrobactrum	0.5	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Pantoea	0.0	0.0	0.4	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Pasteurella	0.0	1.6	11.3	0.4	1.9	0.0	-	0.3	0.3	1.5	0.0	0.0
	Patulibacter	0.5	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Phyllobacterium	0.0	0.0	0.9	0.0	2.0	0.0	-	0.0	0.0	0.3	0.0	0.0
	Planomicrobium	0.0	0.0	0.3	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Pseudomonas	0.0	0.0	0.3	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Rhizobium	2.3	0.0	0.0	0.0	0.0	0.0	-	0.0	13.1	0.0	0.0	0.0
	Rhodobacter	0.4	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.3	0.0	0.0
	Sarcina	0.5	0.4	2.3	2.6	18.0	4.4	-	5.2	0.3	1.3	0.9	5.5
	Sphingomonas	0.7	0.0	0.0	0.0	0.0	0.0	-	0.0	4.1	0.0	0.0	0.0
	Streptococcus	2.2	12.0	2.7	0.0	3.5	0.5	-	0.7	0.0	7.9	1.4	0.6
	Turicibacter	0.0	0.0	0.0	0.0	0.3	0.0	-	0.0	0.0	0.0	0.0	4.7
	Un(Anaerolineaceae)	0.4	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Un(B38)	0.0	0.5	1.8	0.0	7.8	2.3	-	0.0	1.2	7.5	0.0	0.0

	Un (Christensenellaceae)	0.3	0.0	0.0	0.0	0.0	0.0	-	0.0	9.1	0.0	0.0	0.0
	Un(Lachnospiraceae)	0.8	22.6	2.0	8.6	20.5	16.4	-	0.0	17.0	3.8	1.3	30.5
	Un (Peptostreptococcaceae)	0.0	0.0	0.0	1.3	0.4	0.0	-	0.0	0.0	0.0	0.0	2.4
	Un(Phyllobacterium)	1.3	0.0	0.5	0.0	0.0	0.0	-	0.0	0.3	0.0	0.0	0.0
	Un(RF9)	0.3	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
	Weissella	0.0	0.6	0.0	0.0	0.0	0.5	-	0.3	0.0	0.0	0.9	0.0
	Xylanibacter	1.9	0.3	0.7	0.5	0.8	2.1	-	0.4	0.0	0.0	0.0	3.0
CAE	*Actinobacillus*	1.3	0.9	-	0.0	0.0	0.0	-	0.0	1.2	0.3	-	-
	Alistipes	0.5	0.4	-	0.0	0.4	0.0	-	0.5	0.5	0.0	-	-
	Blautia	0.8	0.7	-	0.7	0.4	0.6	-	1.0	0.0	0.0	-	-
	Clostridium	0.4	0.7	-	0.6	1.3	1.3	-	0.7	0.4	5.1	-	-
	Coprococcus	0.0	1.2	-	1.1	1.3	1.4	-	1.2	0.8	1.0	-	-
	Desulfovibrio	1.0	0.5	-	2.3	1.5	0.0	-	1.4	0.5	1.8	-	-
	Escherichia Shigella	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	5.6	-	-
	Fibrobacter	3.5	2.2	-	0.0	0.3	0.0	-	0.7	0.4	0.0	-	-
	hoa5_07d05_gut_group	0.7	0.0	-	0.0	0.0	0.0	-	0.0	0.9	0.0	-	-
	IS(Lachnospiraceae)	0.4	1.9	-	0.9	0.7	0.0	-	0.5	1.7	0.0	-	-
	IS(Ruminococcaceae)	0.4	2.3	-	6.4	0.7	2.0	-	0.8	0.0	0.0	-	-
	Johnsonella	0.5	0.0	-	0.6	0.0	0.0	-	0.0	0.0	0.3	-	-
	Lactobacillus	13.1	11.8	-	11.9	11.9	21.6	-	10.7	12.2	26.5	-	-
	Lactococcus	0.0	0.0	-	0.0	0.6	0.0	-	0.0	0.0	3.5	-	-
	Marvinbryantia	0.5	2.4	-	1.1	0.8	0.4	-	2.0	0.3	0.0	-	-
	Oribacterium	0.4	0.0	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	-
	Paludibacter	1.8	1.2	-	2.5	1.0	0.0	-	1.4	0.7	0.0	-	-
	Phasolarctobacterium	1.5	0.0	-	0.9	1.2	0.0	-	0.0	0.0	2.8	-	-
	Prevotella	2.3	1.1	-	3.8	1.6	2.7	-	1.2	4.1	2.9	-	-
	Pseudobutyrivibrio	0.4	0.6	-	0.6	0.4	0.0	-	1.2	1.1	0.0	-	-
	RC9_gut_group	3.3	0.5	-	1.3	0.0	1.2	-	2.1	0.4	2.0	-	-
	Ruminococcus	7.4	6.8	-	7.4	5.5	4.5	-	6.6	10.4	5.2	-	-
	Saccharofermentans	0.4	0.0	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-	-
	Sarcina	0.8	1.1	-	0.9	2.0	1.4	-	1.1	1.0	1.0	-	-
	SP3-e08	0.7	0.0	-	0.0	0.0	0.0	-	0.4	0.0	0.0	-	-
	Streptococcus	0.0	2.4	-	0.4	0.9	0.8	-	0.0	0.4	0.0	-	-
	Thalassospira	0.0	0.0	-	0.0	0.0	0.0	-	0.5	0.3	0.0	.-	-
	Treponema	3.7	0.4	-	0.9	0.0	0.0	-	3.4	2.4	2.6	-	-
	Un(B38)	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	4.3	-	-
	Un(BS11_gut_group)	1.2	0.6	-	1.7	0.8	0.6	-	0.0	0.0	0.0	-	-
	Un(Christenenellacea)	3.0	2.3	-	1.4	0.8	1.4	-	2.9	2.1	0.0	-	-
	Un(Clostridales)	0.0	0.9	-	0.0	0.0	0.6	-	1.0	0.0	0.0	-	-
	Un(Erysipelotrichaceae)	7.4	1.4	-	5.1	2.3	1.0	-	4.8	7.5	5.4	-	-
	Un(Lachnospiraceae)	14.5	44.3	-	22.4	42.2	42.4	-	10.8	11.2	9.8	-	-
	Un (Peptostreptococcaceae)	0.0	0.0	-	0.0	1.7	0.0	-	0.0	0.0	0.0	-	-
	Un(Prevotellaceae)	0.0	0.7	-	1.7	0.8	0.0	-	0.0	0.9	1.3	-	-
	Un(RF16)	0.4	0.4	-	0.4	0.0	0.0	-	0.9	0.0	0.4	-	-
	Un(RF9)	1.3	0.4	-	1.2	0.0	1.2	-	0.8	3.6	0.0	-	-
	Un(RFP12_gut_group)	2.0	0.5	-	1.1	0.7	0.0	-	0.7	0.0	0.5	-	-
	Un(Ruminococccaceae)	7.7	3.7	-	4.3	3.1	4.9	-	4.6	7.1	3.4	-	-
	Un(S24-7)	10.9	3.1	-	11.9	8.4	4.6	-	9.8	9.7	5.3	-	-

	Un(4C0d-2)	1.3	0.0	-	0.8	0.0	0.0	-	0.5	3.2	0.7	-	-
	Uncultured (Clostridiales)	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.3	0.0	-	-
	Weissella	0.0	0.6	-	0.0	0.3	0.0	-	21.6	0.0	2.9	-	-
	Xylanibacter	4.5	2.1	-	4.1	6.3	5.5	-	4.4	14.7	5.3	-	-
CV	*Acetitomaculum*	0.4	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.5	0.0	0.0	0.0
	Acinetobacter	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.5
	Actinobacillus	0.8	0.7	0.9	0.0	0.0	0.0	0.8	0.8	0.4	0.0	0.0	0.5
	Akkermansia	0.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Alistipes	0.4	0.4	0.0	0.0	0.0	0.0	0.4	0.0	0.3	0.0	0.0	0.0
	Anaerosporobacter	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
	Bacteroides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
	Blautia	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.3	0.7	0.0	0.0	0.4
	Clostridium	0.7	0.0	0.7	0.0	5.9	1.7	0.4	0.3	1.2	0.5	0.0	1.4
	Coprococcus	0.8	0.6	0.0	1.1	0.0	0.4	2.4	2.1	2.0	1.1	0.7	0.0
	Desulfovibrio	1.3	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.9	0.6	0.0	0.9
	Enterobacter	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	0.0	0.7
	Escherichia Shigella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.4
	Fibrobacter	0.8	1.5	0.0	0.0	1.9	1.0	0.0	0.0	0.4	0.7	0.0	0.0
	hoa5_07d05_gut_group	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0
	IS(Lachnospiraceae)	0.5	1.1	4.8	0.8	1.4	0.0	3.0	1.7	0.7	0.0	0.4	1.0
	IS(Ruminococcaceae)	0.0	2.0	2.0	1.9	0.0	1.3	4.1	0.0	1.0	0.0	0.0	1.7
	Johnsonella	1.2	0.0	0.0	1.2	0.0	0.8	0.7	0.0	0.0	0.4	0.6	0.5
	Lachnospira	0.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Lactobacillus	9.9	8.2	10.7	6.2	7.0	22.0	12.8	18.7	11.5	19.7	7.6	36.8
	Lactococcus	0.0	0.0	0.0	0.0	0.0	0.0	0.0.	0.0	0.0	2.7	2.4	0.5
	Marvinbryantia	2.6	0.8	0.6	1.5	0.8	0.3	7.9	4.4	1.0	0.0	0.5	0.0
	Oribacterium	0.9	0.0	0.3	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Paludibacter	2.9	1.1	6.3	1.0	0.7	0.5	0.0	0.0	1.0	0.4	0.0	0.7
	Parasutterella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
	Phasolarctobacterium	3.8	0.0	0.9	2.0	0.9	0.4	1.1	0.0	0.0	11.4	0.0	0.0
	Prevotella	0.0	0.0	0.5	1.1	0.0	0.6	0.0	0.0	2.3	0.4	0.0	1.0
	Pseudobutyrivibrio	0.8	0.0	0.5	0.0	0.6	0.0	0.4	0.0	0.6	0.0	0.0	0.0
	RC9_gut_group	4.4	0.0.	3.5	0.4	7.2	1.4	0.6	0.0	1.3	2.1	0.0	0.5
	Ruminococcus	7.0	4.5	1.9	4.6	0.0	1.7	1.0	3.2	5.7	0.4	3.3	1.7
	Saccharofermentans	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
	Sarcina	1.3	0.5	1.2	0.6	5.9	2.5	0.6	0.4	1.9	1.1	0.3	1.5
	SP3-e08	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
	Streptococcus	0.0	1.9	0.3	1.3	0.0	0.9	0.4	0.3	0.0	0.0	1.7	0.0
	Thalassospira	0.0	0.0	1.3	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	1.0
	Treponema	2.8	4.9	9.1	2.1	2.2	1.5	0.0	1.0	3.9	5.5	0.6	2.6
	Un(B38)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.0	0.6
	Un(Bacteroidales)	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Un(BS11_gut_group)	0.7	0.0	0.0	1.1	0.5	0.5	0.0	0.8	0.0	0.3	0.5	3.7
	Un(Christenenellaceae)	4.2	1.9	9.1	1.7	0.4	0.4	7.5	6.6	4.7	0.4	2.9	1.1
	Un(Clostridales)	0.0	0.6	0.4	1.3	2.4	0.0	2.5	4.8	0.7	0.7	0.7	1.0
	Un(Coriobacteriaceae)	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.3	0.0	0.0
	Un(Erysipelotrichaceae)	1.5	2.4	4.4	0.8	1.0	0.4	0.6	0.8	5.9	1.3	0.0	1.9
	Un(Lachnospiraceae)	18.0	51.0	13.0	43.9	26.1	44.4	26.3	21.9	12.3	16.2	55.9	12.6

	Un (Peptostreptococcaceae)	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Un(Prevotellaceae)	0.0	0.0	0.5	1.2	0.0	0.5	0.0	0.0	1.7	0.7	0.4	1.6
	Un(RF9)	0.5	0.0	0.0	0.0	0.0	0.0	0.6	1.0	0.5	0.0	0.0	0.0
	Un(RF16)	1.0	0.6	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0
	Un(RFP12_gut_group)	3.6	0.4	1.1	0.0	0.6	0.0	0.0	0.0	1.4	0.0	0.0	0.5
	Un(Rhodospirillaceae)	0.0	0.0	1.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Un(Ruminococccaceae)	10.1	5.5	12.0	4.3	14.0	4.5	11.0	10.0	6.8	2.0	7.4	4.5
	Un(S24-7)	9.6	5.0	6.7	13.3	6.5	7.3	4.9	3.5	14.3	4.3	4.1	9.1
	Un(4C0d-2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0
	Uncultured (Clostridiales)	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Weissella	0.0	0.4	0.0	0.5	0.0	0.0	0.0	1.9	0.0	13.8	1.0	4.3
	Xylanibacter	5.6	3.2	4.1	5.7	2.1	5.1	8.9	4.0	10.8	4.7	8.2	4.4
CD	*Acetitomaculum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0
	Actinobacillus	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.1	0.0	0.0	0.5
	Akkermansia	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0
	Alistipes	0.4	0.0	0.0	0.0	0.0	0.0	0.5	0.3	0.0	0.0	0.0	0.0
	Anaerosporobacter	0.0	0.0	0.5	0.0	0.0	1.0	0.0	0.6	0.0	0.0	0.4	0.0
	Anaerovorax	0.0	0.0	1.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Bacteroides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
	Blautia	0.5	0.0	0.0	0.0	0.0	0.4	0.8	0.9	0.4	0.0	0.0	0.6
	Clostridium	0.8	0.4	3.5	0.5	1.0	3.5	0.9	0.6	0.7	0.9	4.3	0.7
	Coprococcus	0.6	0.7	0.0	1.1	0.6	1.2	0.8	1.6	0.0	0.5	0.0	0.4
	Desulfovibrio	3.2	0.0	0.0	0.0	0.0	0.0	1.0	0.9	0.4	0.0	0.0	0.6
	Enterorhabdus	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	Fibrobacter	0.7	0.4	2.4	0.0	0.0	0.7	1.0	0.0	0.7	1.2	0.0	0.0
	hoa5_07d05_gut_group	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
	IS(Lachnospiraceae)	0.9	0.3	1.8	1.4	0.6	0.7	1.3	0.5	2.1	1.3	0.0	0.8
	IS(Ruminococcaceae)	0.0	0.8	0.8	3.3	0.0	0.0	12.0	0.6	0.0	0.0	0.0	1.2
	Johnsonella	0.7	0.4	0.0	0.6	0.0	0.3	1.2	0.4	1.4	0.5	1.5	0.5
	Lachnospira	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
	Lactobacillus	11.9	5.1	6.4	9.5	9.4	24.3	18.8	13.1	9.4	12.4	7.7	42.1
	Lactococcus	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	1.3	1.2	0.0
	Marvinbryantia	0.8	0.5	0.0	2.3	0.4	1.3	8.2	1.7	0.0	0.4	1.3	0.0
	Oribacterium	0.4	0.5	11.1	0.0	0.0	0.0	0.0	0.4	5.5	0.0	0.8	0.0
	Paludibacter	1.0	0.0	1.7	1.1	0.0	0.0	1.3	1.4	1.5	0.4	0.4	0.7
	Parabacteroides	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
	Phasolarctobacterium	3.1	0.0	0.0	3.9	0.4	0.0	0.0	0.0	0.0	9.4	0.0	0.0
	Prevotella	1.8	0.0	0.0	0.7	0.0	0.0	0.0	0.4	0.0	0.0	0.0	1.2
	Pseudobutyrivibrio	0.4	0.0	0.0	0.0	0.0	0.0	0.6	0.6	0.5	0.0	0.0	0.0
	RC9_gut_group	6.7	0.4	4.9	1.8	0.0	0.0	0.6	3.3	18.3	6.6	4.6	1.1
	Roseburia	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
	Ruminococcus	7.5	1.0	0.0	3.0	1.6	0.8	1.6	3.3	1.0	0.4	0.7	6.5
	Saccharofermentans	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Sarcina	1.1	0.8	6.3	0.6	1.4	5.9	1.4	1.1	1.4	0.8	3.5	1.3
	SP3-e08	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Spriochaeta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
	Streptococcus	0.4	2.0	0.0	0.7	1.3	0.6	0.0	0.3	0.4	0.3	1.2	0.0
	Thalassospira	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.7	0.0	0.0	0.7

	Treponema	3.1	2.8	5.5	1.0	0.0	0.4	0.0	6.3	7.6	2.4	1.8	1.9
	Un(Bac 22)	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Un(Bacteroidales)	0.0	0.9	0.9	0.0	0.0	0.0	0.3	0.0	2.7	0.0	0.7	0.0
	Un(BD2-2)	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Un(BS11_gut_group)	1.0	0.0	1.3	1.5	0.0	0.0	0.0	0.0	0.8	0.0	0.4	1.6
	Un(B38)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
	Un(Christenenellaceae)	2.8	0.4	4.0	1.3	0.3	1.1	3.4	1.9	0.0	3.2	6.8	0.9
	Un(Clostridiales)	1.0	0.5	0.0	0.9	0.0	0.0	1.2	1.2	0.4	1.7	1.0	0.6
	Un(Coriobacteriaceae)	0.0	0.4	0.7	0.0	0.0	0.6	0.0	0.0	0.0	0.6	0.8	0.0
	Un(Erysipelotrichaceae)	4.3	0.3	1.9	1.7	0.4	0.0	1.6	2.8	4.8	0.0	0.0	1.9
	Un(Lachnospiraceae)	13.9	70.6	9.5	37.1	70.1	45.9	15.7	18.9	7.1	23.1	36.6	10.7
	Un (Peptostreptococcaceae)	0.0	0.4	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	2.7	0.0
	Un(Prevotellaceae)	0.0	0.4	2.4	0.5	0.0	0.0	0.0	0.0	3.1	0.5	0.4	0.4
	Un(RF16)	0.4	0.4	1.5	0.0	0.0	0.0	0.5	0.4	2.4	0.7	1.7	0.4
	Un(RF9)	1.0	0.0	0.0	0.8	0.0	0.9	0.0	0.3	0.0	0.0	0.0	0.4
	Un(RFP12_gut_group)	1.6	3.4	2.1	0.5	0.0	0.0	1.0	0.6	1.2	0.0	6.7	0.6
	Un(Rhodospirillaceae)	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	1.2	0..´0	0.7	0.0
	Un(Ruminococccaceae)	8.8	2.5	10.9	10.8	1.4	2.4	6.5	7.4	10.3	3.0	1.6	3.8
	Un(S24-7)	7.9	1.0	11.9	9.4	3.1	5.6	8.5	9.0	5.5	7.1	4.3	9.9
	Un(4C0d-2)	0.4	1.0	0.6	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.4	0.0
	Uncultured (Clostridiales)	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0
	Victivallis	0.0	0.6	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	Weissella	0.0	0.0	0.0	0.0	0.7	0.0	0.0	6.6	0.0	20.0	0.5	1.0
	Xylanibacter	9.5	1.3	0.8	3.1	5.0	2.1	8.6	10.3	2.6	0.9	2.9	6.5
CT	*Acetitomaculum*	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	1.3	0.0	0.0	0.5
	Actinobacillus	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.6	0.0	0.0	0.0
	Akkermansia	0.0	0.0	2.0	4.1	0.0	1.7	10.1	0.0	0.0	0.0	3.2	0.0
	Alistipes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	Anaerosporobacter	0.0	0.0	0.0	0.0	0.9	0.0	0.0	1.0	0.0	0.0	0.0	0.0
	Bacteroides	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
	Campylobacter	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1
	Clostridium	0.0	0.4	1.5	0.0	1.1	1.1	1.1	0.0	0.4	0.0	1.2	0.9
	Coprococcus	0.5	0.8	0.0	1.1	0.0	0.4	0.0	0.4	0.0	0.6	0.0	0.5
	Desulfovibrio	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
	Escherichia Shigella	0.0	0.0	1.0	0.0	0.0	0.8	0.4	0.0	0.0	0.0	0.0	0.0
	Fibrobacter	0.0	1.4	7.4	2.8	2.5	2.2	1.5	0.0	3.9	0.8	0.0	0.0
	hoa5_07d05_gut_group	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0
	IS(Anaerovorax)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0
	IS(Clostridiales _Family XIII)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	1.0	1.4	1.0
	IS(Lachnospiraceae)	1.6	0.6	1.6	0.0	0.4	0.7	0.0	0.0	2.4	0.9	0.0	1.9
	IS(Ruminococcaceae)	2.0	1.6	0.8	4.5	0.4	1.2	3.7	0.0	0.7	0.4	1.6	1.6
	Johnsonella	1.3	0.8	0.0	1.2	0.0	0.0	0.4	0.0	0.4	1.0	1.0	0.8
	Lachnospira	0.0	0.0	0.0	0.0	0.9	4.2	0.0	0.0	1.2	0.5	0.5	0.0
	Lactobacillus	5.1	4.7	8.7	12.2	8.6	8.6	8.1	8.5	8.2	10.9	10.9	17.7
	Lactococcus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.2	0.0
	Marvinbryantia	0.0	0.8	0.4	6.3	0.0	0.4	3.7	0.0	0.5	0.0	0.6	0.0
	Oribacterium	7.3	1.0	4.5	0.0	0.6	1.1	0.5	0.6	1.5	0.0	2.5	1.4
	Paludibacter	1.9	0.0	1.7	6.8	1.0	0.5	5.8	1.1	3.4	0.0	0.6	1.1

	Papillibacter	0.0	0.0	1.7	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0
	Phasolarctobacterium	4.6	0.0	0.0	3.2	1.0	2.9	3.0	0.0	0.0	24.2	0.4	0.0
	Prevotella	0.4	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.4	0.5	0.0	1.7
	Pseudobutyrivibrio	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0
	RC9_gut_group	0.4	0.5	5.4	0.0	24.4	8.4	14.9	1.8	10.3	2.9	6.2	1.5
	Ruminococcus	2.1	1.5	0.0	1.1	0.8	0.4	0.4	0.7	4.6	2.1	0.5	1.9
	Saccharofermentans	0.0	0.0	2.0	0.0	6.9	0.0	0.4	0.0	0.0	0.0	0.0	0.0
	Sarcina	0.5	0.7	2.2	0.9	2.0	1.3	1.4	0.5	0.6	0.4	1.3	0.9
	Streptococcus	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.6	0.4
	Thalassospira	0.0	0.0	0.5	0.0	0.5	0.0	0.0	0.0	0.7	0.0	0.0	1.5
	Treponema	6.2	4.3	7.8	1.7	2.1	0.9	1.0	4.7	11.0	3.3	1.3	2.0
	Un(B 38)	0.0	0.0	1.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
	Un(Bacteroidales)	1.0	0.7	0.8	0.0	0.0	0.8	0.0	0.0	0.6	0.8	0.9	3.9
	Un(BD2-2)	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Un(BS11_gut_group)	0.6	0.0	2.6	5.7	4.1	0.8	0.0	0.0	0.9	0.0	0.0	2.0
	Un(Christenenellacea)	9.4	1.3	2.9	0.0	1.8	1.5	3.7	1.1	1.3	5.0	5.8	2.0
	Un(Clostridiales)	1.4	0.4	0.0	1.6	0.9	1.5	0.0	0.0	0.6	0.0	0.0	0.0
	Un(Coriobacteriaceae)	0.8	0.3	0.5	0.0	0.8	0.0	0.8	0.6	0.0	1.1	0.9	0.0
	Un(Erysipelotrichaceae)	3.8	0.5	0.7	1.3	0.4	2.9	1.1	0.6	2.7	1.2	0.0	0.6
	Un(Lachnospiraceae)	11.4	62.7	10.3	16.2	16.9	19.1	11.1	5.2	16.4	13.3	25.4	20.0
	Un (Peptostreptococcaceae)	0.0	0.6	4.4	6.5	0.0	0.4	0.0	0.0	0.0	0.0	5.1	0.0
	Un(Prevotellaceae)	0.0	0.0	3.7	1.1	0.0	0.6	0.0	0.0	1.2	0.7	0.4	0.0
	Un(RF16)	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0
	Un(RFP12_gut_group)	0.6	0.0	0.0	0.0	0.0	0.0	8.8	0.3	0.9	0.0	0.6	0.0
	Un(Rhodospirillaceae)	0.0	0.0	0.0	0.0	0.4	0.6	0.0	0.0	0.6	0.0	0.4	0.9
	Un(Ruminococccaceae)	20.2	4.2	10.6	7.1	12.2	26.2	8.6	2.9	5.4	7.4	12.6	17.9
	Un(S24-7)	3.4	1.9	10.8	14.5	6.4	6.6	4.4	2.9	8.9	6.7	8.4	7.0
	Un(4C0d-2)	0.0	0.0	0.0	0.0	0.4	0.0	0.7	0.0	0.0	0.0	0.0	0.0
	Victivallis	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
	Weissella	0.0	4.9	0.0	0.0	0.0	0.0	0.0	61.5	0.0	7.1	0.9	0.0

CAE (caecum), CD (colon dorsale), CT (colon transversum), CV (colon ventrale), PG = (*pars glandularis*), PN (*pars nonglandularis*) and SI (small intestine) for the CON feeding group (placebo) and the JAM feeding group (Jerusalem artichoke meal)
Un = unknown members
IS = incertae sedis species

Eidesstattliche Erklärung/ Declaration under oath

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wortlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only sources mentioned and included all the citations correctly both in word or content.

Datum / *date* Unterschrift des Antragstellers / *Signature of the applicant*

Curriculum vitae

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02/2014 -04/2017	Research assistant Martin-Luther-University Halle-Wittenberg Institute of Agricultural and Nutritional Sciences Chair of Animal Nutrition
04/2013 – 01/2014	Parental leave substitute “Universitäts- und Landesbibliothek Sachsen Anhalt” Subject specialization: Biology
10/2010 – 09/2012	Master studies of Biology Martin Luther University Halle-Wittenberg Degree: Master of Science (M. Sc.)

10/2007 – 09/2010	Bachelor studies of Biology Martin Luther University Halle-Wittenberg Degree: Bachelor of Science
08/2000 – 07/2007	Burg-Gymnasium Wettin Degree: Allgemeine Hochschulreife (Abitur)

Further qualifications

08/2014 – 10/2014	Research stay Wageningen University, The Netherlands
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List of publications, conference and further education contributions

publications

Glatter, M.; Borewicz, K.; van den Bogert, B.; Wensch-Dorendorf, M.; Bochnia, M.; Greef, J. M.; Bachmann, M.; Smidt, H.; Breves, G.; Zeyner, A. (2019): Modification of the equine gastrointestinal microbiota by Jerusalem artichoke meal. revision submitted to *PLoS ONE*

Bachmann, M., **Glatter, M.,** Bochnia, M., Greef, J. M., Breves, G., Zeyner, A. (2019): Estimating compartmental and total tract apparent digestibility in horses using internal and external markers. *Livestock Science* 223: 16-23.

Bochnia, M.; Scheidemann, W.; Ziegler, J.; Sander, J.; Vollstedt, S.; **Glatter, M.**; Janzen, N.; Terhardt, M.; Zeyner, A. (2018): Predictive value of hypoglycin A and methylen-cyclopropylacetic acid conjugates in a horse with atypical myopathy in comparison to its cograzing partners. *Equine Veterinary Education* 30 (1): 24–28.

Bochnia, M.; Schürer, C.; Gottschalk, J.; Einspanier, A.; Hillegeist, D.; Wensch-Dorendorf, M.; Greef, J. M.; **Glatter, M**.; Zeyner, A. (2017): Effects of isoenergetic quantities of a low-starch muesli feed high in fat and fibre vs. oat grains on the glycemic and insulinemic responses and feed intake patterns in sport ponies. *Journal of Animal Physiology and Animal Nutrition* 101 Suppl 1: 43–50.

Glatter, M.; Bochnia, M.; Goetz, F.; Gottschalk, J.; Koeller, G.; Mielenz, N.; Hillegeist, D.; Greef, J. M.; Einspanier, A.; Zeyner, A. (2017): Glycaemic and insulinaemic responses of adult healthy warm-blooded mares following feeding with JAM. *Journal of Animal Physiology and Animal Nutrition* 101 Suppl 1: 69–78.

Glatter, M.; Wiedner, K.; Hirche, F.; Mielenz, N.; Hillegeist, D.; Bochnia, M.; Cehak, A.; Bachmann, M.; Greef, J. M.; Glaser, B.; Wolf, P.; Breves, G. Zeyner, A. (2016): Fermentation characteristics along the gastrointestinal tract after feeding of JAM to adult healthy warmblood horses. *Journal of Animal Nutrition* 1 (3).

Glatter, M., Breves, G., & Zeyner, A. (2016). Anwendung von Präbiotika in der Ernährung von Pferden. *Tierärztliche. Umschau*, 71: 65-71.

Bachmann, M.; Wensch-Dorendorf, M.; Wulf, M.; **Glatter, M**.; Siebmann, M.; Bierögel, C.; Schumann, S.; Bulang, M.; Aurich, C. Zeyner, A. (2016): Bolus matrix for administration of dietary markers in horses. *Livestock Science* 185: 43–49.

Bochnia, M.; Ziegler, J.; Sander, J.; Uhlig, A.; Schaefer, S.; Vollstedt, S., **Glatter, M**.; Abel, S.; Recknagel, S.; Schusser, G. F.; Wensch-Dorendorf, M.; Zeyner, A. (2015): Hypoglycin A content in Blood and Urine Discriminates Horses with Atypical Myopathy from Clinically Normal Horses Grazing on the Same Pasture. *PLoS ONE* 10 (9): e0136785.

Schöttner, K.; **Schmidt, M**.; Hering, A.; Schatz, J.; Weinert, D. (2012): Short-day response in Djungarian hamster from different circadian phenotypes. *Chronobiology International* 29 (4): 430-442.

conference and further education contributions

Glatter, M.; Zeyner, A. (2019): Prebiotka und Probiotika in der Pferdefütterung. Pferdefütterung- Aktuelles für Fachberater, Burg Warberg, Warberg (Germany), 22nd – 23rd Mai 2019.

Glatter, M.; Borewicz, K.; van den Bogert, B.; Wensch-Dorendorf, M.; Bochnia, M.; Greef, J. M.; Bachmann, M.; Breves, G.; Smidt, H.; Zeyner, A. (2019): Variability of the equine intestinal microbiota after feeding of Jerusalem artichoke meal. 73rd Conference of the Society of Nutrition Physiology, Göttingen (Germany), 13rd -15th March 2019.

Glatter, M.; Bochnia, M.; Wensch-Dorendorf, M.; Greef, J. M.; Zeyner, A. (2019): Variation of the hygienic status of hay due to soaking and steaming as well as subsequent storage at different temperatures. 73rd Conference of the Society of Nutrition Physiology, Göttingen (Germany), 13rd -15th March 2019.

Bachmann, M.; **Glatter, M**.; Bochnia, M.; Greef, J. M.; Breves, G.; Zeyner, A. (2019): Estimating apparent digestibility of nutrients in horses using feed-internal and external markers. 73rd Conference of the Society of Nutrition Physiology, Göttingen (Germany), 13rd - 15th March 2019.

Bochnia, M.; Czetoe, A.; **Glatter, M**.; Schuerer, C.; Bachmann, M.; Gottschalk, J.; Einspanier, A.; Koelle,r G.; Wensch-Dorendorf, M.; Zeyner, A. (2019): Feeding two separate meals of crushed or pelleted oat grains from different varieties and the effect on postprandial blood concentrations of amylase, glucose, GLP-1 and insulin in adult healthy horses. 73rd Conference of the Society of Nutrition Physiology, Göttingen (Germany), 13rd -15th March 2019.

Glatter, M.; Wiedner, K.; Hirche, F.; Mielenz, N.; Bochnia, M.; Cehak, A.; Glaser, B.; Wolf, P.; Breves, G.; Zeyner, A. (2017): Fermentation characteristics along the equine gastrointestinal tract after feeding of JAM. 71th Conference of the Society of Nutrition Physiology, Göttingen (Germany), 14th – 16th March 2017.

Glatter, M.; Bochnia, M.; Goetz, F.; Gottschalk, J.; Koeller, G.; Mielenz, N.; Einspanier, A.; Zeyner, A. (2017): Glycemic and insulinemic response of warm-blooded mares after feeding JAM. 71th Conference of the Society of Nutrition Physiology, Göttingen (Germany), 14th – 16th March 2017.

Glatter, M.; Bochnia, M.; Schmiedel, D.; Sitte, K.; Zeyner, A. (2017): Neue Ergebnisse zur Veränderung des Hygienestatus von Heu durch Wässern und Bedampfen sowie die nachfolgende Lagerung. 18. Pferdeworkshop, Burg Warberg, Warberg (Germany), 7th October 2017.

Bochnia, M.; Ziegler, J.; Sander, J.; Schäfer, S.; Uhlig, A.; Abel, S.; **Glatter, M**.; Recknagel, S.; Schusser, G. F.; Wensch-Dorendorf, M.; Zeyner, A. (2016): Atypische Weidemyopathie beim Pferd- Diagnostik und Prävention, 8. Leipziger Tierärztekongress, Leipzig (Germany), 14th – 16th January 2016.

Goetz, F.; Bochnia, M.; **Glatter, M**.; Gottschalk, J.; Koeller, G.; Mielenz, N.; Einspanier, A.; Zeyner, A. (2016): Einfluss von Topinamburmehl auf die Glukose- und Insulinreaktion sowie den pH-Wert im Kot von Pferden. Netzwerktagung Pferdewissen, Göttingen (Germany), 1st March 2016.

Glatter, M.; Hillegeist, D.; Bochnia, M.; Greef, J. M.; Wolf, P.; Breves, G.; Zeyner, A. (2016): Variation of carbohydrate content and degree of polymerization along the equine gastrointestinal tract. 70th Conference of the Society of Nutrition Physiology, Hannover (Germany), 8th – 10th March 2016.

Bachmann, M.; Wensch-Dorendorf, M.; Wulf, M.; **Glatter, M**.; Siebmann, M.; Bierögel, C.; Schuhmann, E.; Bulang, M.; Aurich, C.; Zeyner, A. (2016): Preparation of boluses for administration of dietary markers to horses and investigations on their suitability. 70th Conference of the Society of Nutrition Physiology, Hannover (Germany), 8th – 10th March 2016.

Bochnia, M.; Ziegler, J.; Sander, J.; Schäfer, S.; Uhlig, A.; **Glatter, M**.; Recknagel, S.; Schusser, G. F.; Abel, S.; Wensch-Dorendorf, M.; Zeyner, A. (2016): Hypoglycin A in bodyfluids of horses with atypical myopathy and their cograzing partners. 70th Conference of the Society of Nutrition Physiology, Hannover (Germany), 8th – 10th March 2016.

Bochnia, M.; Schäfer, S.; Simroth, K.; **Glatter, M**., Goetz, F.; Boesel, M.; Bachmann, M.; Zeyner, A. (2016): Chewing frequency and moved distances of warmblood-type horses all-day grazing on an extensive pasture. 70th Conference of the Society of Nutrition Physiology, Hannover (Germany), 8th – 10th March 2016.

Glatter, M.; Borewicz, K.; Wiedner, K.; Mielenz, N.; Bochnia, M.; Hillegeist, D.; Greef, J. M.; Bachmann, M.; Smidt, H.; Glaser, B.; Breves, G.; Zeyner, A. (2016): Einfluss von Inulin aus Topinamburmehl auf die mikrobielle Fermentation im Magen-Darm-Trakt von Pferden . Pferdefütterung- Aktuelles für Fachberater, Burg Warberg, Warberg (Germany), 2nd – 3rd June 2016.

Glatter, M.; Bochnia, M.; Goetz, F.; Gottschalk, J.; Koeller, G.; Mielenz, N.; Hillegeist, D.; Greef, J. M.; Einspanier, A.; Zeyner, A. (2016): Wirkung von Inulin aus Topinamburmehl auf die postprandiale Glukose- und Insulinreaktion im Blut gesunder Pferde. Pferdefütterung-Aktuelles für Fachberater, Burg Warberg, Warberg (Germany), 2nd – 3rd June 2016.

Glatter, M.; Bochnia, M.; Cehak, A.; Bachmann, M.; Wolf, P.; Breves, G.; Zeyner, A. (2016): Compliance between fermentation characteristics in different segments of the terminal tract of horses fed a hay-oats diet alone or added with JAM. 20th congress of the European Society of Veterinary and Comparative Nutrition, Berlin (Germany), 15th – 17th September 2016.

Bochnia, M.; Schäfer, S.; Simroth, K.; **Glatter, M**.; Goetz, F.; Boesel, M.; Bachmann, M.; Zeyner, A. (2016): The daytime chewing frequencies and activity patterns of permanently grazing warmblood type horses. 20th congress of the European Society of Veterinary and Comparative Nutrition, Berlin (Germany), 15th – 17th September 2016.

Bochnia, M.; Scheidemann, W.; Ziegler, J.; Sander, J.; **Glatter, M**.; Vollstedt, S.; Terhardt, M.; Janzen, N.; Zeyner, A. (2016): Aussagefähigkeit von Blutparametern (HGA, MCPA-Konjugate) im Zusammenhang mit der Atypischen Weidemyopathie von Weidepferden (Ergebnisse einer neuen Fallstudie). 17. Pferdeworkshop, Burg Warberg, Warberg (Germany), 24th September 2016.

Glatter, M.; Bochnia, M.; Bachmann, M.; Wiedner, K.; Mielenz. N.; Breves, G.; Zeyner, A. (2015): Untersuchungen zu Effekten von Topinamburmehl auf das equine gastrointestinale Milieu. Göttinger Pferdetage, Göttingen (Germany), 10th – 11th March 2015.

Bochnia, M.; Ziegler, J.; Sander, J.; Schäfer, S.; Uhlig, A.; **Glatter, M**.; Recknagel, S.; Schusser, G. F.; Wensch-Dorendorf, M.; Zeyner, A. (2015): Nachweis von Hypoglycin A und von Stoffwechselmetaboliten. Göttinger Pferdetage, Göttingen (Germany), 10th – 11th March 2015.

Bochnia, M.; Ziegler, J.; Sander, J.; Schaefer, S.; Uhlig, A.; Abel, S.; **Glatter, M**.; Recknagel, S.; Schusser, G. F.; Wensch-Dorendorf, M.; Zeyner, A. (2015): Verifizierende Laborparameter in der Diagnose atypische Weidemyopathie beim Pferd. Zukunft gestalten-40 Jahre Präventivmedizin, Leipzig (Germany), 19th – 20th June 2015.

Glatter, M.; Bochnia, M.; Bachmann, M.; Wiedner, K.; Mielenz, N.; Breves, G.; Zeyner, A. (2015): Impact of JAM on the metabolites of microbial fermentation in the equine gastrointestinal tract. International Scientific Conference on Probiotics and Prebiotics, Budapest (Hungary), 23th – 25th June 2015.

Glatter, M.; Borewicz, K.; Wiedner, K.; Mielenz, N.; Bochnia, M.; Bachmann, M.; Smidt, H.; Glaser, B.; Breves, G.; Zeyner, A. (2015): Feeding of JAM alters the microbial community and fermentation products in the foregut of horses. 19th congress of the European Society of Veterinary and Comparative Nutrition, Toulouse (France), 17th – 19th September 2015.

Glatter, M.; Bochnia, M.; Goetz, F.; Gottschalk, J.; Koeller, G.; Mielenz, N.; Einspanier, A.; Zeyner, A. (2015): Glycaemic and insulinaemic response of adult healthy warmblood mares following supply of JAM. 19th congress of the European Society of Veterinary and Comparative Nutrition, Toulouse (France), 17th – 19th September 2015.

Glatter, M.; Borewicz, K.; Wiedner, K.; Mielenz, N.; Bochnia, M.; Bachmann, M.; Smidt, H.; Glaser, B.; Breves, G.; Zeyner, A. (2015): Feeding of JAM alters the microbial community and fermentation products in the hindgut of horses. 19th congress of the European Society of Veterinary and Comparative Nutrition, Toulouse (France), 17th – 19th September 2015.

Glatter, M.; Borewicz, K.; Wiedner, K.; Mielenz, N.; Bochnia, M.; Hillegeist, D.; Greef, J.-M.; Bachmann, M.; Smidt, H.; Glaser, B.; Breves, G.; Zeyner, A. (2015): Neue Ergebnisse zur Wirkung von Inulin und anderen Fruktanen aus zugefüttertem Topinamburmehl im Magen, Dünndarm und Dickdarm von Pferden. 16. Pferdeworkshop Burg Warberg, Warberg (Germany), 10th October 2015.

Bochnia, M.; Schaefer, S.; **Glatter, M**.; Simroth, K.; Goetz, F.; Boesel, M.; Bachmann, M.; Zeyner, A. (2015): Aktuelle Ergebnisse aus Kaustudien: Verzehrparameter auf der Weide. 16. Pferdeworkshop Burg Warberg, Warberg (Germany), 10th October 2015.

Bochnia, M.; Ziegler, J.; Abel, S.; Sander, J.; Uhlig, A.; Recknagel, S.; Schusser, G. F.; **Schmidt, M**.; Zeyner, A. (2014): Cases of atypical myopathy in middle Germany in 2013 caused by Hypoglycin A? 18th congress of the European Society of Veterinary and Comparative Nutrition, Utrecht (Netherland), 11th – 13th September 2014.

Schöttner, K.; **Schmidt, M**.; Weinert, D. (2010): 12th International Conference *Rodens et Spatium* on Rodent Biology. Zonguldak, Turkey - July 19-23, 2010