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Effect of various iodine supplementations and species on the iodine transfer into milk and on serum, urinary and faecal iodine of dairy cows fed rations varying in the glucosinolate content

Dissertation

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ABBREVIATIONS

ADF	Acid detergent fibre
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
BfR	Federal Institute for Risk Assessment
BP	Breakpoint
BW	Body weight
cAMP	Cyclic adenosine monophosphate
CP	Crude protein
d	Day
D-A-CH	German-, Austrian- and Swiss Nutrition Society
DDGS	Distillers dried grains with solubles
DDG	Distillers dried grains (without solubles)
DG	Distillers grains
DIM	Days in milk
DIT	Diiodotyrosines
DM	Dry matter
DMI	Dry matter intake
DOM	Digestible organic matter
DRI	Dietary Reference Intake
DVG	German Veterinary Medical Society
DWG	Distillers wet grains
EDDI	Ethylenediamine dihydroiodide
EFSA	European Food Safety Authority
EHPM	European Federation Association of Health Products Manufacturers
EMFEMA	International Association of the European Manufacturers of Major, Trace and Specific Feed Mineral Materials
ERNA	European Responsible Nutrition Alliance
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FLI	Friedrich-Loeffler-Institute
fT_3	Free triiodothyronine
fT_4	Free tetraiodothyronine
GfE	Society of Nutrition Physiology

GSL	Glucosinolate(s)
ICCIDD	International Council for the Control of Iodine Deficiency
ICP-MS	Inductively coupled plasma-mass spectrometry
IDD	Iodine deficiency disorders
IGF-1	Insulin-like growth factor
KIGGS	Kinder- und Jugendgesundheitssurvey
LE	Energy content of milk
LSmeans	Least square means
ME	Metabolizable energy
MIT	Monoiodotyrosines
MRI	Max Rubner-Institute
n.d.	Not determined
NDF	Neutral detergent fibre
NEL	Net energy lactation
NIS	Sodium iodide symporter
NRC	National Research Council
OM	Organic matter
P_i	Phosphate
PBI	Protein bound iodine
PSEM	Pooled standard error of means
RES	Rapsextraktionsschrot
RIA	Radio-immunoassay
RNB	Ruminal nitrogen balance
RSM	Rapeseed meal
rT_3	Reverse triiodothyronine
SCF	Scientific Committee on Food
SCN	Thiocyanate(s)
SEM	Standard error of the mean
SHIP	Study of Health in Pomerania
T_2	Diiodothyronine
T_3	Triiodothyronine
T_4	Tetraiodothyronine/ thyroxine
Tg	Thyroglobulin
TMAH	Tetramethylammonium hydroxide
TMR	Total mixed ration
TPO	Thyroid peroxidase
TRH	Thyreotropin releasing hormone
TSH	Thyreoidea stimulating hormone

TSHR	TSH receptor
uCP	Utilizable crude protein
UDP	Undegraded feed protein
UL	Tolerable Upper Intake Level
US	United States
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten
WHO	World Health Organization
ZMP	Zentrale Markt- und Preisberichtstelle GmbH

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GENERAL DISCUSSION

INTRODUCTION

Iodine is an essential trace element for humans and animals. Worldwide still 13% of the population suffer from the implications of iodine deficiency (FAO, 2004). Therefore the German Federal Institute for Risk Assessment (BfR, 2006) assigned iodine to the supply category "1" which indicates a high risk of deficiency. On the other hand iodine is characterized by a high risk of overdosing (Risk category "High" ; ERNA and EHPM, 2004; BfR, 2006), since the margin between the requirement of 150-200µg/d (D-A-CH, 2000; WHO et al., 2001) and the Tolerable Upper Intake Level (UL) of 500-1000µg/d (WHO, 1994b; D-A-CH, 2000) is narrow.

Besides salt iodization in recent years efforts are underway to increase the iodine content of food of animal origin (milk, eggs, meat) by supplementation of feedstuffs. A high carry over of feed iodine was observed into milk and eggs (Swanson et al., 1990; Richter, 1995; Kaufmann and Rambeck, 1998; Flachowsky et al., 2006; Schöne et al., 2009) while no considerable enrichment in meat seems possible at the permitted maximum level of iodine in feed (Franke et al., 2008; Meyer et al., 2008). Enhanced feed iodine supplementation led to increasing iodine contents of consumers' milk during the last 10 years (Launer and Richter, 2005; Jahreis et al., 2007; Stiftung Warentest, 2007). Results of an investigation of European consumers' milk showing mean concentrations of up to 601µg/L (Rysava et al., 2007) point out that in future implications of excessive iodine intake may gain in importance. In a previous study with low-yielding cows a mean milk iodine concentration of 1215µg/kg was detected at the presently permitted maximum level of iodine in feed (Schöne et al., 2009). Since a declaration of the iodine concentration of food of animal origin seems impossible, the iodine content of milk has to be narrowed down by setting low maximum levels in animal feed. On the other hand they still have to be practicable for feed manufacture. In 2005, the European Food Safety Authority (EFSA) evaluated benefits and risks of iodine supplementations in animal feed and established that more dose-response studies are needed to achieve secure information about the transfer of iodine from feed into food of animal origin.

Like the thyroid the mammary gland possesses an active transport system (sodium iodide symporter, NIS) which allows iodine accumulation from the blood and which is competitively inhibited by thiocyanate (SCN) a degradation product of the glucosinolates (GSL; e.g. in rapeseed feedstuffs; Brown-Grant, 1957; Laurberg et al., 2002). In this way diets containing rapeseed not only diminish the iodine transfer into the thyroid but also into milk (Papas et al., 1979; Schöne et al., 2006). As a result, they also may influence urinary, faecal and blood iodine (Lengemann, 1970; Miller et al., 1975a). The utilization of rapeseed meal (RSM) and rapeseed press cake in animal feed gains in importance due to the expectable increase in by-product availability from bioenergy manufacture. But up to now the quantitative impact of

the nowadays applied 00-rapeseed on the mentioned parameters is poorly investigated. No newer study exists investigating the impact of various iodine supplementations (within the permitted maximum level) and rapeseed application on the iodine status of dairy cows as well as on all main routes of iodine excretion (urine, milk and faeces) in the same trial.

Furthermore, studies indicate that different iodine species may be metabolized differently and therefore as well may vary in the extent they are transferred into milk. While Miller and Swanson (1973) and Swanson et al. (1990) observed higher milk iodine and lower urinary iodine concentrations for ethylenediamine dihydroiodide (EDDI) compared to potassium iodide, studies comparing iodide and iodate are rare and controversial. Lengemann (1969) and Bretthauer (1972) described no difference in milk and plasma iodine after daily application of iodide or iodate. When applying a single iodine dosage a delayed absorption is described for iodate compared to iodide due to its conversion to iodide prior to absorption (Moss and Miller, 1970). In the EU sodium iodide, potassium iodide, calcium iodate hexahydrate and calcium iodate anhydrous are approved for feed supplementation (EU, 2005). Iodides are characterized by a higher instability in presence of oxygen compared to iodates. Up to now it is not sufficiently examined if feed supplementations in the form of iodide may lead to higher storage losses causing a lower iodine supply status of the animal and lower iodine contents of milk, urine and faeces.

The performance of the animals was not expected to be influenced by the iodine supplementations or the different iodine species, since the feed iodine content just seems to exert an influence in dairy cows in long lasting iodine deficiency or strong iodine excesses (Groppel, 1993; Paulikova et al., 2002). Furthermore, ruminants tolerate high levels of GSL (Tripathi and Mishra, 2007), so that the tested RSM application as well should not alter the performance of the animals. In contrast, the equal applicability of high amounts of the applied DDGS in rations of dairy cows compared to RSM is rarely investigated, since it is just extensively utilized in animal nutrition for few years. In general, it is assumed that DDGS feeding results in similar economically relevant performance parameters (Urdl et al., 2006; Spiekers et al., 2006; Ettle, 2007), but the nutrient value differs due to the utilized raw material (maize, wheat, barley, rye, sorghum or mixtures from wheat and barley) and the processing (extent of fermentation and drying process; Belyea et al., 1998; Spiekers et al., 2006).

BACKGROUND

1 Iodine and iodine sources

Iodine is a member of the halogen family and elemental iodine is volatilized in the presence of sunlight and heat. Iodine losses can be minimized by maintaining iodine in alkaline mixture (McDowell, 2003). In nature iodine underlies a permanent cycle (Anke et al., 1993). In soil, plants and water iodine predominately is present in the form of iodide (Hetzel and Maberly, 1986). The iodine is leached out from the deposit in soil by rain, glaciers and floodings and in this way reaches the ocean. At the surface it is oxidized and escapes into the atmosphere. The atmospheric iodine then gets back to the soil by rain or snow. Since this cycle is unbalanced, at present the sea water contains the largest amounts of iodine (50µg/L) on earth while many soils worldwide are poor in iodine. Iodine deficient areas are generally associated with high distance from the ocean, low annual rainfall and/or recent glaciation (McDowell, 2003).

Since iodine seems to be not essential for plants (Schöne and Rajendram, 2009), food and feed of plant origin contain low amounts of iodine. According to the BfR food of plant origin features iodine contents between 0.3 and 5.0µg/100g fresh matter (BfR, 2006) while a recent summarization of iodine in common feedstuffs showed concentrations between 0-1mg/kg DM (EFSA, 2005). Next to the iodine content of plants the iodine concentration of water depends on the region and is quite low in Germany (1-9µg/L; BfR, 2004; Anke, 2007).

Due to the low native contents, iodine has to be supplemented in feed to avoid a deficiency of livestock. The iodine supply of humans in Germany presently consists of iodine from (1) native sources, (2) iodized salt and food produced with iodized salt, (3) food enriched by feed iodine supplementation and (4) dietary supplements.

Considerable native iodine amounts are just found in sea fish (50-560µg/100g) and sea algae (0,5-1100mg/100g T; Höhler et al., 1990; BfR, 2007; Souci et al., 2008). For the improvement of human iodine supply various food vehicles have been enriched with iodine (bread, milk, water and salt) or supplements have been given directly as aqueous solution or iodized oil (intramuscular or oral; WHO, 2004). In the 1990s the World Health Assembly declared that the universal salt iodization (iodization of salt for both human and livestock consumption) seems to be the method of choice, since it provides a cheap, secure and easily implementable possibility to eliminate iodine deficiency disorders (WHO, 2004). Hence, salt at present is the most commonly used vehicle. In Germany, the universal application of iodized table salt is permitted since 1989. It was estimated that complete replacement of normal salt by iodized salt with the current iodine content of 15-25mg/kg could ensure sufficient iodine supply of the German population (Gärtner, 2000). But the application of

iodized salt especially in manufacture which considerably elevates the iodine content of bread, bakery products, cheese, sausage and convenience foods (Großklaus and Jahreis, 2004; Sperrhake, 2005) stagnates since 1995 (Großklaus and Jahreis, 2004; Hampel and Zöllner, 2004).

The application of iodine to livestock as salt blocks or by direct addition to the mineral feed may contribute to improving human iodine supply, since food of animal origin can be enriched with the essential trace element at sufficient iodine supply of the animals. A considerable carry over of iodine from feed into milk and eggs is investigated while the transfer into meat is low (Richter, 1995; Kaufmann and Rambeck, 1998; Franke et al., 2008; Meyer et al., 2008; Schöne et al., 2009).

Iodine supplements are predominately recommended for pregnant or nursing women. Hampel and Zöllner (2004) described an increasing consumption of dietary iodine supplements (containing 50-250µg iodine per tablet) in Germany.

Regarding the various iodine sources, in 2004 it was estimated that the contribution of milk and milk products to human iodine supply approximately amounted to 37% while meat and meat products contribute 24%, bread and bakery product 19%, fruits and vegetable 3% and sea fish about 9% to the supply (Großklaus and Jahreis, 2004).

2 Iodine status

2.1 Iodine status of humans

The urinary iodine is considered as a reliable parameter for the assessment of the human iodine supply (Bourdoux, 1993; WHO, 1994a) whereby a 24h urine collection seems to be the best but mostly not practicable method.

Since the iodine excretion via urine is not actively mediated, urinary iodine just depends on the iodine intake and the quantity of urine. The influence of the urinary quantity can be abandoned by setting the urinary iodine in relation to the constantly excreted creatinine (iodine-creatinine quotient). However, the creatinine excretion may be altered at deficient protein supply and disturbances in the muscle metabolism. Moreover, the iodine-creatinine quotient is strongly age-related (Remer and Manz, 1994), so that many authors provide results without creatinine basis. Bourdoux (1993) established a classification for assessing the iodine supply of a population (**Table 1**, requiring about 50-100 individuals) which next to the identification of a normal iodine status distinguishes between moderate, medium and serious deficiency. According to the WHO (1994b), median urinary iodine concentrations above 100µg/L are a sign of a sufficient supply while a mild iodine deficiency (grade I) is indicated by concentrations between 50-99µg/L, moderate iodine deficiency (grade II) in the range of 20-49µg/L and a serious iodine deficiency (grade III) is found at a median < 20µg/L.

The WHO (2004) estimated that still 35.2% of the worldwide and 56.9% of the European population feature an insufficient iodine intake (urinary iodine < 100µg/L) while the FAO (2004) states a percentage of 13% of the world's population which suffer from iodine deficiency. In Germany a monitoring with a representative number of kids and juveniles in the age of 0-17 years (Kinder- und Jugendgesundheitssurvey, KIGGS-study) in 2003-2006 detected a median urinary iodine concentration of 117µg/L (Thamm et al., 2007). From the results it was concluded that following the classification of the WHO (1994a) Germany no longer is considered as an iodine deficient area but iodine intake is located at the lower limit of a sufficient supply. Within the Study of Health in Pomerania (SHIP) with participants in the age of 20-79 years the urinary iodine excretion in the lower desirable region (median = 124µg/L) was affirmed for adults as well (Völzke et al., 2003). However, special groups of the population like vegetarians or vegans feature an increased risk for iodine deficiency, since important iodine sources like fish, milk or eggs are missing (Remer et al., 1999).

Table 1: Classification for the assessment of the iodine status of a population by analysis of 50-100 randomly collected samples established by Bourdoux (1993)

Urinary iodine [µg/L]	Normal iodine status	Deficiency		
		moderate	medium	serious
≤ 20		20-30%	30-50%	> 50%
≤ 50		≤ 70%	> 70%	> 80%
≤ 100	< 50%	> 50%	> 80%	> 90%

The classification allows an assessment of the supply status of a population (normal iodine status, moderate, medium and serious deficiency) by the assignment of the measured urinary iodine contents of 50-100 randomly collected individuals to diverse urinary iodine levels (≤ 20, ≤ 50 and ≤ 100µg/L)

2.2 Iodine status of dairy cows

Similar to humans, urinary iodine quite well reflects the iodine supply of cattle at low iodine intakes and therefore is used as an indicator for the iodine supply (Kroupova et al., 1996; Herzig et al., 1996). Herzig et al. (1996) tested the applicability of the method for the assessment of the iodine intake of humans (**Table 1**) to dairy cows and found that the number of individuals can be reduced from 100 to 25, since just female organisms with a limited variability in feed and water intake are investigated.

Furthermore, total or protein bound iodine (PBI) in blood is used for the estimation of the iodine status of dairy cow herds whereby total iodine concentrations below 40µg/L indicate a deficient and contents above 200µg/L an excessive iodine supply (Alderman and Stranks, 1967; Launer and Richter, 2005). PBI mainly consists of the thyroid hormones and contents below 30-40µg/L are a sign of deficient supply in adult cattle (McDowell, 2003). The thyroid hormones in blood (especially T_4) can show an existing deficiency (Groppel, 1993) but do not reflect different levels of iodine supply at sufficient iodine intake (Convey et al., 1977; Hillman

and Curtis, 1980; Grace and Waghorn, 2005). Moreover, they are dependent on the functionality of the thyroid gland and therefore are no reliable parameters for the iodine intake.

The good and fast reflectance of the iodine intake in milk allows the utilization of milk iodine as an indicator for the supply as well. It is stated that concentrations below 20-25µg/L indicate a deficient supply (Alderman and Stranks, 1967; Miller et al., 1975b).

Newer studies on the iodine supply of dairy cow herds are rare. Scherer-Herr (2001) investigating the supply of a herd in North Rhine-Westphalia on the basis of urinary iodine, detected a normal iodine status with 55% of the animals featuring concentrations above 100µg/L (see **Table 1**). Launer and Richter (2005) examined the iodine concentration in serum and milk of dairy cows in Saxony from 1997-2003. In the end of the investigation period both serum and milk showed a sufficient supply whereby in some herds the concentrations even indicated an excessive iodine intake. Older investigations by Hamann und Heeschen (1982) ascertained that in Southern Germany urinary iodine of dairy cow herds are lower than in Northern Germany.

3 Iodine metabolism of ruminants

3.1 Iodine absorption

Iodine is predominately absorbed in the form of iodide. Iodate and most organic iodine compounds are converted into iodide by reducing agents prior to absorption (Cohn, 1932; Cavalieri, 1997). For iodide an almost complete absorption from the gastrointestinal tract is described (Cavalieri, 1997; Vandecasteele et al., 2000) which is not diminished at high iodine intakes (Miller, 1975). More recent studies with mice and rats, finding similar sodium iodide symporters in the intestine like in the thyroid indicate that at least in some parts of the gastrointestinal tract the iodine absorption may occur as an active transport (Nicola et al., 2009). Some kinds of organically bound iodine (protein bound iodine, iodinated amino acids, thyroxine) might be absorbed in the bound form but feature a strongly diminished bioavailability of 40-70% (Underwood, 1962; Swanson et al., 1965; Heseker, 1999).

Besides the mentioned similarities, in ruminants iodine absorption and secretion proceed quite different to that in humans due to the differences in the constitution of the gastrointestinal tract. In contrast to monogastric animals, in ruminants 70-80% of the iodine is already absorbed in the rumen and additional 10% in the omasum (Barua et al., 1964; Miller et al., 1975b; Groppel, 1993; Simon, 2008) while less absorption takes place in the abomasum (Miller et al., 1975b). Since iodine is also endogenously secreted back into the gastrointestinal tract (especially into the abomasum), considerable absorption also occurs in the later segments (Barua et al., 1964).

Next to absorption in the gastrointestinal tract, iodine can be absorbed in the lungs or via the skin whereby the latter is of particular importance in cows, since iodine containing teat dips are frequently used and lead to direct transfer of the absorbed iodine into the milk (Flachowsky et al., 2007).

3.2 Distribution and tissue uptake

The absorbed iodide in blood is loosely bound to plasma proteins for transport (Heseker, 1999; McDowell, 2003). A simplified scheme of the iodine distribution in dairy cows established by Miller et al. (1975b) is given in **Figure 1**. The small amount of inorganic iodide in the body mainly is located in the extracellular fluid, but it is also found in red blood cells and the intraluminal fluids of the gastrointestinal tract (Saller et al., 1998).

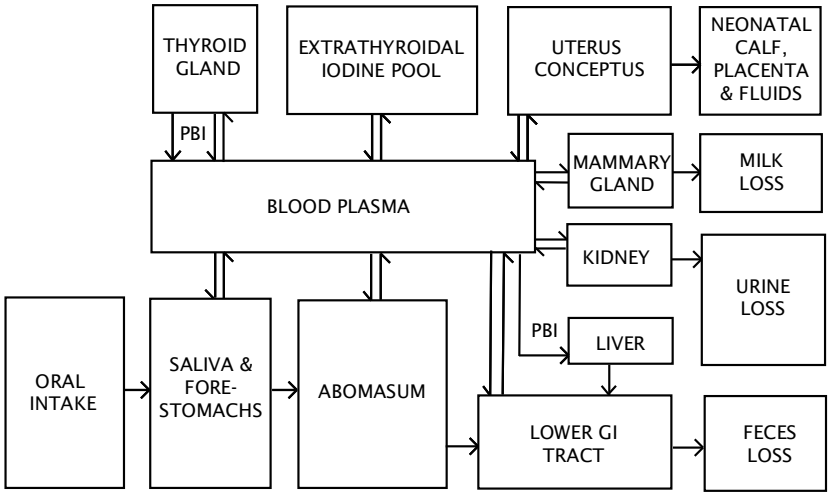

Figure 1: Major physiological compartments of iodine recycling in the cow adapted by Miller et al. (1975b)
PBI = protein bound iodine

Iodide ions can permeate all tissues (McDowell, 2003) but the thyroid as main storage organ for iodine and place for the synthesis of the thyroid hormones triiodothyronine (T_3) and tetraiodothyronine (T_4) actively accumulates iodide by the sodium iodide symporter (NIS). Therefore thyroidal iodine accounts for approximately 90% of the total body iodine (Simon, 2008) and 70-80% of the total inorganic iodide (McDowell, 2003). Accessorily the NIS allows iodide accumulation in the mammary gland, salivary glands, gastric mucosa, choroid plexus and ciliary body of the eye (Carrasco, 1993). From the salivary glands and gastric mucosa iodine again reaches the gastrointestinal tract and afterwards can be reabsorbed. In this way inorganic iodine underlies a turn over several times a day (Saller et al., 1998). Thereby the abomasum is described to be the major site for reentry of iodide into the gastrointestinal tract in the bovine. The endogenous iodine secretion into the abomasum is realized by the chief and mucosal cells of the gastric mucosa (Miller et al., 1975b). A study showed that after

intravenously injection of iodide 65% of the dose was found in the abomasum after 6h (Swanson and Miller, 1973). It is considered that the stomach may serve as an iodine reservoir which causes removal of iodine from the blood and therefore reduces iodine losses via urine (Miller et al., 1975a; Miller et al., 1975b). Although Barua et al. (1964) stated that iodine secretion seems to appear throughout the whole gastrointestinal tract it is not completely clarified if the observed increases in ruminal iodine after subcutaneous injection result from direct secretion through the epithelium or from secretion by the saliva. However, in ruminants the accumulation of iodine by the salivary glands is much lower than in humans (Miller et al., 1975b) and low ruminal iodine contents show that all segments of the gastrointestinal tract prior to the stomach concentrate comparably low amounts of iodine.

In contrast to iodide, the secretion of thyroxine (T_4) into the gastrointestinal tract mainly occurs after uptake in the liver where a part of the T_4 from blood is constantly degraded or conjugated and via bile is secreted into the small intestine (Miller et al., 1975b). Only small amounts pass directly into the stomach, jejunum and colon (Underwood, 1962). Most of the T_4 and organic degradation products secreted into the gastrointestinal tract are not reabsorbed and therefore are excreted by faeces (Miller et al., 1975b). However, the major part of T_4 is deiodinated and the resulting iodine is excreted via urine.

3.3 Thyroid function

In the thyroid the NIS which allows the active ATP dependent transport of iodine into the thyroid is extensively investigated (**Figure 2**; Carrasco, 1993; Schmutzler and Köhrle, 1998; Spitzweg and Morris, 2002). It is located in the basolateral plasma membran of the thyroid follicular cells (thyrocytes). In ruminants, an accumulation from the blood by the factor 10-40 is described under physiological conditions (Wolff, 1964).

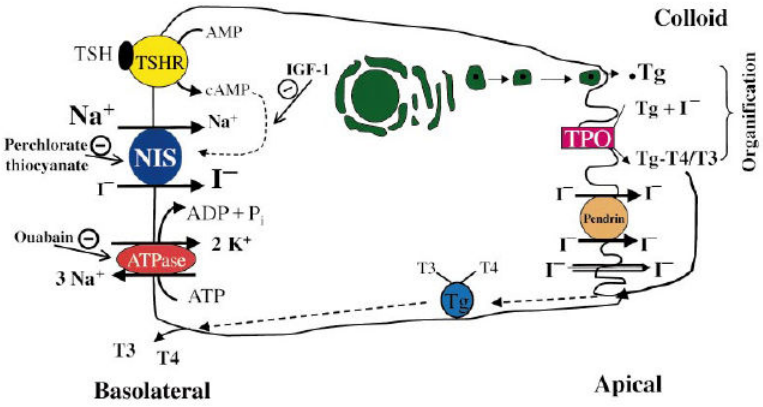

Figure 2: Schematic illustration of the main routes of iodine transport and thyroid hormone synthesis in the thyrocyte by Spitzweg and Morris (2002)
ADP = adenosine diphosphate, AMP = adenosine monophosphate, ATP = adenosine triphosphate, cAMP = cyclic adenosine monophosphate, IGF-1 = Insulin-like growth factor, NIS = sodium iodide symporter, P_i = phosphate, Tg = thyroglobulin, TPO = Thyroid peroxidase, TSH = Thyreoidea stimulating hormone, TSHR = TSH receptor,

Afterwards the iodide is transported passively across the apical membrane into the colloid where it is oxidized to elemental iodine at the cell colloid interface with the aid of hydrogen peroxide (H_2O_2) and catalyzed by the Thyroid peroxidase (TPO). The activated iodine is incorporated into tyrosyl residues within a thyroglobulin leading to monoiodotyrosines (MIT) and diiodotyrosines (DIT) which are subsequently coupled by a TPO regulated process. Depending on the coupled iodothyrosines (MIT+DIT or DIT+DIT) triiodothyronine (T_3) or tetraiodothyronine (T_4) is synthesized (**Figure 3**). The iodinated thyroglobulin is stored in the colloid until its utilization. On demand, the iodinated thyroglobulines enter the thyrocytes by endocytosis and after phagolysosomal hydrolysis of this complex T_3 and T_4 are secreted into the blood. Non utilized MIT and DIT is metabolized by iodothyrosine dehalogenase to tyrosin and iodide for reuse in the thyroid or release into blood ("iodide leak"), e.g. in chronic iodine excess or special disorders (Cavalieri, 1997).

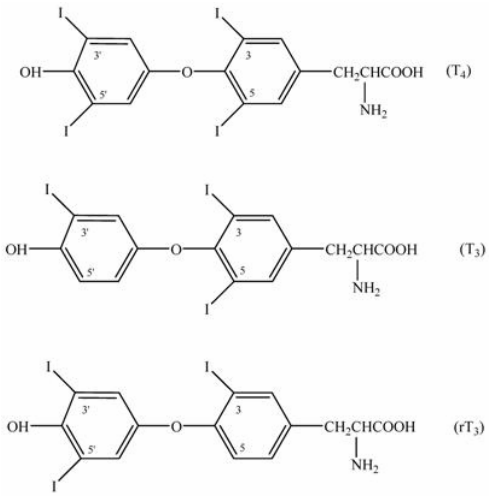

Figure 3: Structures of the thyroid hormones triiodothyronine (T_3), tetraiodothyronine (T_4) and the inactive form reverse triiodothyronine (rT_3)

In the blood the thyroid hormones are predominately bound to transport proteins whereby the most important are thyroxine binding globuline, transthyretin and albumin (Cavalieri, 1997). The protein bound hormones are inactive. With about 0.05% the active, unbound forms of T_3 and T_4 (fT_3 and fT_4) represent a relative small proportion in blood (Leirer et al., 1983). The transport of the thyroid hormones into the cell is carried out in the unbound form. Although T_3 is already built in the thyroid the main part of the more active form (at least ten times the activity of T_4) is evolved from the degradation of T_4 in the target cell by selenium-dependent iodothyronine deiodinases. Furthermore, the inactive form rT_3 (**Figure 3**) can be built from T_4 for the regulation of the thyroid hormone status. Different isoenzymes of deiodinases have been identified (Köhrle, 1994; Larsen and Berry, 1995). Type I mainly found in liver, kidney and thyroid is described to produce most of the circulating T_3, type II mainly is existent in the

central nervous system, brown fat tissue and pituitary gland primarily is responsible for deiodination of T_4 to T_3 for local use and type III mainly occurring in brain and plazenta catalyzes the conversion of T_4 to rT_3 and of T_3 to T_2 (McDowell, 2003). In this way the T_3 content of the cell is regulated by the rate of cellular uptake, the transformation of T_4 into T_3 and the T_3 efflux. The released iodine from the conversion of T_4 into T_3 and rT_3 or from further degradation reenters the circulation or is excreted. The active hormones in the cell regulate the induction of the transcription and hereby influence the synthesis of proteins concerning growth and maturation of the brain and the skeletal system (Heseker, 1999).

3.4 Hormonal regulation and autoregulation of thyroid function

The iodine and thyroid hormone metabolism are regulated by the amount of free thyroid hormones in the blood whereby low fT_3 and fT_4 levels provoke the release of Thyreotropin releasing hormone (TRH) from the hypothalamus which induces the secretion of Thyreoidea stimulating hormone (TSH) by the pituitary gland. TSH stimulates the iodine uptake into the thyroid by the NIS and all phases of biosynthesis and secretion of the thyroid hormones (Schmutzler and Köhrle, 1998; McDowell, 2003). With regard to the iodine uptake, an activation of existent, inactive NIS, the transport of NIS to the plasma membrane, a fortified NIS expression as well as an increase of ATP production and ATPase activity by TSH is described (Cavalieri, 1997; Saller et al., 1998). Within the hormone synthesis TSH provokes an elevated synthesis of TPO and thyroglobulin as well as an increased H_2O_2 production whereat in the process of thyroid hormone secretion a stimulation of endocytosis from colloid into the thyrocyte and proteolysis of the thyroglobulin by TSH was ascertained (Cavalieri, 1997; Saller et al., 1998). Intrathyroidal iodine regulates the formation of the thyroid hormones and the sensitivity of the thyroid towards TSH which is called “autoregulation “ (Ingbar, 1972; Nagataki and Yokohama, 1996; Cavalieri, 1997).

3.5 Excretion

The main routes of iodine elimination in dairy cows are milk, urine and faeces. Important iodine losses via sweat just occur in tropical areas with low dietary iodine supply (McDowell, 2003).

3.5.1 Milk

3.5.1.1 Iodine transfer into milk

Comparable to humans (10-15%; Saller et al., 1998), it is stated for dairy cows that a proportion of 10% of the total iodine is excreted via milk (Simon, 2008). However, strong differences in the distribution to the different routes of iodine excretion (urine, faeces, milk)

occur due to cow individuality (Phillips et al., 1935). Schöne et al. (2009) found that 35-47% of the supplemented iodine was excreted via milk. Hence, next to urine the iodine excretion via milk plays an important role in iodine clearance from the blood in lactating dairy cows (Swanson et al., 1990; Schöne et al., 2009). Like described above the lactating mammary gland comparable to the thyroid accumulates iodide by the NIS (Cavalieri, 1997; Laurberg et al., 2002). Studies with rats show that in the mammary gland, a part of the iodide can be bound to tyrosyl residues of caseins and other milk proteins due to the existence of peroxidase activity (Strum, 1978). However, the predominant amount of iodine in milk is inorganic (Underwood and Suttle, 1999). While Miller et al. (1975b) state that about 10% of the milk iodine is present in the organically bound form Bretthauer (1972) detected that 97% was inorganic.

3.5.1.2 Factors affecting milk iodine

With regard to the milk iodine concentration as an indicator for the iodine supply of the animal, next to the iodine intake (see section 4.1) the most important influencing factor seems to be the utilization of iodine containing teat dips (Hemken, 1979; Flachowsky et al., 2007). Most teat dips (applied before or after milking) contain iodine whereby the content varies between 1-10g/L. Considerable amounts of iodine are absorbed through the skin and thereby get into the milk (Hemken, 1979). Several studies summarized by Flachowsky et al. (2007) showed that the milk iodine concentration is increased by teat dipping by 11 -150µg/kg. Moreover, the improperly use of iodine sanitizing agents for cleaning the milking parlour and teat cups can cause strong increases of milk iodine (Hemken, 1980; Hamann and Heeschen, 1982).

Furthermore, milk iodine is increased by iodine containing drugs (e.g. for treatment of foot-rot and soft tissue lumpy jaw) and in goats an elevation with rising temperature was observed (Lengemann, 1979). In contrast, feed containing goitrogens (like GSL in rapeseed) causes diminished milk iodine concentrations (see section 4.3). Milk iodine as well varies due to the breed (Franke et al., 1983) and the applied iodine species (see section 4.2). Older studies describe an inverse relationship between milk yield and milk iodine (Miller et al., 1963; Iwarsson, 1973) but later studies rather ascribe this effect to the different stages of lactation (Franke et al., 1983; Larson et al., 1983). Regarding the impact of the lactation, it was shown that colostrum generally features considerably higher milk iodine contents than later milk (Lewis and Ralston, 1953; Groppel, 1993). Apart from that, data are controversial. Franke et al. (1983) detected increases of milk iodine with rising stage of lactation while Scherer-Herr (2001) showed decreased concentrations and Kroupova et al. (1996) found no differences.

3.5.1.3 Importance for human nutrition

The high carry over of feed iodine into milk (Swanson et al., 1990; Kaufmann and Rambeck, 1998; Flachowsky et al., 2006; Schöne et al., 2009) leads to considerable iodine contents of this food of animal origin. Since 90% of the milk iodine seems to be available, milk and its products provide an important iodine source for humans (Jahreis et al., 2007). At present their contribution to human iodine intake in Germany is estimated at 40% (Jahreis et al., 2007).

The milk iodine seems to adapt to an altered iodine supply within 7-10 days after the start of daily supplementation (Miller et al., 1975b). The enhanced application of iodine in feedstuffs led to rises of the iodine content of consumers' milk in the last 10 years (Bader et al., 2005; Launer and Richter, 2005; Jahreis et al., 2007). While the German Nutrition table of Souci et al. (2008) for unskimmed milk still states an iodine concentration of 27µg/L, more recent analysis of consumers' milk in Germany (**Table 2**) predominately showed mean milk iodine concentrations in the magnitude of 100 up to 200µg/L. Thereby iodine concentrations are lower in organic than in conventionally produced milk (Jahreis et al., 2007; Stiftung Warentest, 2007). For consumers' milk from the Czech Republic, Rysava et al. (2007) even ascertained mean milk iodine concentrations up to 601µg/kg.

Table 2: Newer analyses of iodine concentration of consumers' milk in Germany by different authors

Reference	Milk iodine mean (minimum-maximum) [µg/kg or µg/L]		Comments
Gärtner (2009)	98	(25-264)	35 samples
Jahreis et al. (1999)	91		30 samples
Jahreis et al. (2007)	178		37 trademarks 2005-2007
	169		conventional (2007)
	112		organic (2007)
Preiss et al. (1997)	115		28 Bavarian dairies sampled over 1 year
Rysava et al. (2007)	130	(93-159)	4 trademarks
Schöne et al. (2003)	94	(9-189)	23 raw bulk milk samples
Stiftung Warentest (2007)	93	(29-178)	36 trademarks
	101	(54-178)	conventional (n = 29)
	56	(29-77)	organic (n = 7)

The consumers' per capita consumption of milk in Germany in 2006 amounted to 62.6kg/year or 172g/d; inclusive milk products about 130kg/year or 356g/d were consumed (ZMP, 2008).

3.5.2 Urine

Excessive iodine predominately is excreted as inorganic iodide via urine (Underwood and Suttle, 1999; McDowell, 2003). Thus, next to the iodine uptake by the thyroid, the kidneys are responsible for the major clearance of iodine from the blood. While for humans it is stated that urinary iodine approximately amounts to 95% of the total iodine excretion, for ruminants a lower proportion of about 40% is described (Simon, 2008). Although Miller and Swanson (1973) investigated a proportional increase of urinary iodine with rising blood iodine, renal iodine excretion is described to be dependent on glomerular filtration, not on blood iodine (Saller et al., 1998). Reabsorption of iodide occurs from the urinary bladder as well as from the renal tubules whereby the process seems to be predominately passive (Miller et al., 1975b).

3.5.3 Faeces

Faecal iodine is composed of endogenously secreted and not absorbed iodine as well as iodine contained in the exfoliated intestinal cells. Therefore both, hormonal and inorganic iodine are important sources of faecal iodine whereby the major part seems to be in a bound or not exchangeable form (Miller et al., 1975b). Due to reabsorption of endogenously secreted iodine, the iodine excretion via faeces in humans with approximately 1% of the total body iodine loss is described to be very low and relatively constant (Leirer et al., 1983; Cavalieri, 1997; Saller et al., 1998; Anke, 2007). In contrast, in ruminants a proportion of 30% is stated (Simon, 2008). Miller et al. (1975b) recovered between 24-46% of the daily orally administered iodine in faeces.

4 Factors affecting iodine metabolism

4.1 Feed iodine supplementation

Since iodine regulates the thyroid function by mediators, the iodine intake has a fundamental impact on iodine metabolism. This impact is of particular importance in deficient and excessive iodine supply (described in sections 7 and 8). Since the native iodine content of feedstuffs is low (EFSA, 2005), feed iodine supplementations are necessary to achieve a sufficient supply of the animals.

As iodine is almost completely absorbed higher iodine supplementations result in constant increases in blood iodine (**Table 3**) whereby no plateau seems to be reached. Effects on thyroid hormone level are just expected in iodine deficiency or excess (sections 7 and 8).

Table 3: Serum iodine concentration at various iodine intakes and species in dairy cows by different authors

Reference	Iodine intake [mg/d]	Iodine species	Serum iodine [µg/L]	Comments
Miller and Swanson (1973)	0[1]	-	40	AP: Serum B: Holsteins AM: alkaline incineration • Previous low iodine ration
	106	KI	236	
	50	EDDI	142	
	100	EDDI	197	
	200	EDDI	398	
	500	EDDI	1351	
	1000	EDDI	1971	
Rogers and Mee (1996)	0[1]		4	AP: plasma inorganic iodine • heifers
	16		65	
	32		138	
	64		264	
Schöne et al. (2009)	3.0	-	48	AP: serum total iodine MPS: Soybean meal B: German Holsteins AM: ICP-MS
	16.8	$Ca(IO_3)_2$	66	
	72.3	$Ca(IO_3)_2$	131	
	134	$Ca(IO_3)_2$	290	
Swanson et al. (1990)	13.7	-	114	AP: Plasma total iodine MPS: soybean meal B: Holsteins AM: dry alkaline ashing
	30.8	KI	116	
	41.7	KI	196	
	74.0	KI	296	
	13.7	-	109	
	30.8	EDDI	157	
	41.7	EDDI	189	
	74.0	EDDI	311	

AP = analyzed parameter, MPS = main protein source, B = breed, AM = analyzing method,
EDDI = ethylenediamine dihydroiodide
[1] without iodine supplementation, intake of natively contained iodine not stated

Furthermore, iodine excretion via all main routes of iodine elimination (milk, urine and faeces) is elevated. The impact of iodine supplementation on milk iodine has been extensively investigated (**Table 4**). Milk iodine proportionally increases with rising feed iodine concentration and therefore with rising iodine intake of the cow. Thereby no upper limit seems to exist for the iodine concentration of milk. Hillman and Curtis (1980) and Schöne et al. (2009) testing daily iodine dosages of 164mg and 134mg detected mean milk iodine concentrations of approximately 2100 and 2762µg/kg with a maximum of about 6200µg/kg.

Table 4: Milk iodine concentration at various iodine intakes and species in dairy cows by different authors

Reference	Iodine intake [mg/d]	Iodine species	Milk iodine [µg/kg or µg/L]	Comments
Hemken et al. (1972)	0[1]	-	8	MPS: soybean meal B: Holstein
	6.8	KI	81	
	68	KI	694	
Herzig et al. (1999)	0[1]	-	20	MPS: wheat and barley B: Bohemian Pied Cattle AM: Sandell-Kolthoff
	3.8	KI	50	
	7.6	KI	80	
	11.5	KI	173	
	0[1]	-	20	
	3.8	EDDI	37	
	7.6	EDDI	64	
	11.5	EDDI	121	
	72.3	$Ca(IO_3)_2$	1215	
	134	$Ca(IO_3)_2$	2762	
Hillman and Curtis (1980)	16	EDDI	370	B: Holsteins AM: polarographic technique
	164	EDDI	2160	
Kaufmann et al. (1997)	0[1]		129	B: Holstein Friesian
	20		167	
	60		439	
	100		493	
Kaufmann and Rambeck (1998)	0[1]	-	≈130	B: Holstein Friesian AM: gas chromatography
	20	KIO_3	≈170	
	60	KIO_3	≈420	
	150	KIO_3	≈460	
Miller and Swanson (1973)	0[1]	-	8	B: Holsteins AM: alkaline incineration • Previous low iodine ration
	106	KI	379	
	50	EDDI	361	
	100	EDDI	895	
	200	EDDI	1559	
	500	EDDI	2036	
	1000	EDDI	2393	
Schöne et al. (2009)	3.0	-	101	MPS: Soybean meal B: German Holsteins AM: ICP-MS • ∅ milk yield: 19.8kg
	16.8	$Ca(IO_3)_2$	343	
	72.3	$Ca(IO_3)_2$	1215	
	134	$Ca(IO_3)_2$	2762	
Swanson et al. (1990)	13.7	-	200	MPS: soybean meal B: Holsteins AM: dry alkaline ashing
	30.8	KI	414	
	41.7	KI	477	
	74.0	KI	692	
	13.7	-	211	
	30.8	EDDI	447	
	41.7	EDDI	535	
	74.0	EDDI	831	

MPS = main protein source, B = breed, AM = analyzing method, EDDI = ethylenediamine dihydroiodide
[1] without iodine supplementation, intake of natively contained iodine not stated

Studies which investigated urinary iodine and showed a linear increase with rising iodine intake in dairy cows (**Table 5**) indicate that no upper limit for the transfer of iodine into the kidneys exists as well.

Table 5: Urinary iodine concentration at various iodine intakes and species in dairy cows by different authors

Reference	Iodine intake [mg/d]	Iodine species	Urinary iodine [µg/L]	Comments
Herzig et al. (1999)	0[1]	-	44	B: Bohemian Pied Cattle AM: Sandell-Kolthoff • wheat and barley based concentrate
	3.8	KI	108	
	7.6	KI	154	
	11.5	KI	321	
	0[1]	-	44	
	3.8	EDDI	98	
	7.6	EDDI	157	
	11.5	EDDI	346	
Hillman and Curtis (1980)	16	EDDI	1870	B: Holsteins AM: polarographic technique
	164	EDDI	6810	
Miller and Swanson (1973)	0[1]	-	49	B: Holsteins AM: alkaline incineration • Previous low iodine ration
	106	KI	1017	
	50	EDDI	292	
	100	EDDI	404	
	200	EDDI	1468	
	500	EDDI	6143	
	1000	EDDI	10238	

MPS = main protein source, B = breed, AM = analyzing method, EDDI = ethylenediamine dihydroiodide
[1] without iodine supplementation, intake of natively contained iodine not stated

Studies on the impact of iodine supplementation on faecal iodine concentration in cattle are rare. To the authors' knowledge only one study investigated faecal iodine concentrations at various iodine supplementations in dairy cows (**Table 6**). In concordance with studies with ponies and pigs (Wehr et al., 2002; Franke et al., 2008) an elevation of faecal iodine with rising iodine intake was observed. However, lower iodine concentrations are reached compared to the elevation in urine (**Table 5**).

Table 6: Faecal iodine concentration at various iodine intakes and species in dairy cows by different authors

Reference	Iodine intake [mg/d]	Iodine species	Faecal iodine [µg/kg]	Comments
Miller and Swanson (1973)	0[1]	-	54	B: Holsteins AM: alkaline incineration • Previous low iodine ration
	106	KI	169	
	50	EDDI	143	
	100	EDDI	176	
	200	EDDI	467	
	500	EDDI	933	
	1000	EDDI	1626	

MPS = main protein source, B = breed, AM = analyzing method, EDDI = ethylenediamine dihydroiodide
[1] without iodine supplementation, intake of natively contained iodine not stated

4.2 Iodine species

Differences in iodine excretion via urine and milk were seen for the application of EDDI compared to potassium iodide (**Tables 4 and 5**) showing higher milk iodine and lower urinary iodine for the organic form (Miller and Swanson, 1973; Swanson et al., 1990). Ammerman and Miller (1972) described similar availabilities of calcium iodate, sodium and potassium iodide, diiododithymol and pentacalcium orthoperiodate while diiodosalicylic acid is readily absorbed but also excreted very fast with low release of iodine in the body. Miller et al. (1968) investigating blood, urinary and faecal iodine, described an equal utilization of sodium iodide and calcium iodate as well. On the contrary, Moss and Miller (1970) detected an initially delayed uptake of calcium iodate into blood as well as initially lower urinary and faecal iodine excretion compared to sodium iodide when applying a single iodine dose into the rumen or the abomasum. In addition they investigated that iodate was absorbed more rapidly when applied in the abomasum compared to the rumen. From their results it was concluded that due to the conversion of iodate to iodide before absorption iodate may be absorbed slower and possibly in later segments of the gastrointestinal tract (Miller et al., 1975b). However, differences between iodide and iodate at the latest disappeared 48h after applying the single dose and do not seem to appear at daily iodine supplementation. Lengemann (1969) showed that initially lower plasma and milk iodine concentrations were achieved with single sodium iodate dosages compared to sodium iodide whereas no effects of the iodine species were observed at daily oral iodine supplementation. Besides, he found initially lower urinary and faecal iodine for iodate application at single dosing. In contrast, Leskova (1969) described higher serum and milk iodine contents as well as a longer excretion time following oral application of potassium iodate compared to potassium iodide.
Furthermore, iodides are characterized by higher instability in presence of oxygen than iodates. In table salt, iodine losses of about 30% were detected at 60% relative air humidity and unlimited air access after 30 days of storage (Waszkowiak and Szymandera-Buszka, 2007). At exclusion of light, humidity and high temperatures, however, most of the initially added potassium iodide was conserved for several months (Voudouris, 1975). To the authors' knowledge, until now iodine losses from feed were not examined.

4.3 Goitrogens

Agents that interfere with the thyroid and therefore may cause thyroid enlargement are called goitrogens. They may either influence (1) the iodine uptake into the thyroid (e.g. SCN, nitrate, perchlorate), (2) the oxidation of iodide or the synthesis of the thyroid hormones (e.g. flavonoids, goitrin, resorcinol and phenolics) or (3) the proteolysis or release of the thyroid hormones (e.g. iodide, Lithium; Gaitan, 1990). The goitrogenic potential of feed- and foodstuffs mainly is derived from the contained thioglucosides (cabbage, mustard, rape) or

cyanogenic glycosides (cassava, maize, sorghum). Both, thioglucosides and cyanogenic glycosides are precursors of SCN. Thereby the extent of goitrogenicity seems different in the various plants.

With regard to the goitrogenic potential, rapeseed feedstuffs are of major importance in animal nutrition in Germany. Primarily their goitrogenic activity results from the contained GSL and their degradation product SCN, but rape as well accumulates nitrate (Herzig et al., 1996) which may enhance this effects. The utilization of rapeseed meal and rapeseed press cake in animal feed gains in importance due to the expectable increase in by-product availability from bioenergy manufacture. At present 00-RSM is commonly applied which features low contents of erucic acid and GSL. But the remaining GSL content still leads to considerable GSL intakes which influence iodine metabolism when high amounts of RSM are applied (Schöne et al., 2006).

4.3.1 Glucosinolates (GSL) and its degradation products

GSL are a large class of secondary plant metabolites which occur in almost all plants of the order Brassicales. In animal nutrition, fodder and seed meals of the genus Brassica (e.g. rape, mustard, cabbage) are the main sources of GSL. **Figure 4** shows the general structure of the GSL (amino-acid derived thioglucosides) which consists of a glucose molecule (glycone), a sulphuric acid moiety and a sulphurous group with a variable side chain which is obtained from phenylalanine, methionine or tryptophan (R, aglycone).

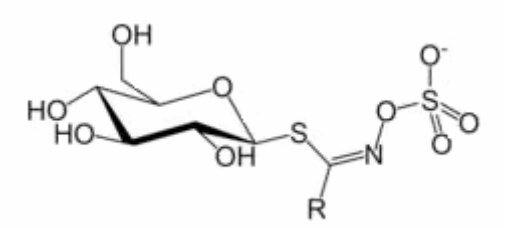

Figure 4: General structure of the glucosinolates (side group R varies)

The plant as well contains the GSL cleaving enzyme myrosinase (thioglucosidase) which is stored separately from the GSL in the cell. The spatial separation is abolished by mechanical actions like chewing or cutting. Furthermore, GSL can be degraded by microbial enzymes in the gastrointestinal tract of the animals (Mandiki et al., 2002). Major degradation products of the enzymatic hydrolysis of the GSL are SCN, isothiocyanates, nitriles and oxazolidinethiones. Tripathi and Mishra (2007) stated that SCN and oxazolidithione interfere with the iodine availability to the thyroid whereas 5-vinyl-2-oxazolidinethione causes morphological and physiological changes of the thyroid.

The best investigated and probably most important degradation product with regard to the influence on iodine metabolism is SCN. SCN due to its similar charge and size to iodide competitively inhibits the iodine concentrating mechanism of the thyroid which can be overcome by iodine application (Gaitan, 1990; Tonacchera et al., 2004). Higher SCN concentrations as well seem to inhibit thyroid hormone synthesis by interfering with thyroid peroxidase (Laurberg et al., 2002). Studies investigating the impact of perchlorate or SCN on blood iodine in cows found enhanced or unchanged plasma iodine levels (Lengemann, 1973; Miller et al., 1975a). SCN application at single iodine dosages caused an initial increase of plasma iodine whereby within some hours (~70-90h) the blood iodine concentration converged to that of the non glucosinolate group (Miller et al., 1969; Miller et al., 1975a). It was shown that the T_4 level of blood was reduced by high GSL contents of feed (Papas et al., 1979; Vincent et al., 1988; Tripathi et al., 2001), but not by RSM with low GSL contents (Papas et al., 1979; Zech et al., 1993; Ehlers et al., 1994). T_3 showed no change due to RSM application (Papas et al., 1979).

NIS inhibiting anions not only diminish the iodine uptake into the thyroid but also into the mammary gland and the other NIS containing organs or tissues (Brown-Grant, 1957). In this way rapeseed feeding leads to lower iodine excretion via milk (Piironen and Virtanen, 1963; Iwarsson, 1973; Papas et al., 1979; Schöne et al., 2006). At GSL intakes of 78.7 and 9.2mmol/d, Papas et al. (1979) and Schöne et al. (2006) showed reductions in milk iodine concentration of 78 and 54% compared to a glucosinolate free ration. Due to the existence of the NIS in salivary glands, gastric mucosa and enterocytes, NIS inhibiting anions like SCN also seem to influence the iodine absorption in the intestine (Nicola et al., 2009) as well as endogenous secretion into the gastrointestinal tract (Miller et al., 1975a) and therefore may alter faecal iodine excretion. Miller et al. (1975a) and Moss et al. (1968) observed a reduced transfer of radioiodine into the faeces of non lactating cattle at SCN application and oral or intravenous iodine dosages (Miller et al., 1975a). The impact of SCN on urinary iodine is not fully understood. While Papas et al. (1979) ascertained no influence, Miller et al. (1975a) observed an increase in urinary iodine at SCN application in cows. In goats, Lengemann (1970) found higher iodine contents in urine when applying perchlorate.

Ruminants are quite tolerant to SCN exposure whereby adults are less sensitive than young animals. A tolerance level of 1.5-4.22mmol/kg diet is described (Tripathi and Mishra, 2007). At high GSL intakes for a long time different adverse effects like goitrogenicity, elevated blood SCN, decreased plasma thyroxin, depressed fertility, thyroid disturbances, reduced feed intake and milk yield were observed in dairy cows (Waldern, 1973; Ingalls and Sharma, 1975; Laarveld et al., 1981; Vincent et al., 1988; Ahlin et al., 1994).

4.3.2 Other possible goitrogens

The flavonoids genistein and daidzein contained in soya or clover have been described to inhibit the activity of TPO and therefore the synthesis of thyroid hormones (Divi et al., 1997; Doerge and Sheehan, 2002; Teas et al., 2007).

Furthermore the synthesis of the thyroid hormones is reliant on the presence of iron, since TPO is a heme-containing enzyme with iron as the central part (Gärtner, 2007). Iron deficiency (ferritin < 20µg/L) seems to disturb iodide utilization and therefore may contribute to the development of goiter. Selenium, as constituent of the iodothyronine deiodinases may inhibit the formation of T_3 at severe selenium deficiency. Furthermore, selenium as constituent of the antioxidative enzyme glutathione peroxidase contributes to the degradation of excessive H_2O_2 of the iodine oxidation process in the thyroid. In this way already slight deficiency seems to provoke thyroid inflammation and increases the incidence of autoimmune thyroiditis (Gärtner, 2007). McDowell (2003) additionally described that high dietary arsenic, fluorine or calcium levels, deficient or high cobalt levels and low manganese levels interfere with iodine metabolism.

4.4 Other influencing factors

Thyroid activity was observed to be lower in summer than in winter and spring (Miller et al., 1975b) which corresponds with the results of Thompson et al. (1963) who described lower thyroidal T_4 secretion in Holstein heifers at high environmental temperatures (24-35°C) in comparison to temperatures between 3-18°C. Furthermore, thyroid activity seems to decrease with growth in calves and is greater in Jerseys compared to Holsteins or Brown Swiss (Johnson and Ragsdale, 1959). Moreover, in lactating cows the iodine uptake into the thyroid was observed to be lower than in non lactating animals (Flamboe and Reineke, 1959) whereby thyroidal iodine uptake seems to be highest in the beginning of lactation (Swanson et al., 1957).

5 Iodine requirement and maximum level

5.1 Iodine requirement and maximum level of humans

For humans an iodine requirement of 1µg/kg body weight is calculated (Großklaus, 1999). With regard to individual variation and environmental factors, the iodine intake recommended by different scientific societies is higher (**Table 7**) and varies depending on age and physiological stage. For adult humans a requirement between 150-200µg/d is stated while the Tolerable Upper Intake Level (UL) is set between 500 and 1000µg/d (WHO, 1994b;

D-A-CH, 2000; SCF, 2002). Higher iodine intakes are recommended for pregnant and lactating women.

Table 7: Iodine requirement of humans (µg/d) by various scientific societies

Age / physiological stage	Scientific society WHO et al. (2001)	USA DRI (2001)	D-A-CH (2000)
0 – 1 year		110 – 130	40 – 80
0 – 6 years	90		
1 – 8 years		90	
1 – 15 years			100 – 200
6 – 12 years	120		
9 – 13 years		120	
14 – 18 years/ adults	150	150	180 – 200
Pregnancy		220	200-230
Pregnancy/ Lactating women	200		
Lactating women		290	260

5.2 Iodine requirement and maximum level of dairy cows

Groppel (1993) considers feed iodine contents of 0.2-0.3mg/kg DM in lactating cows as sufficient. The German Society of Nutrition Physiology (GfE, 2001) states a requirement of 0.5mg/kg DM for dairy cows if the feed does not contain considerable amounts of goitrogenic agents while iodine supplementation should be twice the recommendation if the feed contains more than 20% of feedstuffs with goitrogenic components. The NRC (2001) has set the requirements for lactating cows at 0.6mg/kg DM (including a safety factor for feeding diets containing goitrogens).

The maximum level for iodine in feed of dairy cows in the EU is set at 5mg/kg diet with 88% DM (EU, 2005) while in the United States (US) a supplementation (EDDI) of just 10mg/d is permitted (NRC, 2001).

6 Impact of iodine on the performance of dairy cattle

Studies show that at sufficient iodine supply of the animal further iodine supplementations have no impact on the dry matter intake, milk yield and milk composition (fat, protein, lactose, urea) of dairy cows (Hemken et al., 1972; Kaufmann et al., 1997; Grace and Waghorn, 2005). Moreover, an insufficient iodine intake first causes changes in animal performance after some months (Potter et al., 1980), since iodine from the storage in the thyroid compensates the deficient intake (Delange and Hetzel, 2003). Groppel (1993) described reduced milk yields in dairy cows next to other adverse effects of iodine deficiency (see section 7).

On the other hand Hemken et al. (1972) showed that daily iodine intakes of 68mg (approximately seven times the requirement) did not influence feed intake, milk yield and milk composition and Kaufmann (1997) demonstrated that milk composition was not even altered

by iodine dosages of 100mg/d. However, reduced feed intake and milk yields are decribed as a result of iodine toxicity (see section 8.2).

7 Iodine deficiency

The signs of iodine deficiency are similar in humans and animals. A deficiency may result from an insufficient iodine intake but also from the inhibition of the iodine uptake into the thyroid or from a disrupt of the thyroid hormone synthesis or -release (see section 4.3). An insufficient iodine concentration in the thyroid leads to a diminished production of thyroid hormones (hypothyroidism) whereby first the T_4 level of blood is affected. The low thyroid hormone level of blood results in increased secretion of thyroid-stimulating hormone (TSH) to maximize iodine uptake into the thyroid. TSH stimulates a multiplication (hyperplasia) and an increase (hypertrophy) of the thyrocytes and therefore leads to an enlargement of the thyroid (goiter). A chronic iodine deficiency causes alterations in the thyroid tissue leading to hot and cold nodules. In cold nodules the cells lost their ability to produce thyroid hormones while in hot nodules cells produce thyroid hormones but independently from the demand now (autonomous nodules). However, the development of a goiter and nodules partly seems to be genetically determined. Furthermore, women seem to feature a higher risk for the development of nodules than men (Schilddrüseninitiative Papillon, 2004).

Iodine deficiency causes a number of adverse effects termed as iodine deficiency disorders (IDD). For humans they were summarized by Delange (1994), for animals by Pitman and Pitman (1997). The most apparent consequence is the goiter. The most serious effects result from a deficient iodine supply in pregnancy and include an increased risk of stillbirths and abortions as well as congenital abnormalities, diminished growth, mental retardation, skeletal deformations of the newborn (cretinism; Groppel, 1993; Delange, 1994). In humans a deficient iodine supply was shown to be linked to a higher incidence of breast and thyroid cancer (Venturi, 2001; Smyth, 2003; Arroyo-Helguera et al., 2006; Dal Maso et al., 2009).

In dairy cows further indications of an iodine deficiency are a decreased milk yield and fertility rate (Groppel, 1993). Implications on reproduction were described at feed iodine contents lower than 0.11mg/kg DM while diminished milk yields were observed at 0.09mg I/kg DM.

8 Iodine excess

The impact of excessive iodine on the thyroid function has been extensively investigated. As a result two types of iodine excess are distinguished; the acute and the chronic excess. The acute exposure to high iodine amounts (2-10 milligrams, BfR, 2001) accompanied by an increase of inorganic blood iodine and elevation of total thyroidal iodine leads to an instant stop of the thyroid hormone synthesis which is called Wolff-Chaikoff-effect (Wolff and

Chaikoff, 1948; Saller et al., 1998). When the high iodine supply is maintained (chronic excess) the mRNA expression of NIS and TPO is downregulated resulting in a reduction of the iodine transport into the thyroid and therefore reductions in thyroidal iodine (Braverman and Ingbar, 1963; Uyttersprot et al., 1997). In this way the so-called escape from the Wolff-Chaikoff-effect allows the return to a normal thyroid hormone synthesis (Saller et al., 1998). Intrathyroidal iodine regulates the stimulatory effects of TSH, the inhibitory effects of the Wolff-Chaikoff-effect, but also the escape from this inhibition. This mechanism has been termed thyroid autoregulation. Thereby iodine mediates the inhibition of iodide organification, the increase in iodotyrosine/ iodidtyronine ratio and the inhibition of pinocytosis and proteolysis (resulting in decreased hormone secretion = Plummer effect). Iodinated lipid derivates seem to play an important role in the mediation of thyroid autoregulation mechanisms (Ingbar, 1972; Cavalieri, 1997).

8.1 Iodine excess in humans

Despite the effective regulatory mechanisms, in susceptible subjects thyroid dysfunctions may occur. In humans with chronic high iodine intakes due to seaweed consumption (like in China or Japan) the development of hypothyroidism (indicated by lower T_4 levels) and enlarged thyroids were observed at iodine intakes of approximately 8000µg/d (Namba et al., 1993; Markou et al., 2001). It is stated that iodine intakes up to 1000µg/d do not cause health implications in chronically high supplied individuals without previous deficiency (BfR, 2001). However, in children Zimmermann et al. (2005) found that urinary iodine intakes of > 500µg/L were associated with an increased thyroid volume. Reasons for effects at chronic high iodine intake may be a defective autoregulatory system, an absence of an escape from the Wolff-Chaikoff effect or a decreased thyroid reserve (Saller et al., 1998).

However, implications due to iodine excess predominately seem to occur at iodine supplementations after a previous deficiency whereby much lower iodine intakes cause adverse effects, due to the existence of autonomous nodules. Chronic high iodine intakes after previous deficiency may occur when salt iodization is too high or badly controlled. In Brazil, Algeria, Côte d'Ivoire, Zimbabwe and Uganda median urinary iodine excretions of > 300µg/L, in Chile and Congo even > 500µg/L were detected (Zimmermann et al., 2005). The most common side effect of iodine supplementation programs is the occurence of transient iodine-induced hyperthyroidism (Stanbury et al., 1998; Delange and Lecomte, 2000; Azizi et al., 2005) which even seems not to be avoidable by moderate iodine supplementations (150µg/d; Baltisberger et al., 1995). In regions with replete iodine supply iodine intakes of 1.5-4.5mg/d were shown to cause diminished T_4 contents (Roti and Vagenakis, 2000). In an investigation of Paul et al. (1988), the lowest amount which did not influence the thyroid function was 500µg/d, but another study showed subtle changes

(increases in TSH) at the same intake (Gardner et al., 1988). The higher incidence of hyperthyroidism seems to be transient, disappearing within few years in a population, but it can feature an important health problem including a high risk of tachyarrythmia (Todd et al., 1995).

In addition to the iodine induced hyperthyroidism an elevated incidence of Hashimoto thyroiditis and thyroid cancer is decribed (McConahey et al., 1962; Stanbury et al., 1998; Slowinska-Klencka et al., 2002; Azizi et al., 2005). Although the occurrence of thyroiditis due to iodine supplementation was observed in dogs, chicken and rats, up to now this direct connection is not assured for humans (Delange and Lecomte, 2000). Kahaly (1998) described the developement of thyroid autoantibodies at a supplementation of 500µg/d for 6 months. Acute very high iodine doses (drugs: ≈0.250-0.375mg/dose, x-ray contrast medium: ≈5000mg/dose, sea algae: 0,5-1100mg/100g T) may induce iodine acne or allergies (Heseker, 1999; BfR, 2007).

8.2 Iodine excess in dairy cows

Paulikova (2002) showed that differences in the responsiveness to excessive iodine occur between individual animals of the same species but also between different species. Nagataki (1974) stated that effects also depend on the functional state of the animal and on the amount and time of iodine exposure.

In calves reduced feed intake and body weight gain and other symptoms of iodine excess were first seen at 50mg I/kg diet (Newton et al., 1974). For beef cattle the National Research Council (NRC, 1980) states a maximum tolerable iodine level of 50mg/kg diet. For dairy cows the available data are not sufficiently conclusive to set a maximum level (EFSA, 2005), but first effects of toxicity have already been observed at daily iodine intakes of 50mg (about 5mg/kg DM; NRC, 2001).

The effects of iodine toxicity on ruminants have been reviewed by Paulikova et al. (2002). Coughing, naso-ocular discharge, salivation, pneumonia, reduced milk yield, lacrimation, coryza, conjunctivitis, hair loss, dermatitis and exophtalmus are described as signs of chronic iodine excess in dairy cows. Moreover, an elevation of the metabolic rate shown by increases in body temperature, heart rate, arrhythmia of the heart, nervousness, respiration rate, blood glucose, urinary nitrogen, skin evaporation as well as loss of weight were observed. Others investigated a delayed postcalving estrus and impaired reproductive efficiency as well as immunity in connection with higher mortality of the offspring. With regard to the mineral and vitamin metabolism Soesanto (1979) additionally described reduced vitamin A contents while Blaxter (1948) and Newton et al. (1974) found diminished calcium concentrations in blood. The information on the impact of high iodine dosages on the thyroid in cattle is controversial, since studies with higher supplementations (200-400 and 500 times

higher than the requirement) found no enlargement of the thyroid (Convey et al., 1978; Leung et al., 1980) while iodine amounts which were 10 and 30 times higher than the requirements resulted in increased thyroid weights (Wallace, 1975; Meyer et al., 2008). Regarding the thyroid hormone status, studies with dairy cows (Convey et al., 1977; Hillman and Curtis, 1980; Grace and Waghorn, 2005) indicate that at sufficient iodine supply further supplementations have no impact. However, Hillman and Curtis (1980) described a high variance of the T_4 levels of blood at high iodine intakes (164mg I/d) and suggested that both hyper- and hypothyroidism may have occurred in the cows inhibiting the appearance of an effect.

SCOPE OF THE THESIS

The aim of the present study was to investigate the impact of different iodine supplementations as iodide and iodate up to the permitted maximum level in feed and the influence of RSM in the ration compared to a GSL free diet on the iodine status (serum iodine, T_3 and T_4) and on the iodine content of the main substrates of iodine excretion (urine, faeces and milk) in dairy cows. Thereby especially the possible risk for human nutrition, arising from iodine enrichment of milk should be evaluated concerning prophylactic consumer protection.

DDGS (made from 90% wheat and 10% barley) was used in comparison to the GSL containing RSM, since it seems to possess no goitrogenic potential (in contrast to soybean meal). The impact of the various iodine supplementations on the performance of the cows could not be investigated, due to the constitution of the trial. Moreover, the tested GSL intake by RSM is tolerated by ruminants without effects on the performance. Since performance, however, is elementary for evaluating the impact of the mentioned factors on iodine metabolism, concerning the performance parameters, it was focussed on the impact of the applied DDGS compared to RSM (**Paper I**). The application of DDGS made from wheat and barley in dairy cows is rarely investigated, since it is just extensively utilized in animal nutrition for few years and the nutrient value still varies due to the processing. For DDGS from wheat an equal applicability to other protein feedstuffs was shown, but data regarding high fed DDGS amounts in high yielding cows are scarce. In recent years DDGS, comparable to RSM, gains in importance in animal feeding due to its increasing availability as a by-product of bioenergy manufacture.

Due to its contribution to human iodine supply, milk is of particular importance when examining iodine elimination by the cow. As recent studies indicated that efforts to enrich milk with iodine for improvement of human iodine supply also bare the risk of excessive iodine intakes, the aim of the present study was to detect which milk iodine concentrations will be reached at the highest presently permitted iodine supplementation of feed. Thereby the human iodine supply resulting from an enrichment of milk by the currently allowed iodine supplementations should be evaluated. Since goitrogens considerably counteract the iodine supplementations, their impact on milk iodine was included in the trial by testing a commonly applied 00-RSM. The results at the various feed iodine supplementations with either RSM application or a GSL free diet should establish a basis for evaluating the adequacy of the present maximum level of iodine in feed of dairy cows (**Paper II**).

Iodine enrichment in milk by iodine supplementation is accompanied by a high iodine status of the cow and high iodine losses via urine and faeces leading to economic inefficiency and possible implications on the cows and the environment. Recent studies including both iodine status and iodine excretion via all main routes (urine, faeces, milk) in dairy cows are missing. Therefore this issue was investigated in the present study whereby the impact of goitrogens (in RSM) and two commonly applied iodine species (potassium iodide and calcium iodate anhydrous) was included (**Paper III**).

With regard to their importance the following questions should be investigated in the present study:

1) Will similar milk iodine concentrations be reached in high yielding dairy cows like those found in a previous study with low-yielding cows? Which milk iodine concentrations are reached at the presently permitted maximum level of iodine in feed?
2) Does the presently permitted maximum level avoid an exceeding of the Tolerable Upper Intake Level in human nutrition or does it need re-evaluation?
3) To which extent does a high amount of 00-rapeseed in the ration diminish the milk iodine concentration?
4) Does iodide application lead to lower iodine contents of milk due to higher iodine losses from feed?
5) How is the iodine status and the distribution to the main routes of iodine excretion (urine, faeces and milk) altered by high daily iodine dosages, rapeseed feeding or iodide and iodate application?
6) Does a high proportion of DDGS from wheat and barley allow a similar performance of high yielding dairy cows like RSM feeding?

PAPER I

Evaluation of the applicability of distillers dried grains with solubles compared to rapeseed meal in rations of dairy cows

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ABSTRACT

The aim of the present study was to evaluate if similar performances of high yielding dairy cows can be achieved with a high proportion of distillers dried grains with solubles (DDGS) consisting of 90% wheat and 10% barley compared to rapeseed meal (RSM) in the diet. Of a total of 32 dairy cows, 16 were fed RSM as protein source (16.5% of ration), the others DDGS. Equivalent high dry matter intakes, milk yields, milk fat percentages and milk lactose percentages were observed. Milk protein percentages were lower when DDGS was applied. However, the milk fat, -protein and -lactose yields showed no significant differences between RSM and DDGS application. Since similar performances were reached with DDGS and RSM, the tested DDGS can replace RSM as protein source in feed of high yielding dairy cows up to the tested amount of 16.5% of the ration.

Keywords dairy cows, distillers dried grains, milk yield, milk composition, dry matter intake, rapeseed meal

Short Title: Distillers dried grains in dairy cow rations

INTRODUCTION

With the increase in by-product availability from bioenergy manufacture, the utilization of distillers grains (DGs) as feeding stuffs gains in importance. Due to their high protein and energy contents they may present an alternative to soybean meal, RSM or rapeseed expellers in animal nutrition, but the feed value of DGs still varies considerably due to the utilized raw materials and differences in the production process (Spiekers et al., 2006). Normally DGs obtained from maize, wheat, barley, rye, sorghum or mixtures from wheat and barley are used in animal nutrition. Depending on their further processing, a distinction is drawn between distillers wet grains (DWG) and distillers dried grains with or without solubles (DDGS, DDG). The composition of the distillers dried grains furthermore depends on the extent of the fermentation- and the drying process as well as on the addition of the solubles (Belyea et al., 1998; Spiehs et al., 2002). Due to these differences DDGs feature a high variability in nutrient content and the availability of the contained protein (Powers et al., 1995; Schingoethe, 2006; Kleinschmit et al., 2007). It is not sufficiently investigated if all kinds of DDGS are equally applicable to RSM, rapeseed expellers or soybean meal in rations of high yielding dairy cows, especially when high proportions of DDGS are applied. Investigations on the nutrient value of distillers dried grains mainly originate from the USA and therefore predominately are based on maize as raw material (Palmquist and Conrad, 1982; Van Horn et al., 1985; Powers et al., 1995; Al-Suwaiegh et al., 2002; Anderson et al., 2006; Kleinschmit

et al., 2007; Janicek et al., 2008). Only few studies exist on the equivalent applicability of DDGs from wheat or wheat and barley in dairy cow feeding (Dunkel, 2005; Spiekers et al., 2006; Urdl et al., 2006; Ettle, 2007). Due to usually higher protein and lower fat and energy contents of wheat based DDGS compared to DDGS from corn (Spiekers et al., 2006), the results achieved with corn DDGS cannot simply be transferred to the tested DDGS of the present trial. On the contrary DDGS from wheat or wheat/barley seem to be comparable (Spiekers et al., 2006).

For DDGS from corn it is shown that it can be fed up to 15-20% of the ration to dairy cows without over feeding protein (Mc Kinnon, 2008). For DDGS from wheat or wheat and barley studies investigating the limits of application in high yielding dairy cows are missing (Spiekers et al., 2006).

The aim of the present study was to evaluate if similar performances of high yielding dairy cows can be achieved with distillers dried grains with solubles (DDGS) consisting of 90% wheat and 10% barley at a proportion of 16.5% of the ration compared to rapeseed meal (RSM). The assignment of the animals to four groups was made, since the original aim of the conducted study was to investigate the impact of the two different protein sources (RSM and DDGS) and two different iodine species on the milk iodine content (Franke et al., 2009). Since no impact of the applied iodine species on the performance is expected, the present paper focusses on the comparison between DDGS and RSM application. However, data of all 4 groups is additionally presented to ascertain if differences occurred in both DDGS groups or just in one.

EXPERIMENTAL

Animals and diets

The trial was carried out at the experimental station of the Institute of Animal Nutrition (FLI), Braunschweig with 32 dairy cows of the German Holstein breed in early lactation. The cows were divided equally into four groups of 8 animals each with regard to milk yield, number of lactations and days in milk. Seasonal calving at the experimental station led to similar days in milk for all animals. At the beginning of the trial, the cows on average were 48 $\pm$ 25 days in milk (DIM) and featured an average number of lactations of 2.1 $\pm$ 1.2, a body weight of 582 $\pm$ 74kg and a daily milk yield of 31.3 $\pm$ 6.3kg.

The cows were fed a total mixed ration (TMR) consisting of 50% concentrate, 25% maize silage and 25% grass silage on dry matter (DM) basis for *ad libitum* consumption. The rations were formulated to meet or exceed the nutritional requirements of the cows stated by the German Society of Nutrition Physiology (GfE, 2001). The composition of the concentrates is shown in Table 1. Water as well was provided *ad libitum*. In two groups the cows received

a ration with distillers dried grains with solubles (DDGS) as main protein source (16.5% of TMR), made from 90% wheat and 10% barley. The DDGS was obtained from indirect drying of the stillage in "tubular bundle driers" at 105°C for about 15min. In the other two groups RSM with a low glucosinolate content (3.5mmol/kg DM) was applied. One group of each protein source received iodine in the form of potassium iodide (KI), the other group as calcium iodate anhydrous ($Ca(IO_3)_2$). In the following the diets are mentioned in the way they are shown in Table 1. The trial lasted 147 days. The iodine supplementation of the cows was in the range of the permitted maximum level of iodine in feed.

Table 1: Composition of concentrates [%] in the experimental groups applying distillers dried grains with solubles (DDGS) or rapeseed meal (RSM) as protein source

	DDGS[1]/iodide	DDGS[1]/iodate	RSM[1]/iodide	RSM[1]/iodate
DDGS[1]	33.0	33.0	-	-
RSM[1]	-	-	33.0	33.0
Wheat	25.0	25.0	25.0	25.0
Maize	25.0	25.0	25.0	25.0
Dried sugar beet pulp	13.5	13.5	13.5	13.5
Calcium Carbonate	0.5	0.5	0.5	0.5
Soybean oil	1.0	1.0	1.0	1.0
Mineral feed[2]	2.0	2.0	2.0	2.0

[1] DDGS = distillers dried grains with solubles; RSM = rapeseed meal

[2] without iodine supplementation; per kg mineral feed: 140g Ca, 120g Na, 70g P, 40g Mg, 6g Zn; 5.4g Mn; 1g Cu; 40mg Se; 25mg Co; 1000000IU vitamin A; 100000IU vitamin D_3; 1500mg vitamin E

The assignment of the animals to four groups was made, since the original aim of the conducted study was to investigate the impact of RSM, DDGS, iodide and iodate on milk iodine (Franke et al., 2009).

Recorded parameters and analysis

The individual feed intake was recorded continuously by self-feeding stations (Type RIC, Insentec, B.V., Marknesse, The Netherlands) and ear transponders. In each group, 7 self-feeding stations were available for the cows. The cows were housed in group pens according to their feeding group. The pens were equipped with a slatted floor and cubicles covered with rubber mats and straw dust. Milking took place twice a day at 5.30 in the morning and 15.30 in the afternoon. The milk yield was recorded with automatic milk counters at each milking. The body weight was automatically recorded when leaving the milking parlour twice daily.

Representative concentrate samples were taken once, and silage samples twice, a week. Silage samples were dried at 60°C for 72 hours. All samples were ground to pass through a sieve with 1mm pore size. The crude nutrients in all feed samples were analyzed according to the methods of the “Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten“ (VDLUFA, Bassler, 1976). The determination of ADF and NDF was done following Goering and Van Soest (1970) without the use of sodium sulfite. In the concentrates due to the high starch contents α-amylase was applied. The total glucosinolate

content of the RSM was analyzed by HPLC according to the international standard DIN EN ISO 9167-1 (1995).

To ascertain the Metabolizable Energy (ME) and Net Energy Lactation (NEL), balance studies with four wethers each were carried out for both applied concentrates (differing in the protein source) and for maize- and grass silage, following the standard procedure described by the GfE (1991).

Milk samples for the analysis of fat, protein, lactose and urea contents were taken twice a week. The samples were preserved with bronopol and stored at 8 °C until the analysis. Fat, protein and lactose in milk were analyzed by an infrared milk analyzer (Milkoscan FT 6000 combined with a Fossomatic 5000, Foss Electric, Hillerød, Denmark). From the analyzed data of morning and evening milk a weighed mean (considering the milk yields) was calculated for the percentages and yields of milk fat, -protein, -lactose and -urea.

Calculations and statistics

The concentrations of the crude nutrients, ADF and NDF of the TMR were established on dry matter basis from the analyzed concentrations in the concentrates, and the silages with regard to the percentage of the components of the TMR.

The utilizable Crude Protein (uCP) describes the amount of protein which is available at the duodenum and consists of microbial and undegraded protein. It was calculated as follows (GfE, 2001):

$$\text{uCP [g]} = (187.7 - (115.4 * (\text{undegraded feed protein [g]} / \text{feed crude protein [g]}))) * \text{digestible organic matter[kg]} + 1.03 * \text{undegraded feed protein[g]} \quad (1)$$

The undegraded feed protein (UDP) is based on data of the DLG (1997). For the calculation of digestible organic matter (DOM), ME, NEL and Ruminal Nitrogen Balance (RNB) as well as for calculation of the uCP demand the formulas of the GfE (2001) were used. The difference between the uCP demand and the uCP intake demonstrates the protein balance of the animals. The energy balance was calculated by subtracting the daily requirement for maintenance and the energy content of milk from the daily energy intake. For the calculation of the energy content of milk, the formula of the GfE (2001) was applied.

Statistical analysis was performed by the SAS-software package (version 9.1, SAS Institute, Cary, NC, USA). The experimental unit was the animal during the whole study. All parameters were tested for normal distribution by the Kolmogorov-Smirnov test.

All data were subjected to analysis of variance (ANOVA) according to a two-factorial design:

$y_{ijk} = \mu + a_i + b_j + (a * b)_{ij} + e_{ijk}$

where $y_{ij} = k^{th}$ observation related to the "protein source" i and the "iodine species" j; μ = overall mean; a_i = effect of the "protein source" (DDGS or RSM); b_j = effect of the "iodine species" (iodide or iodate); $(a * b)_{ij}$ = interactions between "protein source" and "iodine species" and e_{ijk} = error term.

Thus, the model included the "protein source", the "iodine species" and the interaction for "protein source" by "iodine species". Differences were considered to be significant at $P < 0.05$.

RESULTS

Feed composition

The applied protein sources DDGS and RSM showed similar contents of crude protein (Table 2). They provided 37.3% of the total crude protein of the ration in case of DDGS and 37.9% in case of RSM (Table 3). The DDGS featured slightly higher concentrations of crude fat and NDF while ADF was slightly lower than in RSM (Table 2).

Table 2: Analysis of composition of the applied distillers dried grains with solubles (DDGS) and the rapeseed meal (RSM)

	DDGS[1]	RSM[1]
Dry matter [g/kg]	923	898
Crude Nutrients [g/kg DM]		
Crude ash	58	75
Crude protein	367	369
Crude fat	62	52
ADF	159	208
NDF	496	408

[1] DDGS = distillers dried grains with solubles; RSM = rapeseed meal

The total mixed rations (TMR) similarly to the main protein sources contained slightly higher crude fat and less ADF when DDGS was applied compared to RSM (Table 3), but NDF was similar in all rations. Resulting from the higher fat concentration the diets with DDGS featured a marginally higher ME and NEL content.

The GSL concentration of the TMRs with RSM amounted to 0.58mmol/kg DM. The resulting mean daily glucosinolate intake in the RSM groups is shown in Table 4.

Table 3: Analysis of composition of the total mixed ration (TMR)

	DDGS[1]/iodide	DDGS[1]/iodate	RSM[1]/iodide	RSM[1]/iodate
Dry matter [g/kg]	618	618	611	612
Crude Nutrients [g/kg DM]				
Crude ash	63	63	67	67
Crude protein	164	164	162	162
Thereof: % protein from by-product	37.3	37.3	37.9	37.9
Crude fat	42	42	37	38
ADF	179	181	187	186
NDF	353	358	353	355
Glucosinolate content [mmol/kg DM]	n.d.	n.d.	0.58	0.58
Energy [MJ/kg DM]				
ME	11.5	11.5	11.3	11.3
NEL	7.03	7.03	6.92	6.92

n.d. not detected

[1] DDGS = distillers dried grains with solubles; RSM = rapeseed meal

The assignment of the animals to four groups was made, since the original aim of the conducted study was to investigate the impact of RSM, DDGS, iodide and iodate on milk iodine (Franke et al., 2009).

Body weight

Means and standard derivations of the body weight are shown in Table 4. No significant impact of either the “protein source” or the “iodine species” on the body weight and as well no interaction was observed. During the whole trial the cows of the DDGS/iodate group showed a slightly but not significantly lower body weight than the other groups, which was already existent at the beginning of the trial.

Dry matter intake (DMI)

The averaged DMI of the cows amounted to 21.3 $\pm$ 2.5kg/d. The DMI at DDGS application was not significantly different from the DMI at RSM application (Table 4). Numerically higher DMIs were observed in the RSM/iodide group, being up to 2.2kg/d higher than in the other groups. Although the other three groups showed similar DMIs this fact caused slightly higher means for RSM compared to DDGS application.

Table 4: Body weight, dry matter intake (DMI) and energy and protein supply as well as milk yield and milk composition in the experimental groups (means)

	DDGS[1]	RSM[1]	DDGS[1]/iodide	DDGS[1]/iodate	RSM[1]/iodide	RSM[1]/iodate	PSEM[2]	Probability		
								Protein source	Iodine species	Protein source∗ Iodine species
Body weight [kg]	625	638	632	617	629	647	24.35	0.595	0.958	0.505
Dry matter intake [kg/d]	20.8	21.9	20.5	21.1	22.7	21.0	0.681	0.137	0.402	0.092
Concentrate intake [kg DM/d]	10.6	11.0	10.4	10.7	11.5	10.6	0.346	0.185	0.411	0.091
Maize silage intake [kg DM/d]	4.9	5.2	4.8	5.0	5.4	4.9	0.159	0.100	0.376	0.082
Grass silage intake [kg DM/d]	5.4	5.7	5.3	5.4	5.9	5.4	0.176	0.096	0.409	0.104
Crude protein intake [kg/d]	3.4	3.6	3.4	3.5	3.7	3.4	0.111	0.098	0.261	0.410
ME intake [MJ/d]	239	247	235	242	257	237	7.782	0.286	0.410	0.093
NEL intake [MJ/d]	146	151	144	148	157	145	4.763	0.313	0.411	0.093
UDP [g/d][3]	972	918	958	986	955	881	30.70	0.088	0.464	0.109
uCP [g/d][4]	3383	3469	3333	3432	3608	3329	109.8	0.439	0.419	0.096
Milk yield [kg/d]	34.9	34.0	36.1	33.6	34.5	33.5	1.879	0.650	0.371	0.679
Milk fat [%]	3.26	3.53	3.20	3.32	3.64	3.42	0.233	0.269	0.836	0.467
Milk fat [kg/d]	1.14	1.18	1.17	1.10	1.25	1.12	0.082	0.550	0.264	0.696
Milk protein [%]	3.11[a]	3.29[b]	3.07	3.15	3.24	3.34	0.080	0.031	0.285	0.915
Milk protein [kg/d]	1.09	1.12	1.12	1.06	1.11	1.12	0.053	0.624	0.592	0.560
Milk lactose [%]	4.85	4.80	4.87	4.82	4.78	4.82	0.035	0.210	0.857	0.192
Milk lactose [kg/d]	1.70	1.64	1.77	1.63	1.66	1.63	0.089	0.520	0.330	0.566
Milk urea [mg/kg]	182	195	179	185	199	191	9.378	0.169	0.934	0.449
Milk urea [g/d]	6.38	6.66	6.54	6.22	6.92	6.41	0.435	0.525	0.354	0.830

[ab] Values with different superscripts within a row are significantly different ($P < 0.05$)

[1] DDGS = distillers dried grains with solubles; RSM = rapeseed meal

[2] Pooled standard error of means

[3] UDP = undegraded feed protein, UDP is based on data of the DLG (1997)

[4] uCP = utilizable crude protein, uCP [g] = (187.7 - (115.4 ∗ (UDP [g] / feed crude protein [g]))) ∗ digestible organic matter [kg] + 1.03 ∗ UDP[g] (GfE, 2001)

The assignment of the animals to four groups was made since the original aim of the conducted study was to investigate the impact of RSM, DDGS, iodide and iodate on milk iodine (Franke et al., 2009).

Energy and protein supply

Similar results were observed for the ME, NEL and XP intakes, showing no significant differences but slightly lower values for DDGS application due to higher feed intakes in the RSM/iodide group (Table 4). The energy balance was positive in all experimental groups (5.4, 16.5, 17.7 and 9.9MJ NEL/d for DDGS/iodide, DDGS/iodate, RSM/iodide and RSM/iodate).

The daily intake of protein which is not degraded in the rumen (UDP) was slightly higher for DDGS feeding (Table 4) but as well showed no significance.

The average RNB was positive in all experimental groups (6.7, 6.4, 13.5 and 12.8g/d in the DDGS/iodide, DDGS/iodate, RSM/iodide and RSM/iodate group).

The calculated daily intake of available protein at the duodenum (uCP, consisting of microbial protein and UDP) showed no significant differences between DDGS and RSM application. Although the UDP was slightly higher when DDGS was applied, numerically lower uCP were observed for DDGS application, resulting from a numerically but not significantly higher uCP in the RSM/iodide group while the other groups showed similar values. The protein balance (calculated from the daily uCP intake and demand) was positive for all experimental groups (248, 462, 469 and 231g/d for DDGS/iodide, DDGS/iodate, RSM/iodide and RSM/iodate).

Milk yield

The average milk yield was 34.2 $\pm$ 5.7kg/d. No significant differences in milk yield were found between application of DDGS and RSM (Table 4). However, the daily milk yield on average was 0.9kg higher when feeding DDGS. With regard to the single group means it becomes obvious that this fact mainly result from differences in case of iodide application.

Milk composition

Milk fat percentage and milk fat yield were neither significantly influenced by the “protein source” nor by the “iodine species” and as well no interaction was observed (Table 4). However, numerically lower contents were seen for DDGS application, which were apparent in both DDGS groups.

A significantly lower milk protein percentage was detected for DDGS application (Table 4). The “iodine species” showed no significant impact and no interaction was observed between both tested impact factors. Considerable differences were detected for both DDGS groups (Table 4). The milk protein yield per day was slightly lower at DDGS application but the difference was not significant. Regarding the single group means, a numerically lower milk protein yield just occurred at iodate application.

Milk lactose percentage and –yield as well as milk urea content were not significantly altered by the “protein source” or the “iodine species” (Table 4).

DISCUSSION

Feed composition

The tested DDGS batch reflects the usually found high NDF contents of distillers grains and shows that DDGS from wheat and barley similarly to DDGS from wheat feature equal protein and higher fat concentrations compared to RSM (Schingoethe, 2006; Wiedner, 2008). The crude protein, crude fat, NDF and ADF concentrations of the DDGS were in the middle of previously measured contents of DDGS from wheat summarized by Schingoethe (2006) which ranged from 30.5-44.7% for protein, 3.1-9.9% for fat, 33.4-57.0% for NDF and 11.1-24.3% for ADF. In addition similar crude ash, protein and fat contents were described for DDGS from wheat and barley in Germany (Spiekers et al., 2006; Wiedner, 2008).

The detected GSL contents of the TMR were far below the described tolerance level for ruminants of 1.5-4.22mmol/kg diet (Tripathi and Mishra, 2007).

Dry matter intake

The high DMIs in the DDGS groups showed the good acceptance of the applied kind and amount of DDGS. Former studies investigating the substitution of soybean meal, a mixture of soybean meal and rapeseed cake, RSM or rapeseed cake by DDGS from wheat as well established no differences in DMI (Dunkel, 2005; Urdl et al., 2006; Ettle, 2007). No explanation could be found for the higher DMIs observed in the RSM/iodide group in the present study.

Energy and protein supply

Although no significant differences were detected for the daily UDP intake, a P-value of < 0.1 for the impact of the “protein source” and numerically lower values in the RSM/iodate groups indicate that only the high DMI in the RSM/iodide group may have prevented significance.

Despite the slightly higher UDP the microbes in the rumen were also sufficiently supplied with N when DDGS was fed, indicated by a positive RNB.

The similar uCP intake in both DDGS groups and the RSM/iodate group at simultaneously numerically higher uCP intake in the RSM/iodide group show that differences mainly occur due to the numerically higher DMI and therefore higher intake of crude protein in the latter group (Table 4). The positive protein balance (uCP intake - uCP demand) indicates a sufficient supply of the animals with protein, but the uCP does not make a statement about the availability of this protein. Therefore it cannot be assessed from this parameter if a heat damage of the protein of the tested DDGS has occurred during the drying process.

Milk yield

The present study affirms results of former studies where similar milk yields were observed with wheat DDGS in comparison with either RSM or soybean meal (Ettle, 2007) or a mixture

of soybean meal and rapeseed press cake (Urdl et al., 2006). Dunkel (2005) detected lower milk yields at DDGS application and suggested that an overheating of the DDGS resulting in lower protein and energy availability or a lower energy content of feed may have caused this effect. The short drying at relatively low temperatures in the present trial seems to have prevented a heat damage of the proteins in the DDGS of the present trial allowing high protein availability.

Milk composition

In former studies with wheat DDGS, no effect of the DDGS application on the milk fat was observed (Dunkel, 2005; Urdl et al., 2006; Ettle, 2007). Although the difference in the present study was not significant the results show the problem of feeding high amounts of DDGS. The increasing fat content of the diet with rising proportion of DDGS is accompanied by a risk of milk fat depression (Kononoff and Christensen, 2007). However, this problem can be avoided when the high fat contents are considered at formulation of the ration.

No differences in milk protein percentage were observed by Urdl et al. (2006), Dunkel (2005) and Ettle (2007). In the present study the lower milk protein percentage at DDGS application may mainly result from slightly but not significantly higher milk yields, since no significant differences were established for the milk protein yield. Schingoethe (2006) as well stated that milk protein depression due to DDGS feeding is hardly observed but may appear due to limiting protein or lysine intake or due to higher fat contents of the diet. The positive protein balance and unchanged milk yields in the present trial indicate a sufficient protein supply of the animal, but lysine content was not detected. However, the slightly higher fat concentration of the DDGS compared to RSM were observed and may have contributed to the lower milk protein percentages.

CONCLUSIONS

Equivalent high performances were achieved when applying DDGS, made from 90% wheat and 10% barley, as protein source compared to RSM. Therefore the results of this study show that the tested DDGS can replace RSM in dairy cow feed up to the tested amount of 16.5% of the ration (37% of total protein).

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PAPER II

Influence of various iodine supplementations and two different iodine species on the iodine content of milk of cows fed with rapeseed meal or distillers dried grains with solubles as protein source

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Abstract

The iodine supplementation of animal feed influences the iodine content of milk and therefore, in addition to salt iodination, provides another possibility to improve human iodine supply. On the other hand, an excessive iodine intake of men by milk has to be avoided. Furthermore, the iodine content of milk varies depending on the presence of iodine antagonists in feed (e.g., glucosinolates in rapeseed), and the applied iodine species. The study should evaluate the impact of various feed iodine supplementations up to the permitted maximum level, the effect of applying rapeseed compared to a glucosinolate free ration and the impact of two different iodine species on the iodine content of milk. A total of 32 dairy cows were divided into 4 groups with 8 animals each. Two groups received distillers dried grains with solubles (DDGS) as protein source, the others rapeseed meal (RSM, 16.5% of total diet). In each case half of the animals received feed supplemented with iodine in the form of potassium iodide, the other half as calcium iodate. Iodine supplementations of 0, 0.5, 1, 2, 3, 4 and 5mg/kg DM were tested in consecutive periods of 21 days each. The milk iodine concentration increased with rising iodine supplementation of feed. RSM in the ration (0.58mmol glucosinolates/kg diet DM) diminished the milk iodine concentration by one half up to one third of the concentration achieved by DDGS. At iodine supplementations of 2mg/kg DM and higher, the differences were significant. The application of iodate predominantly resulted in higher milk iodine concentrations compared to iodide but was not significant in any period. At the highest tested iodine supplementation (5mg/kg DM), the milk iodine concentration rose up to 1464 (iodide) and 1578µg/kg (iodate) when feeding DDGS and up to 718 (iodide) and 620µg/kg (iodate) in the RSM groups. The carry over of iodine from feed into milk amounted to 30-56% when using DDGS, and 11-25% when applying RSM. The currently allowed maximum level of iodine in feed of dairy cows in Europe may lead to high milk iodine concentrations. As a result the Tolerable Upper Intake Level (UL) in human nutrition may be exceeded. Therefore this maximum level has to be reevaluated. In addition to the iodine supplementation, the application of RSM in the ration has to be considered when estimating the iodine content of milk.

Keywords dairy cow, iodine, milk, rapeseed meal

Introduction

Worldwide 13% of the population still suffer from iodine deficiency. In addition to the salt iodination, the iodine supplementation of animal feed can contribute to improving human iodine supply because iodine is one of the rare trace elements whose concentration in food of animal origin (milk, eggs) can be altered considerably by its content in animal feed (Kaufmann and Rambeck, 1998; Flachowsky et al., 2006; Schöne et al., 2009). On the other hand, iodine in human nutrition is also characterized by a high risk of overdosing (ERNA and

EHPM, 2004) because the margin between the requirement of 150-200µg/d (D-A-CH, 2000; WHO et al., 2001) and the Tolerable Upper Intake Level (UL) of 500µg/d (D-A-CH, 2000) up to 1mg/d (WHO, 1994) is narrow for adult humans. A declaration of the iodine content of food of animal origin seems impossible. Therefore the enrichment of iodine in consumers' milk should to limited by setting low maximum levels for iodine in animal feed which are still practicable. In 2005, the European Food Safety Authority (EFSA) evaluated benefits and risks of the level of iodine supplementation in animal feed concluding that data concerning the iodine transfer from feed into food of animal origin are insufficient (EFSA, 2005). In this regard milk is of particular importance because in contrast to meat (Franke et al., 2008; Meyer et al., 2008), it is characterized by a high carry over of iodine from feed into food of animal origin (Flachowsky et al., 2006) and therefore may lead to high iodine concentrations of milk (Hillman and Curtis, 1980; Schöne et al., 2009; Rysava et al., 2007). Besides the thyroid and other extrathyroidal tissues (salivary glands, gastric mucosa, choroid plexus and ciliary body of the eye) iodine is actively accumulated in the lactating mammary gland by the sodium iodide symporter (NIS; Cavalieri, 1997; Laurberg et al., 2002). Due to the enhanced application of iodine in feeding stuffs, the iodine content of consumers' milk rose in the last 10 years (Launer and Richter, 2005; Jahreis et al., 2007). At present the contribution of milk and its products to human iodine intake in Germany is estimated at 40% (Jahreis et al., 2007). Furthermore, milk is an alternative indicator to urine for the iodine supply of the animal due to its good and fast reflectance of the iodine intake (Binnerts, 1989; Herzig et al., 1996).

Next to the primary impact of the feed iodine on the iodine concentration of milk, there are further influencing factors like goitrogens in feed, the breed, the applied iodine species, the stage of lactation, utilization of iodized udder disinfection solutions, iodine containing drugs and possibly the milk yield (Iwarsson, 1973; Papas et al., 1979; Hemken, 1979; Franke et al., 1983; Swanson et al., 1990; Flachowsky et al., 2007). Due to the expectable increase in by-product availability from bioenergy manufacture, the utilization of rapeseed meal (**RSM**) and rapeseed press cake in animal feed gains in importance. The degradation product thiocyanate of the contained glucosinolates (**GSL**) competitively inhibits iodine uptake by the NIS into the thyroid (Brown-Grant, 1957) and also seems to reduce the transfer of iodine into the milk (Papas et al., 1979; Laurberg et al., 2002; Schöne et al., 2006). In the EU sodium iodide, potassium iodide, calcium iodate hexahydrate and calcium iodate anhydrous are accredited for the iodination of feeding stuffs (EU, 2005). Because iodide is characterized by instability in presence of oxygen, storage losses may also result in lower iodine contents of milk.

In the present cow study, the milk iodine concentration at various feed iodine supplementations up to the permitted maximum level in feed (5mg/kg; EU, 2005) should be investigated. Furthermore, the impact of a high percentage of RSM in the ration and of the applied iodine species (iodide, iodate) on the iodine content of milk should be evaluated.

Materials and Methods

Experimental Design and Conditions

The trial was carried out at the experimental station of the Institute of Animal Nutrition (FLI), Braunschweig. Thirty-two dairy cows of the German Holstein breed in the first stage of lactation were divided equally into four groups of 8 animals each, considering the milk yield, age and days in lactation. At the beginning of the trial, on average the cows were 48 ± 25 days in milk and featured an average number of lactations of 2.1 ± 1.2, a body weight of 582 ± 74kg and a daily milk yield of 31.3 ± 6.3kg. The cows were fed a TMR which was formulated to meet the nutritional requirements of dairy cows stated by the German Society of Nutrition Physiology (GfE, 2001) and which on DM basis consisted of 50% concentrate, 25% maize silage and 25% grass silage. The precise composition is presented in Table 1. Feed and water were provided *ad libitum*. In two groups RSM containing 3.5µmol GSL/g DM was applied as protein source (16.5% of TMR), in the other 2 groups the cows received distillers dried grains with solubles (**DDGS**) made from 90% wheat and 10% barley. One group of each protein source received iodine in the form of potassium iodide (KI), the other group as calcium iodate anhydrous ($Ca(IO_3)_2$). In the following, the groups are mentioned in the way they are shown in Table 1. In 7 periods of 21 days each, iodine supplementations of 0, 0.5, 1, 2, 3, 4 and 5mg/kg DM were tested, equally in all groups.

Table 1: Composition of the concentrates and the total mixed ration (TMR) in the experimental groups

		DDGS[1]/iodide	DDGS[1]/iodate	RSM[1]/iodide	RSM[1]/iodate
Calculated composition of concentrate					
DDGS[1]	[%]	33.0	33.0	-	-
RSM[1]	[%]	-	-	33.0	33.0
Wheat	[%]	25.0	25.0	25.0	25.0
Maize	[%]	25.0	25.0	25.0	25.0
Dried sugar beet pulp	[%]	13.5	13.5	13.5	13.5
Calcium Carbonate	[%]	0.5	0.5	0.5	0.5
Soybean oil	[%]	1.0	1.0	1.0	1.0
Mineral feed[2]	[%]	2.0	2.0	2.0	2.0
Analyzed composition of TMR					
Dry matter	[g/kg]	618	618	611	612
Crude ash	[g/kg DM]	63	63	67	67
Crude protein	[g/kg DM]	164	164	162	162
Crude fat	[g/kg DM]	42	42	37	38
Crude fibre	[g/kg DM]	150	150	159	160
ADF[1]	[g/kg DM]	179	181	187	186
NDF[1]	[g/kg DM]	353	358	353	355
ME[1]	[MJ/kg DM]	11.5	11.5	11.3	11.3
NEL[1]	[MJ/kg DM]	7.03	7.03	6.92	6.92
Glucosinolate content	[mmol/kg DM]	n.d.	n.d.	0.58	0.58

[1] DDGS = distillers dried grains with solubles, RSM = rapeseed meal, NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ME = Metabolizable Energy, NEL = Net Energy Lactation

[2] without iodine supplementation; per kg mineral feed: 140g Ca, 120g Na, 70g P, 40g Mg, 6g Zn; 5.4g Mn; 1g Cu; 40mg Se; 25mg Co; 1000000 IU vitamin A; 100000 IU vitamin D_3; 1500mg vitamin E

n.d. not detected

Iodine addition was realized by making a premix of ground wheat and the appropriate iodine amount for each iodine species and supplementation level. The adequate amount of wheat in the concentrate was exchanged with this premix. The feed was mixed few days before the start of each period. The feed intake was recorded continuously for each cow by self-feeding stations (Type RIC, Insentec B.V., Marknesse, The Netherlands) and ear transponders. According to their feeding group the cows were housed in group pens which were equipped with a slatted floor and cubicles covered with rubber mats and straw dust. Milking took place twice a day at 5.30 and 15.30 whereat milk yield was recorded with automatic milk counters. Teats were dipped with iodine free teatdip (Natidine, Hypred GmbH, Bornheim-Sechtern, Germany) after each milking.

Sample Collection, Preparation and Analysis

Representative concentrate samples were taken once, and silage samples twice a week. Before analyzing, the feed samples were dried, ground and homogenized. For disintegration, the silages were spiked with a 1% ammonia solution at a ratio of 1:100 and the concentrates at a ratio of 1:200 up to 1:1000 depending on the expected iodine content (comparable to Franke et al., 2001). The dilutions were boiled for 30 minutes and afterwards filtrated. After addition of 0.5mL tellurium (100µg/L, Alfa Aesar, Johnson Matthey GmbH, Karlsruhe) as internal standard to 10mL filtrate the samples were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500c). The GSL content of the RSM was analyzed by HPLC according to the international Standard DIN EN ISO 9167-1 (1995). Milk samples were taken on Days 1, 4, 11, 19, 20 and 21 of each period to evaluate the achievement of a steady state after the changed iodine supply. Samples were stored at -20°C. Before analyzing, the milk samples were heated up to 50°C and homogenized by Ultra Turrax treatment. The appropriate morning and evening milk were mixed according to their milk yields. The milk was diluted (1:10 or 1:25, depending on feed iodine supplementation) with a solution of Tetramethylammonium hydroxide (0.07mol/L, TMAH, Alfa Aesar GmbH Co KG, Karlsruhe, Germany) according to Fecher et al. (1998). After addition of 0.5mL tellurium as internal standard to 10mL of the dilution, the samples were analyzed by ICP-MS according to Sturup and Buchert (1996).

Calculations and Statistics

The iodine concentration of the TMR was established from the analyzed concentrations in the single feed components (concentrate, maize silage, grass silage) with regard to the proportion of the component of the TMR.

In each period the day was identified at which a steady state of the milk iodine concentration was reached (Breakpoint, BP), using the following model:

$$\text{Milk iodine} = (a + b * \text{Day}) * (\text{Day} \leq \text{BP}) + (a + b * \text{BP}) * (\text{Day} > \text{BP})$$

The established Breakpoints showed that at sampling Days 19, 20 and 21 definitely a steady state of the milk iodine concentration was reached independently from the period. Therefore only the values of those days were used for calculations and statistical evaluation.
The carry over represents the total amount of iodine in the milk per day (milk yield multiplied by milk iodine concentration) related to the respective daily iodine intake (feed intake multiplied by feed iodine concentration) given as percent.
Milk iodine reduction per mmol ingested GSL was calculated by dividing the differences in milk iodine concentration between DDGS and RSM by the mean daily GSL intake.

Statistical analysis was performed with the SAS-software package (version 9.1, SAS Institute, Cary, NC, USA). The individual cow was the experimental unit. Normal distribution was tested by the Kolmogorov-Smirnov test. The tested variables milk iodine concentration, -amount and carry over were subjected to ANOVA applying the GLM-procedure with a three-factorial design. Fixed effects were “supplementation” (0, 0.5, 1, 2, 3, 4 and 5mg I/kg DM), “protein source” (DDGS, RSM) and “iodine species” (iodide, iodate). As co-variable the “lactation number” (first lactation/heifers = 1; two or more lactations = 2) was taken into account for all three variables, for milk iodine concentration accessorily the “milk yield.” The Tukey-Kramer procedure was used for the multiple comparisons of the LSmeans of the experimental groups. LSmeans and standard errors of the mean of the milk iodine concentration and –amount are reported. Differences were considered to be significant at $P < 0.05$.

Results and Discussion

Iodine and GSL Content of Feed

The analyzed iodine concentrations of the TMR nearly corresponded with the intended feed iodine supplementations (Table 2). The iodine concentrations in the period without supplementation were below the recommendations for dairy cows of 0.5mg/kg DM (GfE, 2001). Iodine concentrations of the concentrates in the groups with iodide were not consistently lower than in the groups with iodate. Hence, the analyzed iodine contents of the feed show that no larger losses of iodine occurred when adding potassium iodide compared to calcium iodate at the storage time of 3-4 weeks. In table salt iodine losses of about 30% were investigated at 60% relative air humidity and unlimited air access after 30 days of

storage (Waszkowiak and Szymandera-Buszka, 2007) while at exclusion of light, humidity and high temperatures, most of the initially added potassium iodide was conserved during storage for several months (Voudouris, 1975).

As a consequence in the present study possible differences in the tested variables due to iodide and iodate application cannot be ascribed to iodine losses in feed but have to be traced back to differences in metabolism.

The utilized RSM showed a low GSL concentration of 3.5mmol/kg DM. The resulting GSL content of the TMR of 0.58mmol/kg DM (Table 1) led to mean daily glucosinolate intakes between 11.0 and 13.7mmol.

Table 2: Analyzed iodine concentrations of the TMR and daily iodine intakes (means ± SD, n = 8) in the experimental groups at the various iodine supplementation levels

Iodine supplementation [mg/kg DM]	DDGS/iodide[1]	DDGS/iodate[1]	RSM/iodide[1]	RSM/iodate[1]
Iodine concentration of TMR [mg/kg DM]				
0	0.18	0.18	0.16	0.16
0.5	0.61	0.71	0.70	0.61
1	1.37	1.37	1.38	1.38
2	1.98	1.84	1.92	1.97
3	2.69	2.98	2.65	3.00
4	3.93	3.58	3.37	3.92
5	4.81	4.78	5.17	5.38
Iodine intake [mg/d]				
0	3.7 ± 0.5	3.9 ± 0.5	3.7 ± 0.4	3.2 ± 0.4
0.5	12.1 ± 1.7	13.6 ± 2.5	15.9 ± 1.7	12.2 ± 0.9
1	26.3 ± 3.5	25.8 ± 3.5	26.6 ± 5.2	26.3 ± 3.6
2	40.9 ± 4.2	38.5 ± 4.3	43.4 ± 4.8	43.0 ± 3.5
3	55.4 ± 7.6	63.5 ± 7.5	62.5 ± 5.4	62.7 ± 4.7
4	84.8 ± 9.6	81.6 ± 8.6	79.9 ± 7.7	87.4 ± 6.1
5	102.9 ± 3.5	108.7 ± 9.6	119.7 ± 0.4	119.1 ± 8.9

[1] DDGS = distillers dried grains with solubles; RSM = rapeseed meal

Performance

The cows featured an average milk yield of 34.2 ± 5.7kg/d at a mean dry matter intake of 21.3 ± 2.5kg/d and a mean body weight of 633 ± 70kg. The cows of the different experimental groups featured similar performances (Franke et al., unpublished data).

Milk Iodine Concentration

Milk iodine concentration adapted to the changed iodine supply within a few days (breakpoints between 3rd and 11th day). Consequently the milk iodine concentrations of Day 19, 20 and 21 of each period were independent of the iodine supplementation of the previous period. Figure 1 shows the course of the milk iodine concentration in the periods with rising iodine supplementation of feed.

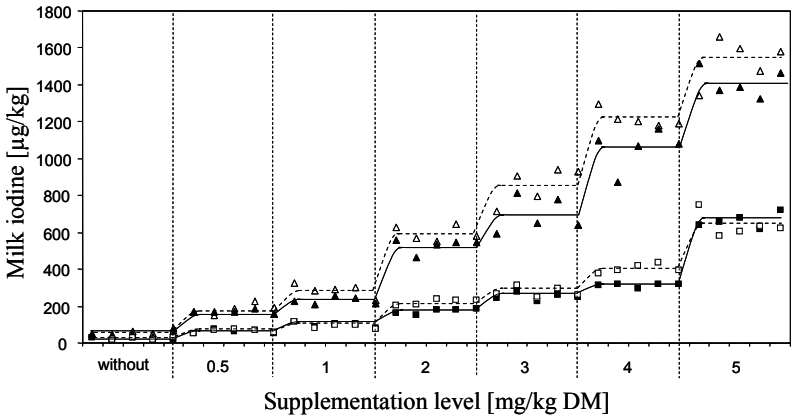

Figure 1: Milk iodine concentration within the periods of increasing iodine supplementation of the different experimental groups (▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate). The 5 parts` graduation within each period represents the means of the sampling Days 4, 11, 19, 20 and 21 (n = 8).
DDGS = distillers dried grains with solubles; RSM = rapeseed meal

The "supplementation" and the "protein source" showed a significant influence ($P < 0.05$) on the iodine concentration of milk (Tables 3 and 4). Rising iodine supplementations of feed caused increased milk iodine concentrations in all groups (Table 3). The application of RSM led to strongly diminished milk iodine concentrations compared to the groups fed DDGS. On the one hand, the significant interaction between "supplementation" and "protein source" pointed out that differences between the LSmeans of the RSM and DDGS groups were just significant at iodine supplementations of 2mg/kg DM and higher. Conversely, significant differences between supplementation levels first were investigated at feed iodine supplementations of 2mg/kg DM and higher in the groups with DDGS, and only at iodine supplementations of 4 and 5mg/kg DM in the RSM groups. Less significant differences in the case of RSM may be explained by generally lower iodine concentrations at a relatively high individual cow variation.

Table 3: Iodine concentration of milk (LSmeans ± SEM of values from Day 19, 20 and 21, n = 24)

Iodine supplementation [mg/kg DM]	DDGS/iodide[1] [µg/kg]	DDGS/iodate[1] [µg/kg]	RSM/iodide[1] [µg/kg]	RSM/iodate[1] [µg/kg]
0	$83^{D} \pm 5$	$72^{D} \pm 3$	$18^{C} \pm 2$	$27^{C} \pm 2$
0.5	$158^{D} \pm 8$	$188^{D} \pm 9$	$51^{AB} \pm 4$	$59^{C} \pm 5$
1	$214^{D} \pm 12$	$231^{D} \pm 14$	$82^{AB} \pm 7$	$73^{C} \pm 6$
2	$550^{aC} \pm 28$	$584^{aC} \pm 30$	$186^{bAB} \pm 11$	$234^{bBC} \pm 15$
3	$638^{aC} \pm 39$	$930^{aB} \pm 44$	$249^{bAB} \pm 17$	$262^{bBC} \pm 17$
4	$1085^{aB} \pm 54$	$1188^{aB} \pm 50$	$321^{bB} \pm 24$	$393^{bAB} \pm 27$
5	$1464^{aA} \pm 67$	$1578^{aA} \pm 52$	$718^{bA} \pm 46$	$620^{bA} \pm 29$

ab Values with different superscripts within a row are significantly different ($P < 0.05$)
ABCD Values with different superscripts within a column are significantly different
1 DDGS = distillers dried grains with solubles; RSM = rapeseed meal

In almost all periods milk iodine concentrations were slightly higher in the iodate groups compared to the appropriate iodide groups (Table 3). The ANOVA (Table 4) showed a significant influence of the "iodine species" on the milk iodine concentration but no significant differences were observed in any period comparing the respective groups (Table 3).

Table 4: P-values for the influence of the "iodine supplementation", "protein source", "iodine species" of the ration and their interaction as well as the impact of the co-variables "lactation" and "milk yield" on different milk parameters

	Iodine concentration	Iodine amount	Carry over rate
Supplementation	< 0.001	< 0.001	< 0.001
Protein source	< 0.001	< 0.001	< 0.001
Iodine species	0.027	0.350	0.588
Supplementation ∗ Protein source	< 0.001	< 0.001	0.014
Supplementation ∗ Iodine species	0.306	0.495	0.869
Protein source ∗ Iodine species	0.053	0.423	0.431
Supplementation ∗ Protein source ∗ Iodine species	0.249	0.219	0.713
Lactation number	0.375	0.011	0.274
Milk yield	0.758	-	-

The iodine concentration of milk showed a linear dependence on the feed iodine supplementation (Figure 2). In the region of the highest permitted feed iodine concentration (5mg/kg) the milk iodine concentration at DDGS application rose up to 1464 and 1578µg/kg when applying iodide and iodate, respectively. This lack of a steady state which would limit the attainable iodine content of milk was already found in former studies (Swanson et al., 1990; Schöne et al., 2009). Mean milk iodine concentrations of 2762 and approximately 2160µg/kg with a maximum of about 6200µg/kg were detected (Hillman and Curtis, 1980; Schöne et al., 2009).

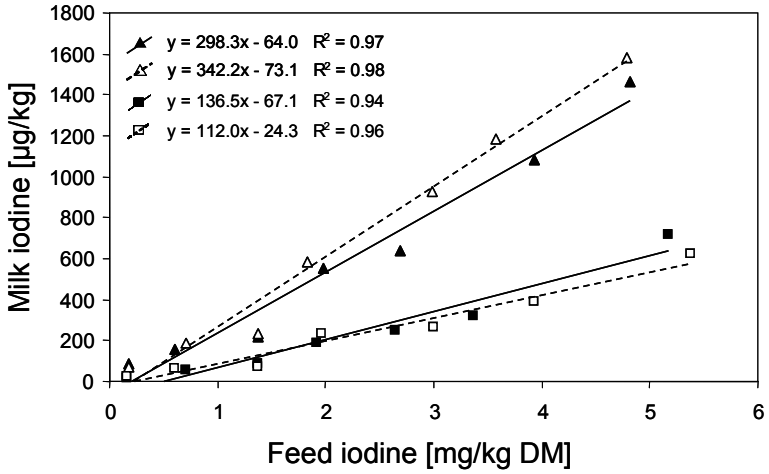

Figure 2: Dependence of the milk iodine concentration (LSmeans; n = 8) on the feed iodine supplementation in the experimental groups (▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate).
DDGS = distillers dried grains with solubles; RSM = rapeseed meal

In contrast, at the highest supplementation level the milk iodine concentrations in the RSM groups just amounted to 718 and 620µg/kg for iodide and iodate, respectively. The distribution of all values of this supplementation level after reaching the steady state showed that in the DDGS groups no sample contained less than the recommended maximum value for milk of 500µg/kg (Hamann and Heeschen, 1982), whereas in the RSM groups 32% of the values were below this concentration. In the period without iodine supplementation, the milk iodine concentrations (Table 3) in the DDGS groups were far above the critical concentration of 20 or 25µg/L, indicating a deficient supply of the cow (Alderman and Stranks, 1967; Miller et al., 1975b) while in the RSM groups concentrations were barely above this critical level in case of iodate and slightly below in case of iodide. With regard to the various impact factors on the milk iodine concentration mentioned in the introduction and the resulting different test conditions of the various trials (Hemken, 1979; Franke et al., 1983; Flachowsky et al., 2007), comparing results of different studies seems to be difficult. The high milk iodine concentrations at the highest tested supplementation of feed (5mg/kg DM) in the magnitude correspond to the results found in a preliminary investigation of our institute (Schöne et al., 2009) in which at a feed iodine supplementation of 5.5mg/kg DM, a milk iodine content of 1215µg/kg was determined. The slightly lower milk iodine contents in the previous trial result from lower feed intakes and therefore lower iodine intakes of those animals. At comparable iodine intakes, and without rapeseed in the ration, Swanson et al. (1990) and Herzig et al. (1999) investigated milk iodine concentrations on the magnitude of those determined in the present study in the DDGS groups while Kaufmann and Rambeck (1998) determined slightly lower concentrations than in the RSM groups. No information is given about the feed composition in this trial. A study by Hillman and Curtis (1980) at comparable iodine intakes showed higher milk iodine concentrations, possibly due to the utilization of ethylenediamine dihydroiodide (EDDI) as iodine species, which seems to allow higher carry over of feed iodine into milk compared to KI (Swanson et al., 1990). The method for analyzing iodine may as well be partly responsible for investigated differences (Leiterer et al., 2001).

Former studies also show a reducing effect of rapeseed in the ration on the iodine content of milk even at low GSL contents (Piironen and Virtanen, 1963; Iwarsson, 1973; Papas et al., 1979; Schöne et al., 2006). Papas et al. (1979) and Schöne et al. (2006) investigated reductions in milk iodine concentration of 78 and 54% at GSL intakes of 78.7 and 9.2mmol/d, respectively. In the present study, the average milk iodine concentrations in the groups fed RSM were reduced by 51 up to 78% compared to the appropriate DDGS groups, showing no dependency on the level of iodine supplementation. This percentage corresponded to a reduction of the milk iodine concentration by 45-958µg/kg or 4-75µg/kg per mmol ingested GSL respectively at the applied type and amount of RSM (3.5µmol GSL/g, 16.5% of ration DM) increasing linearly with rising iodine supplementation of feed. The effect of further GSL

contents of feed could not be derived from this trial. High iodine supplementations at RSM application increased the iodine concentration of milk but could not completely abolish the inhibition of the NIS. The mammary gland similarly to the thyroid responds to a glucosinolate exposure with a reduced iodine uptake. Therefore milk iodine better reflects the availability of the ingested iodine for the animal than the urinary iodine which may be elevated at glucosinolate intake (Miller et al., 1975a). The German Society of Nutrition Physiology (GfE, 2001) suggests that iodine supplementation should be twice the recommendation if the ration contains RSM. At the recommendation for dairy cows (0.5mg/kg DM; GfE, 2001) in the DDGS groups, a milk iodine concentration of 85.2µg/kg would be expected in case of iodide and 98µg/kg in case of iodate (calculated from the regression equation, Figure 2). So milk iodine concentrations on this magnitude seem to signify a sufficient iodine supply for the animal. When applying RSM, similar milk iodine contents would be reached at feed iodine concentrations of 1.1mg/kg DM both in iodide and iodate, which corresponds quite well with the recommended doubling of the iodine supplementation when applying RSM.

Differences in iodine content of milk due to the utilized iodine species were shown for potassium iodide (KI) and EDDI (Swanson et al., 1990). Studies comparing the effect of potassium iodide and calcium iodate on milk iodine show controversial results. Whereas Leskova (1969) investigated a stronger increase in milk iodine when using iodate compared to iodide, Lengemann (1969) and Bretthauer et al. (1972) ascertained no significant differences at daily oral iodine application. The ANOVA of the present study indicates that higher iodine concentrations in milk may be reached at calcium iodate application compared to potassium iodide. But the almost significant interaction between “protein source” and “iodine species” and the following comparison of the single group LSmeans do not support this finding.

Total Milk Iodine Output

Differences in the amount of iodine (Table 5) between the experimental groups and supplementation levels were comparable to the iodine concentrations (Table 3). The impact of the “supplementation” and the “protein source” (Table 4, Table 5) were significant whereas the “iodine species” showed no significant influence. The DDGS and RSM groups were significantly different at iodine supplementations of 2mg/kg DM and higher. Significant differences between the supplementation levels were observed at feed supplementations of 1mg/kg DM and higher in the case of DDGS, and starting at 2mg/kg DM in case of RSM (Table 5). Comparable to the milk iodine concentration the daily amount of iodine excreted with milk showed a linear dependence on the daily iodine intake of the cow.

Table 5: Total iodine amounts of milk (LSmeans ± SEM of values from Day 19, 20 and 21, n = 24)

Iodine supplementation [mg/kg DM]	DDGS/iodide[1] [mg/d]	DDGS/iodate[1] [mg/d]	RSM/iodide[1] [mg/d]	RSM/iodate[1] [mg/d]
0	$1.90^{E} \pm 0.19$	$2.03^{F} \pm 0.08$	$0.73^{E} \pm 0.07$	$0.87^{D} \pm 0.08$
0.5	$5.80^{DE} \pm 0.35$	$6.09^{EF} \pm 0.26$	$1.99^{DE} \pm 0.17$	$2.17^{D} \pm 0.21$
1	$8.51^{D} \pm 0.57$	$9.00^{E} \pm 0.45$	$3.31^{CDE} \pm 0.31$	$3.13^{CD} \pm 0.20$
2	$21.9^{aC} \pm 0.95$	$21.1^{aD} \pm 0.90$	$7.29^{bBCD} \pm 0.61$	$8.42^{bBC} \pm 0.49$
3	$25.5^{aC} \pm 1.43$	$30.1^{aC} \pm 1.60$	$9.39^{bBC} \pm 0.96$	$8.91^{bBC} \pm 0.66$
4	$41.1^{aB} \pm 2.33$	$41.3^{aB} \pm 1.67$	$11.5^{bB} \pm 1.28$	$14.2^{bAB} \pm 1.10$
5	$47.5^{aA} \pm 3.01$	$47.8^{aA} \pm 1.72$	$21.9^{bA} \pm 2.18$	$18.8^{bA} \pm 0.85$

ab Values with different superscripts within a row are significantly different ($P < 0.05$)
ABCD Values with different superscripts within a column are significantly different
1 DDGS = distillers dried grains with solubles; RSM = rapeseed meal

Carry Over

The carry over was significantly influenced by the "supplementation" and the "protein source" but not by the "iodine species" (Table 4, Table 6). The carry over showed no constant increase or decrease with iodine supplementation. In all groups the lowest carry over was observed at a feed iodine supplementation of 1mg/kg DM. Differences between DDGS and RSM were significant in all supplementation levels (Table 6). In cows with low milk yields and feeding a glucosinolate free ration Schöne et al. (2009) found a comparable carry over of supplemented iodine between 35 and 47%. Compared to meat and eggs (Flachowsky et al., 2006; Franke et al., 2008; Meyer et al., 2008; Röttger et al., 2008), milk shows the highest carry over of iodine from feed into food of animal origin.

Table 6: Carry over[1] of iodine from feed into milk (LSmeans ± SEM of values from Day 19, 20 and 21, n = 24 as well as overall LSmeans for the protein source and iodine species)

Iodine supplementation [mg/kg DM]	DDGS/iodide[2,3] [%]	DDGS/iodate[2,3] [%]	RSM/iodide[2,3] [%]	RSM/iodate[2,3] [%]
0	$48.2^{aAB} \pm 4.7$	$48.6^{aAB} \pm 2.9$	$18.6^{b} \pm 2.7$	$24.6^{b} \pm 3.2$
0.5	$51.6^{aA} \pm 5.7$	$45.1^{aAB} \pm 3.3$	$13.1^{b} \pm 1.7$	$18.3^{b} \pm 2.2$
1	$30.4^{aB} \pm 2.4$	$34.3^{aB} \pm 2.8$	$11.8^{b} \pm 1.4$	$10.7^{b} \pm 0.7$
2	$55.6^{aA} \pm 4.8$	$55.5^{aA} \pm 4.9$	$18.1^{b} \pm 2.2$	$19.9^{b} \pm 2.3$
3	$44.4^{aAB} \pm 4.4$	$45.2^{aAB} \pm 4.4$	$14.0^{b} \pm 2.0$	$13.8^{b} \pm 1.9$
4	$51.0^{aA} \pm 5.1$	$54.2^{aA} \pm 4.4$	$14.1^{b} \pm 2.4$	$16.8^{b} \pm 2.4$
5	$47.9^{aAB} \pm 4.5$	$44.1^{aAB} \pm 3.3$	$18.5^{b} \pm 2.9$	$16.0^{b} \pm 1.3$

	DDGS[2]	RSM[2]
0-5	$46.9^{a} \pm 1.3$	$16.3^{b} \pm 0.6$

	Iodide[3]	Iodate[3]
0-5	31.2 ± 1.8	31.9 ± 1.7

ab Values with different superscripts within a row are significantly different ($P < 0.05$)
ABCD Values with different superscripts within a column are significantly different
1 The carry over represents the total amount of iodine in the milk per day related to the respective daily iodine intake given as percent.
2 protein sources: DDGS = distillers dried grains with solubles; RSM = rapeseed meal
3 iodine species

Milk Iodine Regarding the Consumers Need

Considering the consumers' per capita consumption of milk in 2006 in Germany (62.6kg/year or 172g/d; ZMP, 2008), milk produced by the highest presently permitted feed iodine supplementation (5mg/kg) and DDGS as protein source, would already lead to an iodine intake in the range of 250-270µg/d. This amount corresponds to 126-180% of the recommendation and 25-54% of the UL for adults (WHO, 1994; D-A-CH, 2000; WHO et al., 2001). Drinking more milk or just the connection with iodine intake from other food sources (e.g., eggs, milk products, bread or sausage produced with iodized salt, sea fish, oceanic algae and iodized salt in the household) may lead to an exceeding of the UL in human nutrition.

At present in Germany, very high feed iodine supplementations (< 3mg/kg DM) are not applied in practice. In mixed feed for dairy cows Grünewald et al. (2006) detected a mean iodine content (minimum-maximum) of 0.79mg/kg DM (0.45–3.04mg/kg DM). The expectable iodine concentration in consumers' milk in Germany was estimated from the regression (Figure 2). In the case of feeding DDGS, it amounted to 184µg/kg (76–905µg/kg), and in case of RSM, to 52µg/kg (10–332µg/kg). The means are in the dimension of milk iodine concentrations currently found in consumers' milk (Rysava et al., 2007; Jahreis et al., 2007). Regarding those mean milk iodine concentrations and the per head consumption, consumers' milk presently contributes approximately 16-22% to human iodine demand when feeding DDGS, and 5-6% with the tested RSM in the ration. Taking into account that milk from different producers is mixed in practice, an occurrence of the estimated maximum concentrations in milk in Germany seems improbable. Nevertheless, an analysis of consumers' milk in the Czech Republic (Kursa et al., 2004; Rysava et al., 2007) shows that much higher iodine concentrations than the mean of 184µg/kg may occur due to regional or country-specific differences in feed iodine supplementation or manufacturing practices.

Consequences for the Maximum Level of Iodine in Feed

Regarding the cited maximum level of 500µg I/kg milk (Hamann and Heeschen, 1982) and the regression of milk iodine on iodine in the DDGS ration (Figure 2), a maximum level of iodine in dairy cow feed below 2mg/kg DM would have to be recommended (see Table 3). On the other hand, the detected milk iodine concentrations confirm the estimation of the GfE (2001) that in dairy cow feed containing RSM as protein source, an iodine supplementation of at least 1mg/kg DM is recommendable. Such a narrow range between recommendation and maximum level seems not to be practicable because different feeding practices for dairy cows may easily result in an exceeding of the maximum level. Hence, with regard to the higher requirements at the application of rapeseed the maximum level should not be lowered under 3mg/kg.

Conclusions

Iodine supplementation of feed can considerably increase milk iodine and therefore may contribute to improving the human iodine supply. On the other hand the high transfer of iodine from feed into milk may cause an exceeding of the UL in human nutrition at the currently permitted maximum level of iodine in feed in Europe (5mg/kg). Therefore the maximum level for iodine in dairy cows' feed has to be reevaluated. Because the iodine supplementations presently applied in practice in Germany are far below this maximum level, no acute risk of overdose exists at the moment. When estimating the iodine content of milk from the iodine concentration of feed, the utilization of RSM in the ration has to be considered. Milk iodine concentrations may vary due to potassium iodide or calcium iodate application resulting from metabolic differences. The impact of storage losses could not be investigated in this trial.

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PAPER III

Effect of various iodine supplementations, rapeseed meal application and two different iodine species on the iodine status and iodine excretion of dairy cows

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Abstract

The aim of the present study was to investigate the impact of various feed iodine supplementations up to the permitted maximum level in the EU, the effect of applying rapeseed meal (RSM) compared to a glucosinolate (GSL) free ration and the impact of two different iodine species (iodide, iodate) on milk, urinary, faecal and blood serum iodine as well as on T_3 and T_4 levels of blood. The results of the milk iodine are not completely shown but partly discussed in this paper. The study was conducted with 32 dairy cows, divided into 4 groups with 8 animals each. In two groups the cows were fed distillers dried grains with solubles (DDGS) as main protein source (16.5% of ration DM), in the other groups rapeseed meal (3.5mmol GSL/kg) was applied. In each case half of the animals received feed with iodine in the form of potassium iodide, the other half as calcium iodate. Iodine supplementations of 0, 0.5, 1, 2, 3, 4 and 5mg/kg DM were tested in consecutive periods of 21 days each. The supplementary iodine increased iodine contents of serum, urine and faeces. RSM application resulted in consistently higher iodine contents in the mentioned matrices just displaying significant differences at high supplementation levels. When feeding DDGS, at high iodine supplementations iodide caused higher serum and faecal iodine than iodate. Besides, the iodine species showed no consistent impact on the tested parameters. At the highest tested iodine supplementation (5mg/kg DM) in the experimental groups (DDGS/iodide, DDGS/iodate, RSM/iodide, RSM/iodate) the iodine concentration of serum amounted to 234, 157, 334 and 361µg/L, of urine to 1134, 1020, 2341 and 2513µg/L and of faeces to 673, 354, 715 and 790µg/kg fresh matter. At the same supplementation level T_4 was significantly lowered. No impact was shown for the RSM application and the iodine species on T_3 and T_4. The results of the present study indicate that high iodine intakes not only cause strong increases in milk and urinary iodine but also lead to a considerable rise of iodine excretion via faeces. RSM in feed causes a shift of iodine normally excreted via milk to an excretion via urine and faeces accompanied by higher serum iodine.

Keywords dairy cow – feces – iodine – rapeseed meal – serum – urine

Introduction

Iodine is added to dairy cow feed to prevent iodine deficiency disorders. The trace element is essential for men and animals and as constituent of the thyroid hormones regulates important metabolic processes like growth, reproduction and development of brain function. Furthermore, iodine directly regulates thyroid function (Cavalieri, 1997). Urine next to milk is used as an indicator for the iodine supply of cattle (Herzig et al., 1996). As a consequence most of the few existing studies which detected urinary iodine of dairy cows are confined to low or not defined iodine intakes while iodine excretion via faeces is hardly investigated. Moreover, to the authors' knowledge just one study (Miller and Swanson, 1973) exists

investigating the impact of various determined oral iodine dosages on the iodine concentration of all main routes of iodine excretion (urine, faeces, milk) as well as on iodine of blood serum of dairy cows in the same trial.

A main influencing factor on iodine metabolism seems to be the application of anions which inhibit the sodium iodide symporter (NIS). This active transport system extensively investigated in the thyroid also seems to allow iodine accumulation in other extrathyroidal tissues like the mammary gland, salivary glands, gastric mucosa, choroid plexus and ciliary body of the eye (Cavalieri, 1997). The most important inhibiting anions in animal nutrition are thiocyanates (SCN), a degradation product of the glucosinolates (GSL, e.g. in rapeseed feedstuffs). Since the uptake of iodine in the thyroid and the mammary gland is considerably reduced by SCN feeding (Papas et al., 1979; Cavalieri, 1997), rapeseed also ought to influence the blood, urinary and faecal iodine. Studies indicate an increased iodine excretion via urine when either perchlorate or SCN were applied (Lengemann, 1970; Miller et al., 1975a).

Furthermore, several studies showed an effect of different iodine species on the iodine excretion and blood iodine of cows (Leskova, 1969; Moss and Miller, 1970; Miller and Swanson, 1973). While organically bound iodine like ethylenediamine dihydroiodide (EDDI) seems to pass from blood into organs and tissue differently to inorganic iodine (Miller and Swanson, 1973), for iodate a delayed absorption is described compared to iodide due to its conversion to iodide prior to absorption (Moss and Miller, 1970). In the EU sodium iodide, potassium iodide, calcium iodate hexahydrate and calcium iodate anhydrous are accredited for application to animal feed (EU, 2005). Furthermore, the utilization of iodide may also cause lower iodine contents of blood, urine and faeces due to higher storage losses compared to iodate (Waszkowiak and Szymandera-Buszka, 2007).

The aim of the study was to evaluate the iodine status (serum iodine, T_3, T_4) and the iodine concentration of all main routes of elimination (urine, faeces, milk) of dairy cows at feed iodine supplementations ranging from a non supplemented ration up to the permitted maximum level of feed. Data of milk iodine due to its importance for human nutrition are presented separately by Franke et al. (unpublished data). Furthermore, the effect of applying rapeseed meal (RSM) compared to a GSL free ration and the impact of either applying iodide or iodate on these parameters should be investigated.

Materials and Methods

Animals and diets

The trial was conducted with thirty-two dairy cows (German Holsteins) in the first stage of lactation at the experimental station of the Institute of Animal Nutrition (FLI), Braunschweig in accordance with the Code of Ethics for animal experiments (Directive 86/609/EEC). With regard to the milk yield, age and days in lactation the animals were divided equally into four

groups of 8 animals each. At the beginning of the trial, the cows were on average 48 ± 25 days in milk and featured a mean number of lactations of 2.1 ± 1.2. The animals received a total mixed ration (TMR) consisting of 50% concentrate, 25% maize silage and 25% grass silage (DM basis). The precise composition is shown in Table 1. Feed and water were provided *ad libitum*. In two groups RSM containing 3.5µmol GSL/g DM was applied as main protein source (16.5% of TMR DM), in the other two groups the cows received distillers dried grains with solubles (DDGS) made from 90% wheat and 10% barley. One group of each protein source received iodine in the form of potassium iodide (KI), the other group as calcium iodate anhydrous ($Ca(IO_3)_2$). In the following, the groups are mentioned in the way they are stated in Table 1.

Table 1: Composition of the concentrates and the total mixed ration (TMR) as well as iodine concentrations of the TMR in the experimental groups

		DDGS[1]/iodide	DDGS[1]/iodate	RSM[1]/iodide	RSM[1]/iodate
Calculated composition of concentrate					
DDGS[1]	[%]	33.0	33.0	-	-
RSM[1]	[%]	-	-	33.0	33.0
Wheat	[%]	25.0	25.0	25.0	25.0
Maize	[%]	25.0	25.0	25.0	25.0
Dried sugar beet pulp	[%]	13.5	13.5	13.5	13.5
Calcium Carbonate	[%]	0.5	0.5	0.5	0.5
Soybean oil	[%]	1.0	1.0	1.0	1.0
Mineral feed[2]	[%]	2.0	2.0	2.0	2.0
Composition of TMR					
Dry matter	[g/kg]	618	618	611	612
Crude ash	[g/kg DM]	63	63	67	67
Crude protein	[g/kg DM]	164	164	162	162
Crude fat	[g/kg DM]	42	42	37	38
Crude fibre	[g/kg DM]	150	150	159	160
ADF[1]	[g/kg DM]	179	181	187	186
NDF[1]	[g/kg DM]	353	358	353	355
ME[1]	[MJ/kg DM]	11.5	11.5	11.3	11.3
NEL[1]	[MJ/kg DM]	7.03	7.03	6.92	6.92
Glucosinolate content	[mmol/kg DM]	n.d.	n.d.	0.58	0.58
Iodine concentration of TMR at the different iodine supplementation levels					
0 mg/kg DM	[mg/kg DM]	0.18	0.18	0.16	0.16
0.5 mg/kg DM	[mg/kg DM]	0.61	0.71	0.70	0.61
1 mg/kg DM	[mg/kg DM]	1.37	1.37	1.38	1.38
2 mg/kg DM	[mg/kg DM]	1.98	1.84	1.92	1.97
3 mg/kg DM	[mg/kg DM]	2.69	2.98	2.65	3.00
4 mg/kg DM	[mg/kg DM]	3.93	3.58	3.37	3.92
5 mg/kg DM	[mg/kg DM]	4.81	4.78	5.17	5.38

[1] DDGS = distillers dried grains with solubles, RSM = rapeseed meal, NDF = Neutral detergent fibre, ADF = Acid detergent fibre, ME = Metabolizable Energy, NEL = Net Energy Lactation

[2] without iodine supplementation; per kg mineral feed: 140g Ca, 120g Na, 70g P, 40g Mg, 6g Zn; 5.4g Mn; 1g Cu; 40mg Se; 25mg Co; 1000000 IU vitamin A; 100000 IU vitamin D_3; 1500mg vitamin E

n.d. not detected

In seven periods of 21 days each, iodine supplementations of 0, 0.5, 1, 2, 3, 4 and 5mg/kg DM were tested, equally in all groups. Iodine was added by making a premix of ground wheat

and the appropriate iodine amount for each iodine species and supplementation level and replacing the adequate amount of wheat in the concentrate by this premix. The feed was mixed few days before the start of each period. The individual feed intake of each cow was recorded continuously by self-feeding stations (Type RIC, Insentec B.V., Marknesse, The Netherlands) and ear transponders. The cows were housed in group pens according to their feeding group which were equipped with a slatted floor and cubicles covered with rubber mats and straw dust.

Sample collection, preparation and analysis

Representative concentrate and silage samples were taken once and twice a week, respectively. All samples of a period were pooled divided by component, dried, ground and homogenized. Blood and faeces samples were taken in the morning (between 8 and 10) of the last day (Day 21) of each period. On this day the cows had no access to any feed between 5.30 (start of milking) until the end of the sampling. Blood was drawn from the *Vena jugularis* and faeces samples were rectally taken from each cow. Tubes with a clotting activator were used for serum and tubes prepared with EDTA as anticoagulant for plasma samples. Within this investigation, iodine concentrations analyzed in serum and plasma were compared. Since no differences were detected, the analyzed serum iodine contents of the present study should be comparable with plasma iodine contents of other studies. Plasma samples were used for T_3 and T_4 analysis. Serum samples were centrifuged at 2000 x g and 15°C for 20min, plasma samples at the same conditions for 10min. Samples of spontaneous urine were obtained between 8 and 12 a.m. on Day 19. In addition blood, urine and faeces samples were taken one day before the start of the trial to detect the initial iodine content.

All samples were stored at -20°C until analysis. Milk samples were taken on Day 19, 20 and 21 of each period whereby morning and evening milk was mixed regarding the milk yields.

The crude nutrients in all feed samples were analyzed following the methods of the “Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten“ (VDLUFA, (Bassler, 1976). ADF and NDF were determined according to Goering and Van Soest (1970). Iodine analysis in all matrices was performed by inductively coupled plasma - mass spectrometry (ICP-MS, Agilent 7500c). For disintegration, the feed samples were boiled for 1/2h in a 1% ammonia solution which was added to the silage samples at a ratio of 1:100 and to the concentrates at a ratio between 1:200 and 1:1000 depending on the iodine supplementation. After filtration the filtrate was used for iodine analysis. The iodine content of serum was determined after 1:10 dilution with a solution of Tetramethylammonium hydroxide (0.07mol/L, TMAH, Alfa Aesar GmbH Co KG, Karlsruhe, Germany). Urine samples were prepared for analysis by putting the defrosted samples in the drying oven for 1h. Afterwards the samples were diluted with a 1% ammonia solution at a ratio of 1:10 up to 1:50 according to the expected iodine content. Faeces samples, similarly to feed, were diluted with a 1%

ammonia solution (1:25 or 1:50), boiled for 1/2h and afterwards filtrated. Before analyzing, 0.5mL tellurium (100µg/L, Alfa Aesar, Johnson Matthey GmbH, Karlsruhe) was added as internal standard to 10mL of all prepared samples. While T_4 was analyzed at all sampling days, T_3 was just detected at four varying supplementation levels (0, 1, 4 and 5mg/kg DM), since changes in thyroid hormones are first reflected in T_4. The contents of total T_3 and T_4 were measured in plasma with radio-immunoassay (RIA) according to the operating procedures for the applied RIA Kits (DSL TT3 RIA, DSL 3100, DSL TT4 RIA, DSL 3200, Beckman Coulter GmbH, Sinsheim, Germany). In the RSM the GSL concentration was analyzed by HPLC according to the international standard DIN EN ISO 9167-1 (1995).

To ascertain the Metabolizable Energy (ME) and Net Energy Lactation (NEL), balance studies with four wethers each were carried out for both concentrates (differing in the protein source) and for the maize- and grass silage applied following the standard procedure described by the GfE (1991).

Calculations and Statistics

For the calculation of the ME and NEL the formulas of the GfE (2001) were applied. The iodine content of the feed (TMR) was established from the analyzed concentrations in concentrate, maize silage and grass silage with regard to the daily proportion of the component of the TMR.

Since the amount of blood, urine and faeces could not be recorded, the iodine amounts in these fractions and therefore the carry over of ingested iodine into these matrices or a balance of iodine output to iodine intake could not be calculated. Thus, for evaluation of differences due to the various impact factors a carry over factor was calculated. The carry over factor of iodine into the different matrices is calculated as follows:

$$\text{Carry over factor} = \frac{\text{iodine concentration of matrix [µg/L or µg/kg]}}{\text{feed iodine concentration [mg/kg DM]}}$$

No differences in statistical evaluation of faecal iodine content in fresh and dry matter were detected. Thus, only the values of fresh matter are presented.

Iodine concentrations of the tested parameters are given as LSmeans and standard error of the mean. Although in the non supplemented period no different iodine species were tested the classification into the different groups was retained. For statistical analysis the SAS-software package (version 9.1, SAS Institute, Cary, NC, USA) was used. Normal distribution was tested by the Kolmogorov-Smirnov test. The tested variables were subjected to ANOVA applying the GLM-procedure with a three-factorial design. The “supplementation” (0, 0.5, 1, 2, 3, 4 and 5mg I/kg DM), “protein source” (DDGS, RSM) and “iodine species” (iodide,

iodate) were applied as fixed effects in the model and the "initial iodine content" of the respective matrix was implicated into the model as a co-variable. For the detection of differences between the single group LSmeans the Tukey-Kramer procedure was used. Differences were considered as significant at $P < 0.05$.

Results

Feed

The determined iodine concentrations of the TMR agreed satisfactorily with the intended supplementations (Table 1). The native iodine content of feed amounted to 0.16-0.18mg/kg DM. In the TMR as well as in the concentrate of the groups supplemented with potassium iodide analogical high iodine contents were found like in the groups with calcium iodate. The GSL content of the RSM amounted to 3.5mmol/kg DM so that the TMR of the RSM groups featured a GSL content of 0.58mmol/kg DM (Table 1) leading to mean daily GSL intakes between 11.0 and 13.7mmol.

Performance

The mean body weight, dry matter intake and milk yield of the cows over the whole trial and the iodine intakes in the various supplementation levels are presented in Table 2. The cows of the different experimental groups featured similar performances (Franke et al., unpublished data).

Table 2: Body weight, dry matter intake, milk yield and iodine intake in the experimental groups (means ± SD, n = 8)

		DDGS[1]/iodide	DDGS[1]/iodate	RSM[1]/iodide	RSM[1]/iodate
Body weight	[kg]	634 ± 75	620 ± 59	630 ± 58	648 ± 85
Dry matter intake	[kg/d]	20.7 ± 2.5	21.1 ± 2.6	22.6 ± 2.5	21.0 ± 1.9
Milk yield	[kg/d]	36.1 ± 5.7	33.4 ± 5.5	34.2 ± 5.6	33.2 ± 5.9
Iodine intake at the different iodine supplementation levels					
0 mg/kg DM	[mg/d]	3.7 ± 0.5	3.9 ± 0.5	3.7 ± 0.4	3.2 ± 0.4
0.5 mg/kg DM	[mg/d]	12.1 ± 1.7	13.6 ± 2.5	15.9 ± 1.7	12.2 ± 0.9
1 mg/kg DM	[mg/d]	26.3 ± 3.5	25.8 ± 3.5	26.6 ± 5.2	26.3 ± 3.6
2 mg/kg DM	[mg/d]	40.9 ± 4.2	38.5 ± 4.3	43.4 ± 4.8	43.0 ± 3.5
3 mg/kg DM	[mg/d]	55.4 ± 7.6	63.5 ± 7.5	62.5 ± 5.4	62.7 ± 4.7
4 mg/kg DM	[mg/d]	84.8 ± 9.6	81.6 ± 8.6	79.9 ± 7.7	87.4 ± 6.1
5 mg/kg DM	[mg/d]	102.9 ± 3.5	108.7 ± 9.6	119.7 ± 0.4	119.1 ± 8.9

[1] DDGS = distillers dried grains with solubles, RSM = rapeseed meal

Serum iodine and thyroid hormones

When iodine was supplemented the serum iodine concentration was lowest compared to urine and faeces whereby urinary iodine was 1.1-8.9 and faecal iodine 1.6-2.9 times higher (increasing with rising iodine supplementation). Milk/serum ratios were lower when RSM was applied. Serum iodine arose with increasing iodine intake of the cow (Table 3, Figure 1A) whereby at high supplementations (3mg/kg DM and higher) lower or even no increases in

serum iodine were observed especially in the DDGS groups. At iodine supplementations of 1mg/kg DM and higher, the serum iodine concentration at RSM application was consistently higher than at DDGS application amounting to 135-153% (iodide) and 133-230% (iodate) of that in the DDGS groups. Significant differences (Table 3) between serum iodine of non supplemented and supplemented animals in the RSM groups were detected at lower supplementations than in the DDGS groups. Differences between RSM and DDGS groups were significant at iodine supplementations of 2mg/kg DM and higher. The application of iodide or iodate just showed consistent differences when applying high iodine supplementations (3, 4 and 5mg/kg DM) and DDGS showing higher values for iodide application (significant at 5mg/kg DM). Figure 2A points out that with rising iodine in serum the increase in milk iodine got more intense and that the same serum iodine concentration resulted in considerably lower milk iodine contents in the RSM compared to the DDGS groups. The highest carry over factors in all experimental groups were detected when no iodine was supplemented. Furthermore, the factors were consistently higher in the RSM groups (0.371 without iodine supplementation, 0.065-0.132 with supplementation) than in the DDGS groups (0.267 without iodine supplementation, 0.029-0.091 with supplementation).

A significant impact on the T_4 content of blood was observed for the “supplementation” but not for the “protein source” or “iodine species” without any interaction between the impact factors (Table 3). The LSmeans of the supplementation levels under inclusion of all groups showed significantly lower T_4 concentrations at supplementations of 5 and 0.5mg I/kg DM whereby at the latter supplementation significance would not have occurred using a level of significance of 0.01. Considering the single groups (Table 3) significant differences were just observed in the DDGS/iodate group. No impact of the tested factors on the T_3 content of blood was investigated.

Table 3: Iodine concentrations of urine, faeces and serum as well as T_3 and T_4 content of plasma (Lsmeans ± SEM, n = 8) at the different supplementation levels in the experimental groups

Group	Supplementation [mg/kg DM]	Urine [µg/L]	Faeces [µg/kg]	Serum [µg/L]	T_3 [nmol/L]	T_4 [nmol/L]
DDGS[1]/iodide	0	54 ± 12^{A}	32 ± 3^{A}	47 ± 2^{A}	2.40 ± 0.48	99 ± 6
	0.5	202 ± 39^{AB}	89 ± 7^{A}	57 ± 3^{A}	n.d.	89 ± 11
	1	366 ± 30^{ABC}	157 ± 11^{AB}	96 ± 5^{AB}	2.13 ± 0.53	92 ± 8
	2	664 ± 102^{ABCD}	314 ± 29^{BC}	122 ± 9^{B,a}	n.d.	86 ± 6
	3	931 ± 178^{BCD}	408 ± 42^{CD,ab}	194 ± 20^{C,a}	n.d.	95 ± 13
	4	1098 ± 146^{CD,a}	594 ± 50^{DE,ab}	205 ± 20^{C,a}	1.34 ± 0.21	97 ± 6
	5	1134 ± 283^{D,a}	673 ± 60^{E,b}	234 ± 23^{C,b}	2.74 ± 0.53	74 ± 7
DDGS[1]/iodate	0	117 ± 20^{A}	57 ± 2^{A}	65 ± 3^{A}	2.57 ± 0.35	107 ± 7^{B}
	0.5	188 ± 17^{A}	107 ± 5^{AB}	66 ± 2^{A}	n.d.	67 ± 7^{AB}
	1	270 ± 43^{A}	166 ± 12^{ABC}	99 ± 4^{AB}	2.36 ± 0.27	100 ± 15^{B}
	2	448 ± 48^{AB}	264 ± 21^{ABCD}	125 ± 8^{ABC,a}	n.d.	96 ± 4^{AB}
	3	1124 ± 234^{C}	305 ± 26^{BCD,a}	162 ± 9^{C,a}	n.d.	97 ± 7^{AB}
	4	1542 ± 229^{C,a}	438 ± 37^{D,a}	172 ± 11^{C,a}	2.52 ± 0.40	107 ± 6^{B}
	5	1020 ± 123^{BC,a}	354 ± 28^{CD,a}	157 ± 11^{BC,a}	3.02 ± 0.45	58 ± 9^{A}
RSM[1]/iodide	0	97 ± 9^{A}	38 ± 3^{A}	40 ± 2^{A}	2.66 ± 0.47	91 ± 6
	0.5	249 ± 27^{A}	114 ± 11^{AB}	59 ± 3^{A}	n.d.	81 ± 8
	1	606 ± 76^{AB}	288 ± 26^{BC}	136 ± 6^{B}	2.93 ± 0.79	98 ± 9
	2	1017 ± 95^{BC}	396 ± 24^{C}	182 ± 10^{B,ab}	n.d.	93 ± 6
	3	1608 ± 173^{CD}	621 ± 48^{D,b}	297 ± 18^{C,b}	n.d.	101 ± 5
	4	1551 ± 144^{C,a}	779 ± 92^{D,b}	277 ± 17^{C,b}	2.84 ± 0.45	93 ± 6
	5	2341 ± 189^{D,b}	715 ± 92^{D,b}	334 ± 29^{C,c}	2.76 ± 0.29	60 ± 6
RSM[1]/iodate	0	120 ± 13^{A}	52 ± 3^{A}	58 ± 2^{A}	2.21 ± 0.12	87 ± 10
	0.5	225 ± 33^{AB}	115 ± 8^{A}	66 ± 3^{A}	n.d.	82 ± 7
	1	725 ± 205^{ABC}	350 ± 38^{B}	132 ± 7^{B}	2.57 ± 0.37	99 ± 10
	2	888 ± 46^{BC}	353 ± 30^{B}	211 ± 13^{C,b}	n.d.	83 ± 8
	3	1422 ± 112^{C}	456 ± 48^{B,ab}	271 ± 15^{CD,b}	n.d.	92 ± 5
	4	2372 ± 306^{D,b}	762 ± 53^{C,b}	308 ± 21^{DE,b}	2.28 ± 0.23	85 ± 6
	5	2513 ± 172^{D,b}	790 ± 70^{C,b}	361 ± 24^{E,c}	2.52 ± 0.28	71 ± 7

Group	Supplementation [mg/kg DM]	Urine [µg/L]	Faeces [µg/kg]	Serum [µg/L]	T_3 [nmol/L]	T_4 [nmol/L]
Probability						
Supplementation		<0.001	<0.001	<0.001	0.481	<0.001
Protein source		<0.001	<0.001	<0.001	0.349	0.257
Iodine species		0.155	0.006	0.681	0.890	0.656
Supplementation ∗ Protein source		<0.001	<0.001	<0.001	0.457	0.512
Supplementation ∗ Iodine species		0.003	0.007	0.024	0.923	0.888
Protein source ∗ Iodine species		0.485	0.019	0.003	0.057	0.611
Supplementation ∗ Protein source ∗ Iodine species		0.551	0.001	0.006	0.806	0.171

[1] DDGS = distillers dried grains with solubles, RSM = rapeseed meal

n.d. not detected

[ABCDE] Values with different superscripts within one experimental group in the columns show significant differences between the various supplementation levels

[abc] Values of the same supplementation level with different superscripts in the columns show significant differences between the experimental groups

Urinary iodine

The iodine concentration of urine at comparable supplementation levels was 1.6-3.7 times higher than that of faeces (Table 3) whereby the factor showed no increase with rising iodine supplementation. Rising iodine intake of the cow increased the iodine concentration of urine (Figure 1C). The RSM groups showed constantly higher urinary iodine concentrations (Table 3) amounting to 123-206% (iodide) and 103-269% (iodate) of that in the DDGS groups. Significant differences were observed at a feed iodine supplementation of 4mg/kg DM in the case of iodate and at a supplementation of 5mg/kg DM both in iodide and iodate. No constantly lower or higher values were found for any of the iodine species. A significantly lower iodine concentration only was observed for the application of iodide at a supplementation of 4mg/kg DM in the case of RSM. Figure 1C visualizes the stronger increase of the urine iodine concentration in the RSM compared to the DDGS groups and the similar rises in the iodide and iodate groups. The carry over factors as well were highest when no iodine was supplemented and they were higher in the groups fed RSM (0.745 without iodine supplementation and 0.347-0.616 with supplementation) than in the DDGS groups (0.396 without iodine supplementation and 0.170-0.427 with supplementation). Figure 2B showed that comparable to milk (Figure 2A) the increase in urinary iodine became more intense with rising serum iodine and that similar serum iodine caused slightly lower urinary iodine in the RSM groups. The relation between urinary and faecal iodine concentration (Figure 2D) was almost linear and showed no consistent difference between RSM and DDGS application.

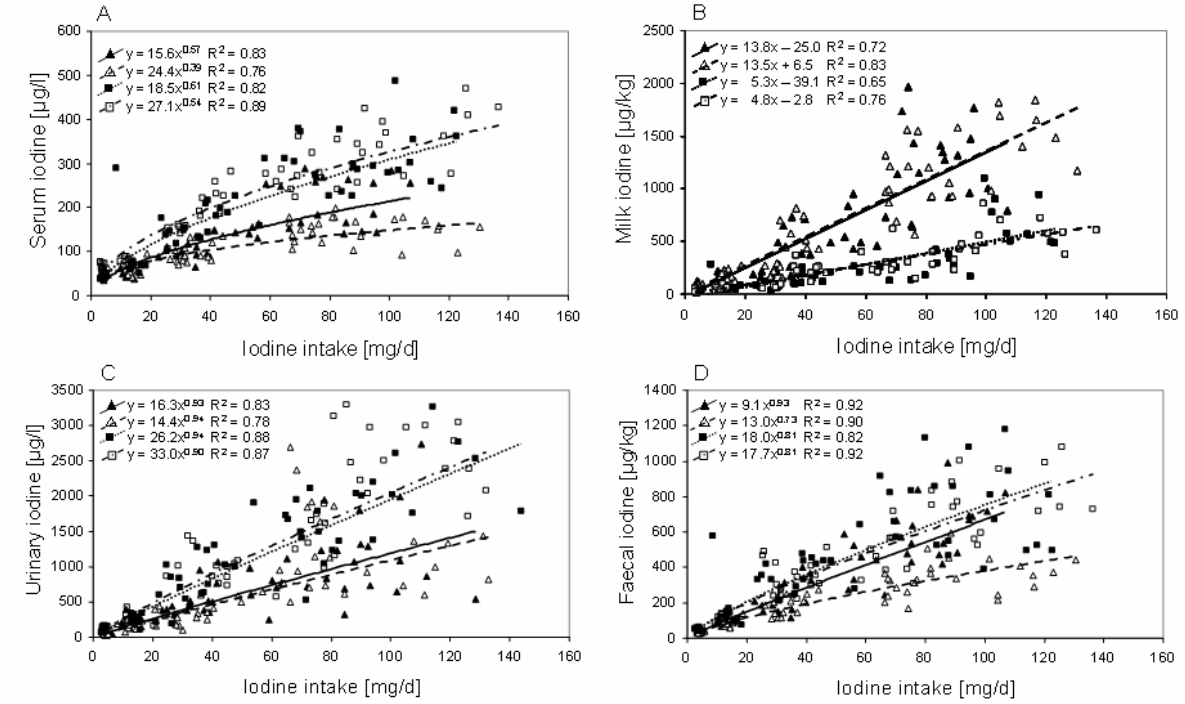

Figure 1: Dependence of iodine concentration of serum (A), milk (B), urine (C) and faeces (D) on iodine intake in the cow groups fed either distillers dried grains with solubles (DDGS) or rapeseed meal (RSM). Milk iodine concentrations are presented separately by Franke et al. (unpublished data).
▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate

Faecal iodine

Faecal iodine increased with rising supplemental iodine (Table 3, Figure 1D) but like in serum at higher iodine supplementations (3mg/kg DM and higher) lower or even no increases were observed. When iodine was supplemented in the RSM groups the faecal iodine concentrations were consistently higher than in the appropriate DDGS groups, amounting to 106-184% of the DDGS concentration in the case of iodide and 107-223% in the case of iodate. Significant differences between RSM and DDGS were just detected for the DDGS/iodate group being lower than in the RSM/iodide group at a supplementation of 3mg I/kg DM and lower than the concentrations of both RSM groups at supplementations of 4 and 5mg/kg DM. Figure 1D visualizes the lower iodine concentration in the DDGS/iodate and as well slightly lower concentrations in the DDGS/iodide group compared to the RSM groups. Comparable to serum iodine, higher concentrations at iodide application were observed at iodine supplementations of 2, 3, 4 and 5mg/kg DM in the case of DDGS showing significance at the highest supplementation level.

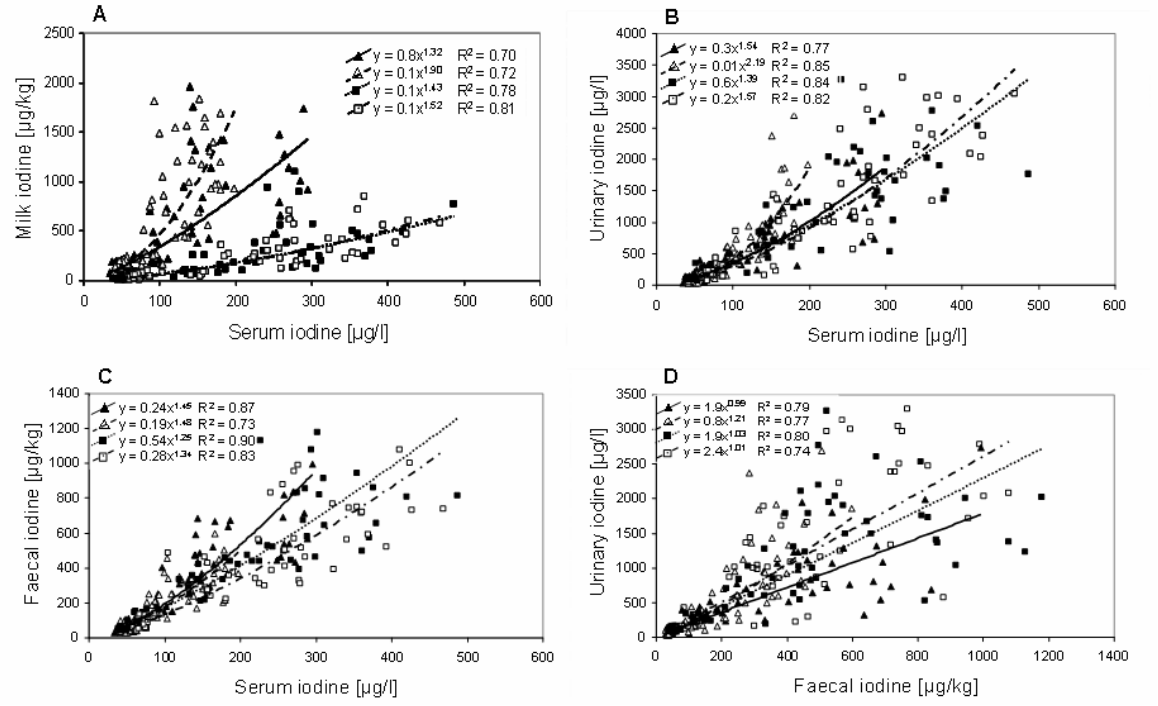

Figure 2: Dependence of iodine concentration of milk (A), urine (B) and faeces (C) on serum iodine as well as correlation between urinary and faecal iodine (D) in the cow groups fed either distillers dried grains with solubles (DDGS) or rapeseed meal (RSM). Milk iodine concentrations are presented separately by Franke et al. (unpublished data).
▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate.

Comparable to milk and urine, Figure 2C pointed out that faecal iodine increased more intensively with rising serum iodine and that the same serum iodine concentrations resulted in slightly lower faecal iodine concentrations in the RSM groups than in the DDGS groups. The carry over factors again were highest when no iodine was supplemented and they as

well were higher in the RSM groups (0.333 without iodine supplementation and 0.143-0.255 with supplementation) than in the DDGS groups (0.211 without iodine supplementation and 0.069-0.162 with supplementation).

Discussion

Feed

The iodine concentrations in the non supplemented rations were below the recommendation for dairy cows of 0.5mg/kg (GfE, 2001), but in the short time of feeding this diet no adverse effects were expected. Since feed iodine concentrations in the groups with potassium iodide were not consistently lower than in the iodate groups, no considerable iodine losses occured at the storage time of maximal 4 weeks and the existing storage conditions. In contrast to studies with salt describing losses of up to 30% at application of potassium iodide (Waszkowiak and Szymandera-Buszka, 2007), the reductive medium of the concentrate or low humidity, temperatures and oxygen access may have avoided iodide losses in the present study. For that reason possible effects of the iodine species may just occur due to differences in metabolism. The GSL content of the feed was far below the described tolerance level for ruminants of 1.5-4.22mmol/kg diet (Tripathi and Mishra, 2007).

Serum iodine and thyroid hormones

When no iodine was supplemented the serum iodine was at or above the concentration of 40µg/L (Alderman and Stranks, 1967) which indicates a sufficient iodine supply of the animal. Rising blood iodine with increasing iodine supplementations was already observed in former studies with cows (Miller and Swanson, 1973; Swanson et al., 1990; Schöne et al., 2009). At comparable iodine intakes Schöne et al. (2009) using soybean meal and Miller and Swanson (1973) established serum iodine concentrations in the magnitude of those of the DDGS groups in the present study while Swanson et al. (1990) using soybean meal found higher contents. The present study shows that the elevation of the serum iodine is fortified by RSM feeding due to the inhibition of the iodine transfer into the thyroid and the mammary gland (Cavalieri, 1997). Studies investigating the impact of perchlorate or SCN found elevated plasma iodine contents in lactating and unchanged levels in non lactating cows (Lengemann, 1973; Miller et al., 1975a). Hence, the present study fortifies the indication that only the reduced transfer of iodine into milk causes considerable increases in blood iodine. Although at low iodine supplementations only low or no differences were observed between DDGS and RSM, serum iodine due to the elevating effect of RSM feeding may pretend a sufficient supply of the animal even if the thyroid function is affected.

With regard to the iodine species the results of the present study in the case of low iodine supplementations (up to 2mg/kg DM) as well as at RSM application confirm a former investigation by Lengemann (1969) who ascertained similar iodine contents in plasma at

sodium iodide and sodium iodate application. Leskova (1969) described higher serum iodine contents at oral application of potassium iodate compared to potassium iodide. The lower serum iodine in the present study in the DDGS/iodate group compared to DDGS/iodide at high iodine supplementations is accompanied by a lower faecal iodine excretion. Moss and Miller (1970) showed that iodate due to its conversion into iodide seems to be absorbed in a later part of the gastrointestinal tract which may cause differences in metabolism when high iodine amounts are applied and the NIS is not considerably inhibited by competitive anions. The fact that without iodine supplementation no reduction of T_4 was observed shows that the animals were sufficiently supplied with iodine. Former studies with dairy cows (Hillman and Curtis, 1980; Grace and Waghorn, 2005) indicate that at sufficient iodine supply further supplementations have no impact on thyroid hormone status of blood. Only Hillman and Curtis (1980) suggested that both hyper- and hypothyroidism may have occurred in the cows inhibiting the appearance of an effect. However, lower T_4 contents at chronically high iodine supplementation were also seen by trend in pigs (Schöne, 1999) and in humans (Paul et al., 1988; Zimmermann et al., 2005) showing that already relatively low daily iodine supplementations may affect thyroid function in the case of sufficient iodine supply. No explanation could be found for the detected lower T_4 levels at iodine supplementation in the magnitude of the requirement (0.5mg/kg DM). The absence of an impact of the RSM application on T_4 is concordant with former studies which as well applied RSM with low GSL contents (Papas et al., 1979; Zech et al., 1993) while high GSL contents of feed seem to cause reductions of T_4 (Papas et al., 1979; Tripathi et al., 2001).

To the authors' knowledge just one study (Swanson et al., 1990) on the impact of the iodine species on the thyroid hormones exists which in concordance with the present trial detected no differences in T_3 and T_4 of plasma when comparing EDDI and KI. Furthermore, the present results confirm former studies (Papas et al., 1979; Grace and Waghorn, 2005) showing no influence of the iodine supplementation or RSM on the T_3 level of blood.

Urinary iodine

Herzig et al. (1996) stated a classification for assessing the iodine supply of dairy cows by urinary iodine whereby approximately 25 cows will be needed. Although in the present trial only 15 or 16 cows were available per protein source this classification was applied in the period without iodine supplementation and indicated that the cows in the RSM groups denoted a normal iodine status (60% ≥ 100µg/L, 9 of 15 cows) while in the DDGS groups cows showed a moderate deficiency (75% ≤ 100µg/L, 12 of 16 cows). Like in Herzig et al. (1999) and Miller and Swanson (1973) in the present study almost linear increases of urinary iodine were investigated with rising feed iodine supplementation. At a comparable iodine intake when applying KI (106mg/d) Miller and Swanson (1973) found a similar urinary iodine content (1017µg/L) like in the present DDGS groups. The application of EDDI in this study

caused lower urinary iodine contents. In contrast, Herzig et al. (1999) feeding a GSL free ration consistently investigated slightly higher concentrations than in the DDGS groups of the present study. In the magnitude of the requirements Herzig et al. (1999) detected urinary iodine concentrations in cows of 321 and 346µg/L applying KI and EDDI, respectively (0.8mg I/kg DM diet) while the present study for iodide and iodate showed mean contents 188 and 202µg/L in the DDGS as well as 225 and 249µg/L in the RSM groups (0.6-0.7mg I/kg DM diet). Next to the slightly higher iodine supplementation, those differences may occur due to the application of a single dose every day in the trial of Herzig at al. (1999) compared to constant iodine application throughout the day in the present trial or due to the method of iodine analysis. Newer studies investigating the effect of RSM feeding on urinary iodine excretion of dairy cattle are rare. While Papas et al. (1979) with low iodine and high GSL content of feed ascertained no impact, Miller et al. (1975a) and Lengemann (1970) confirmed the results of the present study describing an increasing effect of SCN and perchlorate on urinary iodine in cows and goats. The detected increase of urinary iodine at RSM application in the present study points out that similarly to serum iodine the urinary iodine may pretend a better supply of the animal than existent when the feed contains GSL. This assumption is fortified by the apparently better supply of the animals fed RSM compared to those fed DDGS when the feed contained no supplemented iodine.

Iodate, due to its delayed absorption also seems to feature a delayed excretion via urine (Moss and Miller, 1970). The present study indicates that at daily dosing no effect of iodide or iodate application on urinary iodine is expected.

Faecal iodine

Studies investigating the iodine content of faeces of dairy cattle are scarce. However, trials with cows, ponies and pigs (Miller and Swanson, 1973; Schöne et al., 2001; Wehr et al., 2002) confirm the investigated elevation of faecal iodine by iodine supplementation.

At comparable iodine intakes Miller and Swanson (1973) showed much lower values than the present study which may result from a very low iodine supply of these animals (2mg/d) prior to the trial. The higher faecal iodine excretion due to RSM feeding observed in the present study is contrary to a former investigation with non lactating animals describing lower faecal iodine at SCN application (Miller et al., 1975a) due to an inhibition of iodine secretion into the gastrointestinal tract by SCN. However, a recent study with rats and mice indicates that the NIS plays a certain role in iodine absorption in the intestine (Nicola et al., 2009) which may explain the higher faecal iodine excretion at RSM application. Furthermore, in the present study endogenous secretion at RSM application, in contrast to non lactating cows (Miller et al., 1975a) may be higher, since serum iodine in the RSM groups is considerably increased.

Regarding the impact of the iodine species on faecal iodine only studies testing single dosages exist. Lengemann (1969) and Moss and Miller (1970) described an initially lower

iodine concentration at iodate application. As explained for the serum, the differences between iodide and iodate in the DDGS groups at high iodine supplementations are not completely explainable. However, the results should not be overestimated, since the excreted iodine amounts were not detected.

Interrelation

Like already indicated by former studies which investigated single routes of iodine excretion or iodine status of blood, rising iodine supplementations caused almost proportional increases of milk, urinary and faecal iodine concentration and as well considerable rises of serum iodine (Table 3, Figures 1A-D). Swanson and Miller (1973) stated that high iodine intakes do not influence the percentage of the daily iodine dose which is secreted into urine, the abomasum or other parts of the gastrointestinal tract. Although quantities and therefore total iodine amounts of urine and faeces were not determined in the present study, the carry over factors indicate that the distribution of iodine to urine and faeces is not considerably influenced by the iodine supplementation at sufficient supply of the animal. The carry over (% of dose) of iodine into milk as well was not diminished with increasing iodine intake (Franke et al., unpublished data). Higher carry over factors for urine and faeces just seem to be expectable at iodine supplementations below the requirement. Since no considerably higher carry over (% of dose) of iodine into milk was observed in the present study when no iodine was added to the ration (Franke et al., unpublished data), the iodine excretion via milk compared to urine and faeces may be limited best by the organism.

The slightly lower increases of urinary, faecal and especially serum iodine with rising iodine intake (Figures 1A, C and D) at simultaneously constant rise in milk iodine (Figure 1B) may result from a higher transfer of iodine into the thyroid or extrathyroidal tissues and organs or from the time-displaced application of the various iodine dosages.

While to the authors' knowledge no study exists investigating the impact of RSM feeding on all main routes of iodine excretion (milk, urine and faeces) in dairy cows, Schöne et al. (2001) comparably to the present study observed reduced milk iodine accompanied by higher serum, urinary and faecal iodine contents in sows fed rapeseed press cake. Different models demonstrated by Miller et al. (1975b) tried to describe the distribution of iodine to the different compartments of cattle but strong differences seem to occur due to lactating compared to non lactating animals, rapeseed in the ration, stage of lactation and certainly some other factors. The present study demonstrates the changes due to RSM feeding and indicates that differences in the impact of RSM occur in lactating and non lactating cattle.

While at the same serum iodine concentration milk iodine content varied widely (Figure 2A) due to the protein source and cow individuality, the iodine contents of urine and faeces showed just slight differences between the DDGS and RSM groups (Figures 2B and C). However, the figures show that not only the relation between serum and milk iodine but also

to a smaller extent the relation between serum and urine as well as serum and faeces is changed by RSM feeding.

In the present study first effects on thyroid function (reduced T_4) were seen at a supplementation of 5mg/kg DM coming along with milk and urinary iodine concentrations of approximately 1400-1600µg/kg and 1000-1100µg/L in the case of DDGS or 600-700µg/kg and 2300-2500µg/L in the case of RSM. The variation in milk and urinary iodine at similar iodine intakes clarifies the importance of the feed composition (application of rapeseed) not only for recognition of a deficiency but also of an excessive iodine supply of the animals whereby different amounts and GSL contents may influence those parameters to a different degree. Further research is needed especially in the field of iodine absorption and endogenous secretion to explain the differences in iodine metabolism due to interactions between the tested parameters.

Conclusions

Increasing iodine intakes in dairy cows result in higher serum iodine and higher iodine excretion by all main routes of elimination (milk, urine, faeces). When the ration contains RSM the diminished transfer of iodine into milk is compensated by stronger iodine excretion via urine and faeces and also increases in serum iodine. Due to the considerable increases of iodine concentration by RSM, the blood and urinary iodine may pretend a better iodine supply of the animal than existent. Since the mammary gland similarly to the thyroid responds to a GSL exposure with a decreased iodine uptake, the milk iodine concentration seems to be a better indicator of the iodine supply of dairy cows if feed contains rapeseed components. The application of the presently permitted maximum level of iodine in feed of dairy cows may affect the thyroid function. This fact in combination with the cause of high milk iodine recommends a lowering of the maximum level of iodine in feed for dairy cows.

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GENERAL DISCUSSION

It has been shown in low-yielding cows, that iodine supplementations within the presently allowed maximum level in dairy cow feed (EU, 2005) lead to a great variation of the milk iodine concentration (Schöne et al., 2009). Moreover, the high milk iodine concentrations achieved at the maximum permitted iodine supplementation may cause an exceeding of the UL in human nutrition. On the other hand, it is stated that the application of feedstuffs which contain goitrogens (e.g. RSM) considerably reduce milk iodine concentration (Papas et al., 1979; Schöne et al., 2006). However, existing studies allow no estimation of the extent of reduction at varying supplementation levels with the nowadays used 00-rapeseed. The appropriate iodine species for feed supplementation is still discussed, since for example in table salt iodate is preferred due to higher storage stability compared to iodide. Higher storage losses would result in a lower availability of the iodine for the animal and probably as well lower milk iodine excretion.

In comparison to the glucosinolate containing RSM, DDGS from wheat and barley was used because this feedstuff seems to possess no goitrogenic potential. The impact of the applied iodine supplementations on the performance of dairy cows could not be investigated while RSM does not influence the performance at the tested GSL intakes. Since the performance data, however, is necessary for evaluating the impact of the mentioned parameters on iodine metabolism, they were evaluated, focussing on the impact of the applied DDGS (**Paper I**). In general it is assumed that DDGS can replace other protein feedstuffs without consequences on economically relevant performance parameters (Urdl et al., 2006; Spiekers et al., 2006; Ettle, 2007). But since it is just extensively utilized in animal nutrition for few years and its composition is still inconsistent, data are rare.

A declaration of the iodine content of milk seems impossible so that food safety has to be guaranteed by setting low maximum levels for iodine in feed. Since former studies are insufficient for risk assessment and evaluation of the present maximum level (EFSA, 2005), in the present trial the impact of staggered iodine supplementation up to the permitted maximum level of feed (5mg/kg diet) on the milk iodine concentration was investigated (**Paper II**). Thereby feed without goitrogenic potential was compared to feeding a diet with a high proportion (16.5% of ration) of a commonly used RSM with a low GSL content (3.5mmol/kg). Furthermore, the addition of iodine in the form of potassium iodide was compared with calcium iodate application. Changes due to the mentioned impact factors are also expected in blood and in the other main routes of iodine excretion (urine and faeces), but they are poorly elucidated and therefore should be established in the present trial (**Paper III**).

1 Feed intake and performance

Dry matter intake, milk yield and milk composition as well as secretion of fat, protein and lactose change with rising stage in lactation. As a result the influence of the iodine supplementations (which were applied one after another) on the performance could not be evaluated. Nevertheless, the various iodine supplementations were applied consecutively, since former studies showed no effect of similar iodine supplementations on the performance parameters of dairy cows (Hemken et al., 1972; Potter et al., 1980; Kaufmann and Rambeck, 1998; Grace and Waghorn, 2005). Moreover, the tested GSL intake by RSM is tolerated by ruminants without effects on the performance. Thus, regarding the performance parameters, the impact of the applicability of the DDGS compared to RSM was in the centre of attention. The assignment of the animals to four groups was maintained to allow a better association between the performance and the iodine parameters. Comparable to RSM, distillers grains (DG) like DDGS gain in importance in animal nutrition due to their increasing availability at fortified bioenergy manufacture. However, in contrast to RSM, DGs are characterized by a high variability in their nutrient value (Belyea et al., 1998; Spiekers et al., 2006) due to the utilized raw material (maize, wheat, barley, rye, sorghum or mixtures from wheat and barley) and its processing (wet or dried DGs, with or without addition of solubles in dried DGs, extent of fermentation and drying process). It is stated that DDGS from maize is the main DDGS, available in the most areas while DDGS from wheat or barley is just available in some areas of Canada and Europe (Schingoethe, 2006). Therefore investigations on DDGS based on wheat or barley are rare. Some feeding trials tested the application of wheat DDGS in dairy cows (Dunkel, 2005; Urdl et al., 2006; Ettle, 2007), but partly just using low amounts of DDGS (Dunkel, 2005) or low-yielding animals (Urdl et al., 2006) so that limits for application of DDGS from wheat or barley are unclear (Spiekers et al., 2006). Hoffmann and Steinhöfel (2009) state an application limit for dried distillers grains of 3.5kg per animal and day or 35% of the mixed ration at feed intakes up to 10kg, but they give no reason for the restriction. To the authors' knowledge, no study exists on the impact of high proportions of wheat/barley DDGS in the ration on the performance of high yielding cows. Similar crude nutrient contents and digestibilities of wheat and wheat/barley DDGS indicate that their impact on performance is comparable while maize DDGS usually features lower protein and higher fat contents (Spiekers et al., 2006). Urdl et al. (2006) and Ettle (2007) found no difference in DMI, milk yield and milk composition when applying wheat DDGS compared to RES and soybean meal or a mixture of rapeseed press cake and soybean meal. Dunkel (2005) described no impact of wheat DDGS on the DMI, milk fat and milk protein as well, but found lower milk yields. The latter study showed the importance of the drying process of DDGS, since it was suggested that an overheating of the proteins may have caused a lower protein availability leading to diminished milk yields.

Iodide and iodate application resulted in similar DMIs and milk yields as well as contents of milk fat, milk protein, milk lactose and milk urea (**Paper I**). The applied DDGS (16.5% of the ration), based on wheat and barley in concordance with the former studies with wheat DDGS predominately showed no impact on DMI, milk yield and milk composition. Only the milk protein concentration was lower at DDGS application which mainly could be ascribed to slightly but not significantly higher milk yields, since no significant difference was established for the daily milk protein yield. Although a depressed milk protein percentage is rarely seen at DDGS feeding, a limited protein or lysine intake or higher fat contents of the diet are described as possible reasons (Schingoethe, 2006). A positive protein balance (difference between uCP demand and uCP intake) and unchanged or even slighty higher milk yields in the case of DDGS in the present trial (**Paper I**) indicate a sufficient protein supply of the animals, but the lysine content of the diet was not analyzed. Although the differences in the fat content of the applied DDGS and RSM were very low (**Paper I**), it was suggested that the slightly higher fat concentration of the DDGS and therefore slightly higher fat intakes may have contributed to the lower milk protein percentages. Numerically lower milk fat percentages (**Paper I**) fortify this assumption, since a higher fat content of DDGS compared to coarse extraction meals also was shown to cause milk fat depression when the proportion of DDGS of the ration arises (Kononoff and Christensen, 2007). Implications due to a higher fat content of DDGS could easily be avoided by including this aspect in the formulation of the diet.

2 Feed iodine

Iodides are characterized by a higher instability in presence of oxygen, high temperature and high humidity compared to iodates. In table salt, iodine losses of about 30% were investigated at 60% relative air humidity and unlimited air access after 30 days of storage (Waszkowiak and Szymandera-Buszka, 2007). The losses can be minimized by exclusion of light, humidity and high temperatures, shown by a conservation of most of the initially added potassium iodide in table salt at storage for several months (Voudouris, 1975). Since in feed storage, the access of light, heat and humidity cannot completely be excluded, possible losses of iodine from feed at iodide application may cause a lower availability of the added iodine for the animal compared to iodate application. In the present study the appropriate concentrates were mixed some days prior to the beginning of each period, so that storage times of up to 4 weeks were reached (**Paper II**). The iodine concentrations of the concentrates and TMRs were not consistently lower in the groups with iodide than in the groups with iodate, so that no considerable higher iodine losses occurred when adding KI compared to $Ca(IO_3)_2$. In contrast to salt, the reductive medium of the concentrates may avoid considerable iodine losses. As a consequence possible differences in the tested

variables of the present study between iodide and iodate application cannot be ascribed to feed iodine losses, but have to be traced back to differences in metabolism.

The iodine concentrations in the period without supplementation were below the recommendations for dairy cows of 0.5mg/kg DM (GfE, 2001). However, a much longer period of time and probably lower iodine contents would be necessary to induce an iodine deficiency in the animal (Potter et al., 1980).

3 Iodine supply of the animals

In dairy cows serum, urinary and milk iodine concentrations are used as indicators for the iodine supply of the animals whereby predominately minimum levels are stated which should be exceeded for the exclusion of a deficiency. While for blood a limit of 40µg/L and for milk of 20-25µg/L is stated (Alderman and Stranks, 1967; Miller et al., 1975b), Herzig et al. (1996) showed that the complex classification of urinary iodine levels (shown in **Table 1 of the Background**) which was established for humans (Bourdoux, 1993) is applicable to dairy cows as well, even requiring a lower number of individuals.

In the period without iodine supplementation, the serum iodine concentration suggested an iodine intake of the animals at the lower supply level (**Paper III**). The milk iodine concentrations in the DDGS groups (**Paper II**) indicated a sufficient supply whereas in the RSM groups milk iodine was barely above the critical concentration in the case of iodate and slightly below in the case of iodide. In contrast, the iodine concentration of urine denoted a moderate deficiency (75% of values ≤ 100µg/L) in the DDGS groups and a normal iodine status (60% of values ≥ 100µg/L) in the RSM groups. An absent diminishment of T_4 in blood affirmed a sufficient supply of the animals in this period (**Paper III**).

At the highest tested iodine supplementation the iodine contents of serum (**Paper III**) were in the range of the stated level of an excessive supply of 200µg/L (Alderman and Stranks, 1967; Launer and Richter, 2005) in the DDGS groups and far above it in the RSM groups.

4 Iodine status and iodine elimination

4.1 Effect of iodine supplementation

Primarily, the supplementation of various iodine amounts in feed of livestock was investigated to establish the adequate amount for eliminating iodine deficiency disorders. Later their potential in contributing to human iodine supply by iodine transfer into food of animal origin came to the fore. However, the high existing maximum levels and the efforts to enrich food of animal origin rapidly brought up the risks of excessive iodine uptakes in animals and humans. While the problem of high iodine intakes causing high milk iodine concentrations and its risk for animal and human health in the US already was described in

the 1980s (Hemken, 1980; Olson et al., 1984; Berg et al., 1988; Swanson et al., 1990), in Europe such concerns increasingly appeared in recent years (EFSA, 2005). In the US the maximum level for iodine supplementation of EDDI in dairy cows in the meantime is set at 10mg/d (NRC, 2001) which according to the NRC corresponds to a supplementation of approximately 1mg/kg DM whereas in the EU it was recently decreased from 10 to 5mg/kg DM.

Former studies investigating the impact of various feed iodine supplementations mostly are confined to single parameters (predominately milk), but did not investigate the all-embracing influence on the iodine status (blood iodine, T_3 and T_4) and on all main routes of iodine elimination in the cow (milk, urine, faeces). Miller and Swanson (1973) investigating the most parameters described almost linear increases of serum, milk, urinary and faecal iodine concentration by staggered EDDI supplementations up to 1000mg/d. Further studies consistently affirmed the elevating impact on the iodine content of all mentioned matrices (**Table 8**).

Milk iodine concentration was shown to rapidly adapt to changes in iodine intake independently from a rising or decreasing iodine supply (Miller et al., 1975b; Swanson et al., 1990; Flachowsky et al., 2007). In concordance with Miller et al. (1975b), who stated an equilibrium between iodine intake and milk iodine between Day 7 and 10, in the present trial a plateau of the milk iodine concentration was reached between the 3rd and the 11th day after the iodine dosage was changed (**Paper II**). The model for estimating the day at which a steady state was reached is shown on page 45. As an example the estimated functions for the development of the milk iodine concentration in the period with an iodine supplementation of 3mg/kg DM (following the period with 2mg/kg DM) is shown in **Figure 5**.

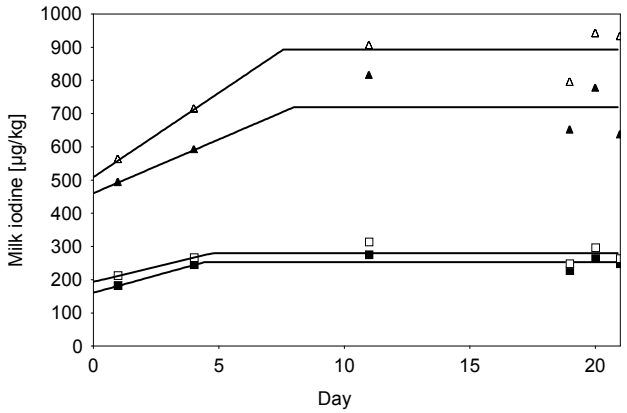

Figure 5: Development of the milk iodine concentration (means of sampling Days 1, 4, 11, 19, 20 and 21) in the period with an iodine supplementation of 3mg/kg DM in the experimental groups (▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate). The day of achievement of a steady state (BP) was calculated by the following model: Milk iodine = (a + b ∗ Day) ∗ (Day ≤ BP) + (a + b ∗ BP) ∗ (Day > BP)
▲ y = (460.6 + 32.4 ∗ x) ∗ (x ≤ 8.00) + (460.6 + 32.4 ∗ 8.00) ∗ (x > 8.00)
△ y = (509.1 + 50.1 ∗ x) ∗ (x ≤ 7.56) + (509.1 + 50.1 ∗ 7.56) ∗ (x > 7.56)
■ y = (161.0 + 20.8 ∗ x) ∗ (x ≤ 4.44) + (161.0 + 20.8 ∗ 4.44) ∗ (x > 4.44)
□ y = (193.8 + 18.1 ∗ x) ∗ (x ≤ 4.73) + (193.8 + 18.1 ∗ 4.73) ∗ (x > 4.73)

Table 8: Impact of rising iodine supplementations, RSM in the ration and iodide application (compared to iodate) on the iodine concentration of blood, milk, urine and faeces in dairy cows investigated by former studies compared to the results of the present trial

Source	Rising iodine supplementation				RSM application				Iodide compared to iodate application				Comments
	blood	milk	urine	faeces	blood	milk	urine	faeces	blood	milk	urine	faeces	
Berg et al. (1988)		↑											
Bretthauer et al. (1972)										↑↓			
Hemingway (2001)	↑												plasma inorganic iodine
Hemken et al. (1972)		↑											
Herzig (1996)			↑										
Herzig et al. (1999)		↑	↑										
Hillman and Curtis (1980)		↑	↑										
Iwarsson (1973)					↑↓	↓							protein bound iodine
Kaufmann et al. (1997)		↑											
Kaufmann and Rambeck (1998)		↑											
Kroupova (1996)			↑										
Lengemann (1969)									↑↓	↑↓			NaI and $NaIO_3$, plasma
Lengemann (1970)					↑	↓	↑	↑					plasma, perchlorate, goats
Lengemann (1973)					↑								plasma, perchlorate, cows
Leskova (1969)									↓	↓			KIO_3 and KI, serum
Miller and Swanson (1973)	↑	↑	↑	↑									serum
Miller et al. (1975a)					↑↓		↑	↓					plasma, cows/calves
Papas et al. (1979)						↓	↑↓						
Piironen and Virtanen (1963)						↓							
Rogers and Mee (1996)	↑												heifers, plasma inorganic iodine
Schöne et al. (2006)						↓							
Schöne et al. (2009)	↑	↑											serum
Swanson et al. (1990)	↑	↑											plasma
present trial	↑	↑	↑	↑	↑	↓	↑	↑	(↑)	↑↓	↑↓	(↑)	

↑ increasing impact on iodine concentration
↓ decreasing impact on iodine concentration
↑↓ no impact observed on iodine concentration

Various former dose-response studies describe a proportional increase of milk iodine with rising iodine intake of the cow (Miller and Swanson, 1973; Swanson et al., 1990; Kaufmann et al., 1997; Herzig et al., 1999; Schöne et al., 2009) leading to high iodine concentrations. While at present in consumers' milk the iodine concentration approximately ranges between 50 and 200µg/L, Miller and Swanson (1073), Hillmann and Curtis (1980) and Schöne et al. (2009) detected mean milk iodine concentrations of 2393, about 2100 and 2762µg/kg with a maximum of about 6200µg/kg. Thus, it is indicated that no saturation for the iodine transfer into milk occurs.

However, at comparable iodine intakes the stated milk iodine concentrations vary considerably. Various factors are described which to a smaller or greater extent may be responsible for the differences, including goitrogens in feed, the breed, the applied iodine species, the stage of lactation, the utilization of iodized udder disinfection solutions, iodine containing drugs, the analyzing method and possibly the milk yield (Iwarsson, 1973; Papas et al., 1979; Hemken, 1979; Franke et al., 1983; Swanson et al., 1990; Leiterer et al., 2001; Flachowsky et al., 2007). Due to the different impact factors it is recommended that studies investigating milk iodine should describe feed composition, details of the utilized animals and dipping methods as detailed as possible to permit comparability.

The milk iodine concentrations detected in the present study in the DDGS groups (**Paper II**) were at the upper level of the previously investigated values except for the study of Schöne et al. (2009) who ascertained still higher contents. The concentrations in the RSM groups were lower than those of most studies except for Kaufmann and Rambeck (1998).

Since the trial of Schöne et al. (2009) like the present study was carried out at the Institute of Animal Nutrition of the Friedrich-Loeffler-Institute, a more detailed comparison was possible. At an iodine supplementation (5.5mg/kg DM) in the magnitude of the presently permitted iodine content of feed for dairy cows (5mg/kg), Schöne et al. (2009) detected a mean milk iodine concentration of 1215µg/kg in cows with low milk yields (average milk yield = 19.8kg/d) and with soybean meal as main protein source. In comparison, in high yielding cows (average milk yield = 34.2kg/d) the milk iodine concentration at DDGS application amounted to 1464 and 1578µg/kg when applying iodide and iodate at a feed iodine supplementation of 5mg/kg DM (**Paper II**). Similar or slightly higher milk iodine concentrations at comparable feed iodine supplementations (**Figure 6A**) in the higher yielding cows mainly result from higher feed intakes (on average 21.3 compared to 13.3kg DM/d in the previous trial) and therefore higher iodine intakes (103 and 109 compared to 72.3mg/d) of the animals. In **Figure 6B** the regressions for the dependence of the milk iodine concentration on the iodine intake in the DDGS groups of the present trial are compared with the regression from the data of the previous trial. At comparable iodine intakes, the higher milk yields result in constantly lower milk iodine concentrations than

observed in the previous trial with lower milk yields. When comparing the milk iodine concentrations at the iodine intake (DDGS/iodide = 102.9, DDGS/iodate = 108.7mg/d) achieved by the maximal permitted iodine concentration in the present trial (5mg/kg DM), they were 28 and 27% lower than in the previous trial.

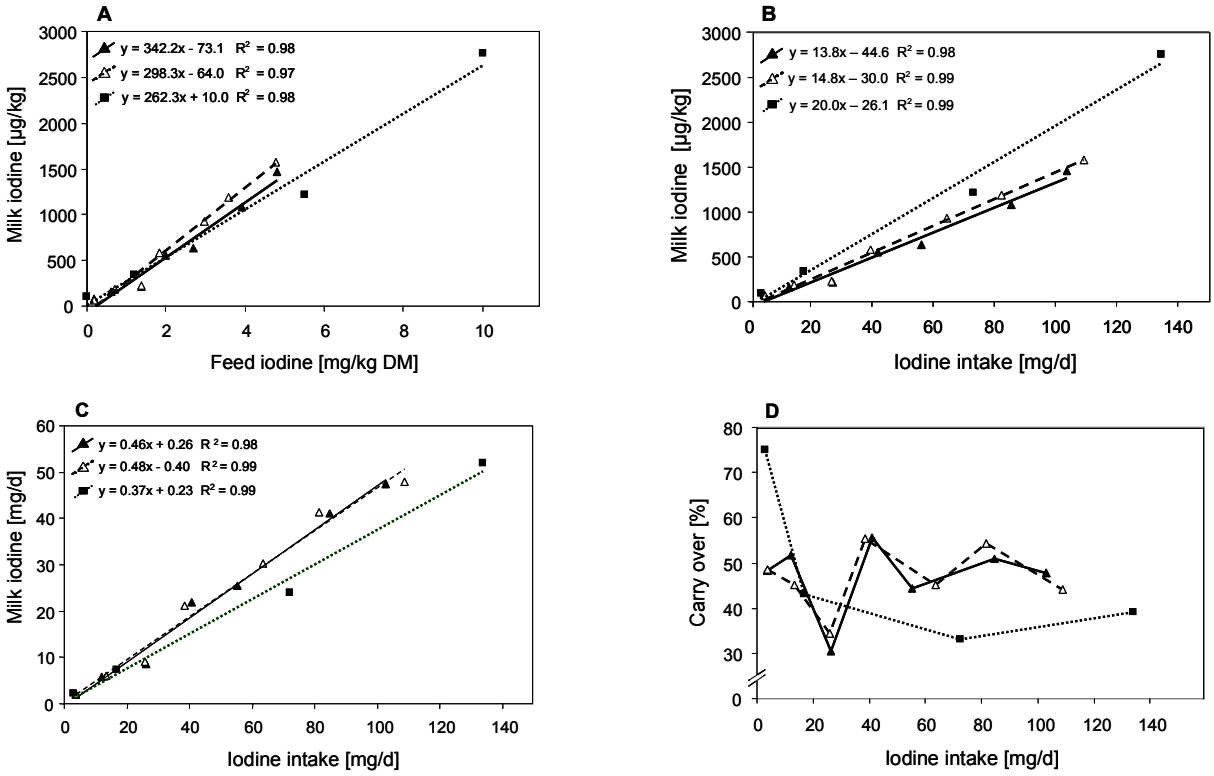

Figure 6: Comparison of the presently detected milk iodine concentrations at various feed iodine concentrations (A) or iodine intakes (B) as well as of the total milk iodine amount (C) and the Carry over (D) at the different iodine intakes in the DDGS groups (▲ DDGS/iodide, △ DDGS/iodate) with the results of a previous study of Schöne et al. (2009, ■)
DDGS = distillers dried grains with solubles

The comparison of the two trials at similar iodine intakes indicates an inverse relationship between milk yield and milk iodine concentration which was also found in older studies (Miller et al., 1963; Iwarsson, 1973). However, a later study, which observed rising milk iodine concentrations with decreasing milk yields as well, rather ascribed this effect to the simultaneously rising stage in lactation (Franke et al., 1983). Larson et al. (1983) and Berg et al. (1988) described no relation between milk yield and milk iodine concentration.

It was stated that higher milk yields are connected with higher total iodine amounts secreted into milk (Miller et al., 1963; Miller et al., 1975b). The comparison of the total excreted milk iodine amounts of the study of Schöne et al. (2009) with the present ones (**Figure 6C)** is concordant with the previous findings, since higher milk iodine amounts were detected in the higher yielding cows (unless no iodine was applied).

The detailed values for the carry over of the study of Schöne et al. (2009) are presented by Bemmann (2005). The comparison of the present results (**Paper II**) with the previous trial showed that the carry over at comparable iodine intakes was slightly higher in the higher yielding cows of the present trial except when no iodine was supplemented (**Figure 6D**). In the present trial a slight but significantly positive correlation was observed between the milk yield and the carry over (**Table 9; Figure 7**). The high carry over in the previous trial when supplementing no iodine leads to a decreasing carry over with rising iodine supplementation, in contrast to the quite constant carry over of the present trial. These high values may appear due to the still lower iodine intakes in the previous trial (3.0mg/d) compared to the present one (3.7-3.9mg/d) at similarly low iodine secretions into milk. The, apart from that, slightly higher carry over in the present trial may contribute to the higher milk iodine concentrations at comparable feed iodine concentrations (**Figure 6A**) compared to Schöne et al. (2009). In addition, differences in the stage of lactation may have influenced the iodine transfer into milk, since the animals of the present study were in early up to mid-lactation while in the previous trial cows in the end of lactation were used. Former studies indicated an increase of the iodine content of milk with rising stage of lactation (Iwarsson, 1973; Franke et al., 1983).
It is shown that for a better evaluation of the impact of the milk yield on milk iodine concentration, looking at the totally excreted iodine amounts is meaningful. Nevertheless, the iodine amounts in most studies are not stated. In the present study differences were obvious, since the ANOVA indicated an impact of the “iodine species” on milk iodine concentration but not on the total iodine amount (**Paper II**).

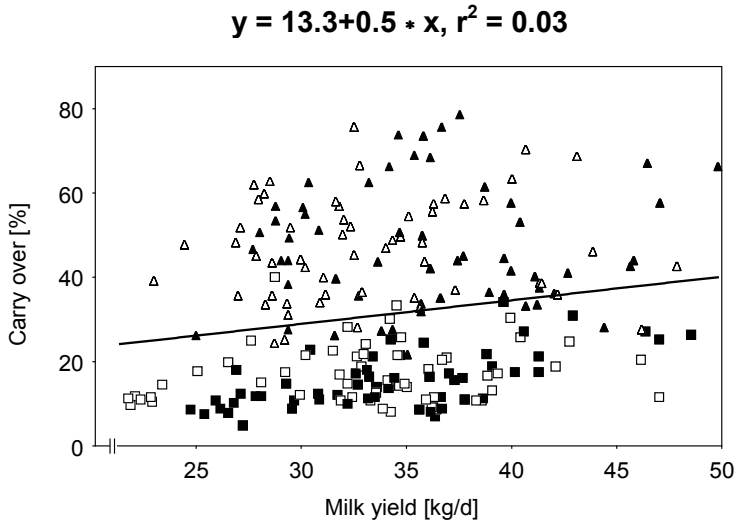

Figure 7: Relationship between the milk yield and the carry over of iodine into milk in the experimental groups (▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate)

In concordance with previous investigations (Miller and Swanson, 1973; Hillman and Curtis, 1980; Swanson et al., 1990; Herzig et al., 1999; Schöne et al., 2009), the results of the

present study demonstrate that next to milk iodine, rising iodine supplementations constantly elevate blood, urinary and faecal iodine concentration without achieving a steady state as well (**Paper III and Table 8**). Significantly positive correlations were detected between the feed iodine concentration or the daily iodine intake and the iodine concentrations of serum, milk, urine and faeces (**Table 9**). However, the high variation in the values due to the different applied protein sources (DDGS, RSM; **Paper III, Figure 1**) shows that the correlation would be better if the same feed is applied.

On the one hand the reason for the similar effect on all matrices is that iodine absorption seems not to be diminished at high iodine intakes (Miller et al., 1975a), on the other hand iodine in blood is not homeostatically kept to a constant level but seems to be equilibrated between the different body compartments (Miller et al., 1975b). Compartment models therefore suggest a quite constant distribution of iodine to the different routes of iodine elimination (Miller et al., 1975b).

Like in humans, the excretion via urine is considered as the main route of iodine elimination in dairy cows, although the proportion of the total iodine excretion is much lower in ruminants (McDowell, 2003; Simon, 2008). Even though faecal iodine in ruminants seems to present a greater part of the total iodine excretion than in humans, it is rarely investigated due to its failing importance as indicator of the iodine supply. However, studies with horses and pigs (Wehr et al., 2002; Franke et al., 2008) affirm the detected considerable increases with rising iodine intake as well. A part of the increase in faecal iodine at higher iodine intakes can be ascribed to the numerically increasing not absorbed iodine at constant rate of absorption. The contribution of changes in endogenous iodine excretion or iodine reabsorption cannot be clarified by the present trial.

While former studies suggest that, like in milk, rising iodine supplementations cause a linear increase of serum, urinary and faecal iodine, the present study showed diminished or even missing increases at high iodine supplementations in some groups (**Paper III**). The lower or missing increase especially was apparent in serum and faeces which is also shown by slightly negative, but significant correlations between the feed iodine content and the carry over factors (for calculation see page 61) of serum and faeces (**Table 9**). In contrast, no significant correlation was shown between feed iodine and the carry over factor for urine and the carry over into milk. These effects may indicate a higher transfer of iodine into the thyroid or extrathyroidal tissues and organs at high iodine intakes. It is imaginable that such effects just occur at a constant application of iodine throughout the whole day, in contrast to the application of a single daily dose in the former trials. Furthermore, an impact of the time-displaced application of the different iodine dosages in the present trial cannot completely be excluded although no relation was found between cows with low iodine concentrations and possible influencing factors like pregnancy or stage of lactation.

Table 9: Correlation coefficients between various parameters (all experimental groups are included) and P-values for significance of correlation

	Iodine intake [mg/d]	Serum iodine [µg/L]	Milk yield [kg/d]	Carry over factor serum	Milk iodine [µg/kg]	Milk iodine [mg/d]	Carry over milk [%]	Urinary iodine [µg/kg]	Carry over factor urine	Faecal iodine [µg/kg]	Carry over factor faeces	Triiodothyronine [nmol/L]	Thyroxine [nmol/L]
Feed iodine [mg/kg DM]	0.98 P < 0.01	0.78 P < 0.01	0.00 P = 0.96	-0.57 P < 0.01	0.75 P < 0.01	0.72 P < 0.01	-0.01 P = 0.89	0.77 P < 0.01	-0.08 P = 0.24	0.80 P < 0.01	-0.42 P < 0.01	0.06 P = 0.54	-0.22 P < 0.01
Iodine intake [mg/d]		0.79 P < 0.01	0.06 P = 0.41	-0.54 P < 0.01	0.71 P < 0.01	0.71 P < 0.01	-0.05 P = 0.48	0.79 P < 0.01	-0.04 P = 0.59	0.80 P < 0.01	-0.40 P < 0.01	0.04 P = 0.64	-0.23 P < 0.01
Milk yield [kg/d]			0.09 P = 0.18	-0.08 P = 0.23	-0.01 P = 0.84	0.15 P = 0.03	0.17 P = 0.01	0.02 P = 0.73	0.00 P = 0.96	0.05 P = 0.48	-0.05 P = 0.43	-0.32 P < 0.01	0.14 P = 0.04
Serum iodine [µg/L]				-0.33 P < 0.01	0.26 P < 0.01	0.27 P < 0.01	-0.39 P < 0.01	0.83 P < 0.01	0.22 P < 0.01	0.85 P < 0.01	-0.08 P = 0.23	-0.02 P = 0.81	-0.14 P = 0.04
Carry over factor serum					-0.52 P < 0.01	-0.50 P < 0.01	-0.15 P = 0.02	-0.36 P < 0.01	0.46 P < 0.01	-0.40 P < 0.01	0.69 P < 0.01	-0.05 P = 0.57	0.12 P = 0.07
Milk iodine [µg/kg]						0.97 P < 0.01	0.52 P < 0.01	0.33 P < 0.01	-0.32 P < 0.01	0.34 P < 0.01	-0.59 P < 0.01	0.06 P = 0.49	-0.12 P = 0.06
Milk iodine [mg/d]							0.54 P < 0.01	0.33 P < 0.01	-0.30 P < 0.01	0.35 P < 0.01	-0.56 P < 0.01	P < 0.01 P = 0.98	-0.10 P = 0.16
Carry over Milk [%]								-0.29 P < 0.01	-0.44 P < 0.01	-0.30 P < 0.01	-0.48 P < 0.01	-0.04 P = 0.67	0.13 P = 0.06
Urinary iodine [µg/kg]									0.40 P < 0.01	0.73 P < 0.01	-0.19 P < 0.01	0.02 P = 0.87	-0.16 P = 0.02
Carry over factor urine										0.07 P = 0.32	0.42 P < 0.01	0.05 P = 0.59	0.01 P = 0.87
Faecal iodine [µg/kg]											0.04 P = 0.54	-0.02 P = 0.79	-0.12 P = 0.07
Carry over factor faeces												-0.10 P = 0.29	0.08 P = 0.21
Triiodothyronine [nmol/L]													-0.12 P = 0.19
Thyroxine [nmol/L]													

The presently ascertained iodine concentrations of serum when feeding a GSL free diet (**Paper III**) agreed with the serum iodine contents of Schöne et al. (2009) using soybean meal and with that of Miller and Swanson (1973). On the contrary, Swanson et al. (1990) applying soybean meal as well and Rogers and Mee (1996) established higher contents in plasma. Although these studies indicate that differences may occur due to the utilization of either serum or plasma, a comparison of analysis of iodine in serum and plasma within our investigation showed no differences, so that serum and plasma iodine should be comparable. The impacts of the season, physiological stage or even different analyzing methods on blood iodine are insufficiently investigated so that the differences cannot completely be explained at the current state of knowledge.

The urinary iodine contents of the present trial (**Paper III**) were in the middle of the previously described contents. However, the results give rise to doubts regarding the reliability of urinary iodine as indicator for the supply of the animals. The urinary iodine contents detected in the magnitude of the requirement of dairy cows in the present trial were compared with a previous investigation (Herzig et al., 1999). Although in both trials glucosinolate free rations were applied, the iodine concentrations in spontaneous urine samples of the present study (188 and 202µg/L at 0.6-0.7mg I/kg DM diet, **Paper III**) were more than 1/3 lower than in the previous trial (321 and 346µg/L at 0.8mg I/kg DM diet). However, Erb et al. (1977) showed that a relation of urinary iodine to creatinine does not provide a better suitable parameter, since creatinine excretion via urine underlies considerable changes within the stage of lactation.

The only study investigating faecal iodine (Miller and Swanson, 1973) detected much lower iodine concentrations than those found in the present trial (**Paper III**). As only study which established the iodine contents of blood and all excretion matrices as well, they simultaneously showed similar serum and urinary iodine contents with lower milk iodine concentrations at KI application. The main differences between the present and the previous trial seems to be that the cows in the study of Miller and Swanson (1973) prior to the trial were fed diets with iodine amounts below the recommendation (2mg/d) for a long time, while the cows in the present study were sufficiently supplied (diets with approximately 1mg/kg DM, corresponding to about 20mg/d). The restock of the thyroidal iodine after a previous deficiency may have caused the comparably lower iodine excretion. It is suggested that this fact may rather affect faecal and milk iodine than urinary iodine, since the transfer of iodine into the gastrointestinal tract (Josefsson, 2009) and into the mammary gland (Laurberg et al., 2002) seems to be regulated actively by the NIS.

Regarding the distribution of iodine to the different routes of elimination, Schöne et al. (2009) described a decrease in the carry over of iodine into milk at rising iodine supplementations

(**Figure 6D**). In contrast, Swanson and Miller (1973) stated that higher iodine intakes do not influence the percentage of the daily applied iodine dose which is secreted into urine and the gastrointestinal tract. Contrary to Schöne et al. (2009), the carry over of iodine into milk (% of dose) in the present study (**Paper II and Figure 6D**) was not diminished with increasing iodine intake. Unfortunately the quantities of urine and faeces and therefore the total iodine amounts could not be determined in the present study. The mean carry over factors indicated that the distribution of iodine to urine and faeces is not considerably influenced by the iodine supplementation at sufficient supply of the animal (**Paper III**), since considerably higher carry over factors just were detected, when diets without iodine supplementation were fed. However, the correlations between the feed iodine concentration or the iodine intake and the carry over factors of urine and faeces (**Table 9**) indicate a slightly stronger increase in the urinary compared to the faecal iodine concentration.

The higher carry over factors in the non supplemented period seem to result from the still existing iodine excretion at low iodine intakes. Since this effect was not observed for milk (**Paper II**), the elimination via this route may be limited best by the organism compared to urine and faeces.

A slightly stronger increase of the milk, urinary and faecal iodine concentrations with rising iodine supplementation of feed compared to the serum iodine content is indicated by rising milk/serum, urine/serum and faeces/serum ratios (**Table 10**) while the milk/urine, milk/faeces and urine/faeces ratios showed no consistent tendency of increase or decrease.

These results suggest that at high iodine intakes the removal of iodine from the blood by iodine excretion proceeds more efficiently. However, the ratios just can indicate trends while the total excreted iodine amounts would be necessary to obtain more detailed information. Moreover, an effect of the consecutive application of the iodine supplementations and therefore an impact of the season or stage of lactation cannot completely be excluded.

Since T_4 is the thyroid hormone that responds first to changes in the iodine supply, the evaluation of thyroid activity in previous studies is mostly confined to this hormone. While for humans and in tendency also for pigs an excessive iodine intake is shown to be accompanied by lowered T_4 levels (Schöne, 1999; Zimmermann et al., 2005), in ruminants predominately no effects are described at iodine supplementations above the requirement (Convey et al., 1977; Hillman and Curtis, 1980; Grace and Waghorn, 2005). Only Hillman and Curtis (1980) suggested that an occurrence of both hypo- and hyperthyroidism may have inhibited the detection of an effect at a mean daily iodine intake of the cows of 164mg. Nowadays, iodine induced hyperthyroidism occurring after previous iodine deficiency (Stanbury et al., 1998) may play a minor role in cows due to the long lasting iodine supplementation of feed (Schöne and Rajendram, 2009). Thus, the sole appearance of

transient hypothyroidism may be the explanation for the lower T_4 values at the highest tested iodine supplementation (5mg/kg DM) in the present study (**Paper III**). A slightly negative, but significant correlation was observed between the T_4 level of blood and feed iodine or iodine intake, serum and urinary iodine content (**Table 9**). Since milk and urinary iodine contents are used as indicators for the iodine supply of the cows, they may also indicate implications on the thyroid. The results of the present trial suggest, that milk iodine contents of approximately 1400-1600µg/kg or urinary concentrations of 1000-1100µg/L may indicate an excessive supply of the animal when no goitrogenic feed is applied. The appropriate concentrations at RSM feeding amount to 600-700µg/kg in milk and 2300-2500µg/L in urine.

Table 10: Ratios for the relation of the iodine concentration of the different matrices in the experimental groups and supplementation levels

Group	Supplementation	Milk/serum	Urine/serum	Faeces/serum	Milk/urine	Milk/faeces	Urine/faeces
DDGS/ iodide	0	1.97	1.37	0.88	1.74	2.30	1.64
	0.5	3.21	3.92	1.79	1.10	1.80	2.25
	1	2.38	4.06	1.77	0.60	1.40	2.37
	2	5.08	5.53	2.74	1.09	1.94	2.08
	3	3.75	4.73	2.31	1.07	1.78	2.34
	4	6.06	5.69	3.08	1.11	1.96	1.88
	5	7.27	4.69	3.10	2.03	2.35	1.68
DDGS/ iodate	0	1.45	1.53	0.73	1.30	1.99	2.23
	0.5	3.69	2.95	1.71	1.36	2.34	1.84
	1	2.77	2.71	1.76	1.20	1.66	1.73
	2	5.65	3.68	2.25	1.62	2.67	1.73
	3	6.56	7.32	1.99	1.15	3.61	3.95
	4	7.93	9.29	2.74	0.96	3.13	3.64
	5	11.75	6.83	2.44	1.79	5.10	3.09
RSM/ iodide	0	0.42	2.12	1.10	0.20	0.40	2.00
	0.5	0.83	3.90	1.93	0.28	0.48	2.03
	1	0.55	4.25	2.14	0.14	0.27	2.12
	2	1.01	5.58	2.17	0.19	0.48	2.58
	3	0.84	5.48	2.12	0.16	0.43	2.74
	4	1.15	5.68	2.85	0.21	0.49	2.34
	5	2.30	7.34	2.19	0.32	1.18	3.67
RSM/ iodate	0	0.41	1.92	0.76	0.22	0.55	2.63
	0.5	0.80	3.33	1.52	0.25	0.57	2.32
	1	0.51	5.29	2.53	0.16	0.23	2.52
	2	1.11	4.20	1.59	0.26	0.73	2.75
	3	0.95	5.23	1.64	0.18	0.62	3.44
	4	1.28	7.66	2.46	0.21	0.55	3.35
	5	1.75	6.97	2.24	0.25	0.85	3.50

4.2 Effect of RSM

In lactation, the mammary gland increasingly produces the NIS which allows active accumulation of iodine from the blood (Cavalieri, 1997; Laurberg et al., 2002). Thus, the mammary gland similarly to the thyroid responds to a glucosinolate exposure with a reduced

iodine uptake. It was shown several times that milk iodine is diminished by RSM feeding (Iwarsson, 1973; Papas et al., 1979; Schöne et al., 2006). However, it is still not completely clarified if the RSM impact on milk iodine depends on the amount of GSL in feed. Laurberg (2002) stated that different experiments in Denmark investigated a negative correlation of milk iodine with GSL intake. However, Papas et al. (1979) tested two kinds of RSM with varying GSL content and detected similar reductions of the milk iodine concentration. Although 00-rapeseed was bred, the commonly used RSM still is not free of GSL. Schöne et al. (2006) showed that high amounts of 00-RSM in the ration still lead to strongly diminished iodine contents of milk. Papas et al. (1979) and Schöne et al. (2006) ascertained reductions of the milk iodine concentration of 78 and 54% compared to a glucosinolate free ration at GSL intakes of approximately 78.7 and 9.2mmol/d. These results fortify the assumption, that higher GSL intakes are connected with a stronger diminishing impact on milk iodine. However, in the present trial quite constant GSL intakes (between 11.0 and 13.7mmol/d) caused reductions of the milk iodine concentration by 51 up to 78% compared to the appropriate groups fed DDGS (**Paper II**). Thus, approximately two thirds of the iodine was inhibited to transfer into milk whereby the percentage of reduction showed no constant rise or decrease with the feed iodine (**Figure 8**).

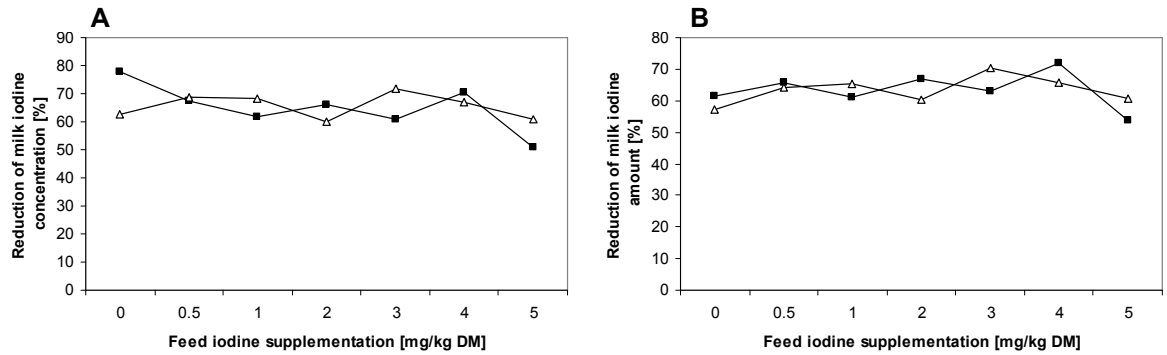

Figure 8: Percentage of reduction of the milk iodine concentration (A) and total milk iodine amount (B) at different iodine supplementations when applying KI (■) or $Ca(IO_3)_2$ (△)

Still diminished totally excreted iodine amounts at high iodine supplementations show that the presence of iodine cannot completely abolish the inhibition of the iodine uptake. This observation is contradictory to a competitive inhibition and may similarly occur in the thyroid. The constant percentages of iodine which transfer into milk even at low iodine supply indicate that next to the uptake by the NIS a part of the iodine additionally gets into the mammary gland by another probably not active transport mechanism.

Due to the relatively constant percentages, rising iodine intake increased the total amount of iodine which is inhibited to transfer into milk. As a result, the reduction of the milk iodine concentration and amount per mmol ingested GSL showed a linear increase (**Figure 9**).

However, the impact of higher or lower GSL intakes on milk iodine can not be derived from these results.

Since the iodine uptake into the thyroid is diminished by RSM feeding as well, the GfE (2001) suggests that the iodine requirement of the animal is doubled if the ration contains RSM. The present study affirms this estimation, since the regressions (**Paper II, Figure 2**) showed that the milk iodine concentrations, reached with DDGS at the requirement (0.5mg/kg DM) would have been achieved with RSM feeding at a feed iodine concentration of 1.1mg/kg DM.

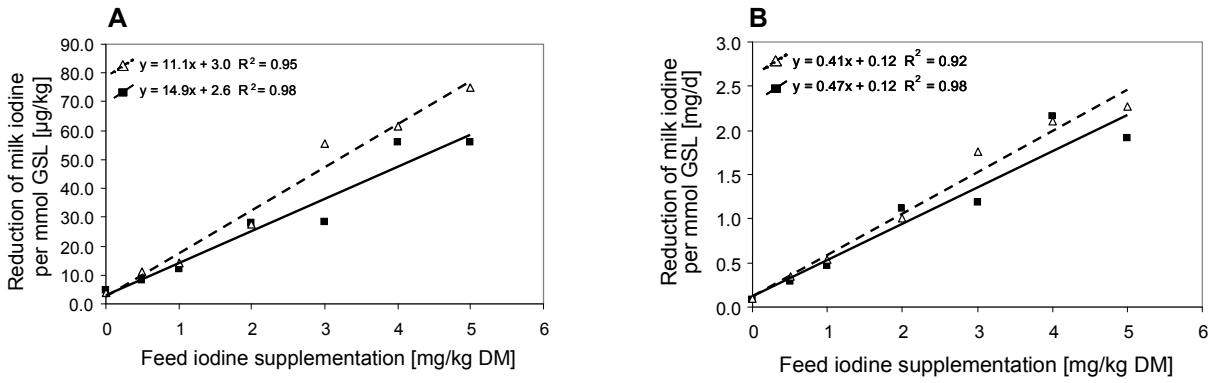

Figure 9: Quantitative reduction of the milk iodine concentration (A) and the total milk iodine amount (B) by the intake of 1mmol GSL at the different iodine supplementations when applying KI (■) or $Ca(IO_3)_2$ (△)

While the reducing impact of RSM on milk of dairy cows due to its importance for human nutrition at least partly is elucidated, the implications on blood, urinary and faecal iodine are poorly investigated. Especially an all embracing study on the iodine status and all main routes of iodine elimination in dairy cows is missing. Comprehensive studies with goats or sows (Lengemann, 1970; Schöne et al., 2001) showed reduced milk iodine concentrations accompanied by higher blood, urinary and faecal iodine contents when applying perchlorate or rapeseed press cake.

Studies concerning the impact of NIS inhibiting anions (SCN, perchlorate) on blood, urinary and faecal iodine with cattle show controversial results (**Table 8**). The application of 00-RSM at varying iodine supplementation levels (**Papers II and III and Table 8**) shows that, like in sows and goats, the diminished milk iodine contents due to RSM feeding are accompanied by higher serum, urinary and faecal iodine concentrations in lactating dairy cows. Next to the increase of the iodine concentration with rising iodine supplementation in all fractions, **Figure 10** visualizes the shift of iodine in milk when feeding DDGS to serum, urine and faeces when applying RSM in the ration. This development is seen by lower milk/serum, milk/urine and milk/faeces ratios in the RSM groups as well (**Table 10**). Unchanged iodine concentrations in previous studies (**Table 8**) may appear due to low iodine supplementations, since at low supplementation levels no significant differences were detected in the present

trial as well (**Papers II and III**). The contrary results concerning the impact on faecal iodine compared to the former study of Miller et al. (1975a) indicate that differences in the impact of RSM may occur in lactating and non lactating cattle.

The detected differences between DDGS and RSM application (**Papers II and III**) demonstrate that feed which contains goitrogens considerably alters the distribution of iodine to the different compartments of cattle so that compartment models like described by Miller et al. (1975b) cannot generally be applied.

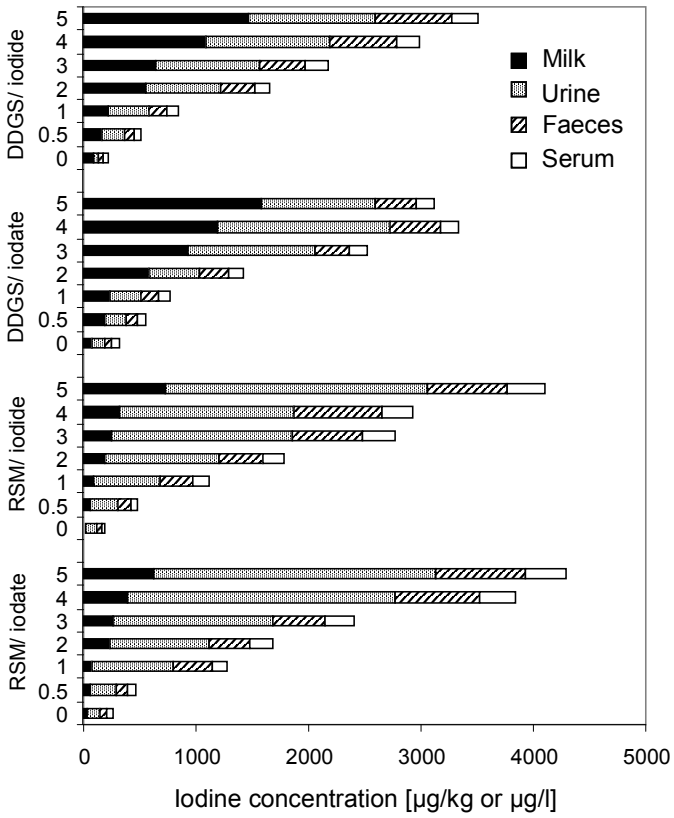

Figure 10: Iodine concentrations in milk, urine, faeces and serum at the different iodine supplementation levels in the experimental groups
DDGS = distillers dried grains with solubles; RSM = rapeseed meal

At similar serum iodine concentrations, RSM application caused considerably lower milk iodine contents (**Paper III, Figure 2A**) and slightly reduced urinary and faecal iodine concentrations (**Paper III, Figures 2B and 2C**) compared to DDGS feeding. Since RSM feeding, to a smaller extent also altered the relation between serum and urinary iodine as well as between serum and faecal iodine, it is indicated that the NIS may be of importance in urinary and faecal iodine excretion as well. The diminished faecal iodine contents at similar serum iodine concentrations when feeding RSM are in concordance with the previously observed decrease in endogenous secretion of iodine into the abomasum and other parts of the gastrointestinal tract at SCN application, described by Moss et al. (1968) and Miller et al.

(1975a). As a result the higher faecal iodine concentrations in the RSM groups at the various supplementation levels of the present study (**Paper III**) just seem to result from the increase in serum iodine due to the diminished iodine transfer into milk in lactating dairy cows. However, from the present study it cannot be concluded if additionally the iodine absorption was inhibited by the NIS inhibiting SCN like described by Nicola et al. (2009) for rats and mice.

The iodine concentrations (regarding all experimental groups) of all iodine excretion matrices as well as serum iodine showed a significant positive correlation with each other (**Table 9**). Scherer-Herr (2001) described a highly significant correlation between milk and urinary iodine (r = 0.90). In contrast, the correlation coefficient in the present investigation was much lower (**Table 9**), but **Figure 11A** visualizes that the relation would be closer if the same feed (with or without GSL) was applied to all animals. The strong variation in the values due to RSM and DDGS application also was detected for the relation between milk and faecal iodine (**Figure 11B**) as well as between milk and serum iodine (**Paper III, Figure 2A**). In contrast, the iodine concentrations of urine and serum, faeces and serum as well as between urine and faeces (**Paper III, Figure 2B-D**) showed a much closer relation, since all these parameters were elevated by RSM feeding. As a result the correlation coefficients of the latter relations were much higher (**Table 9**).

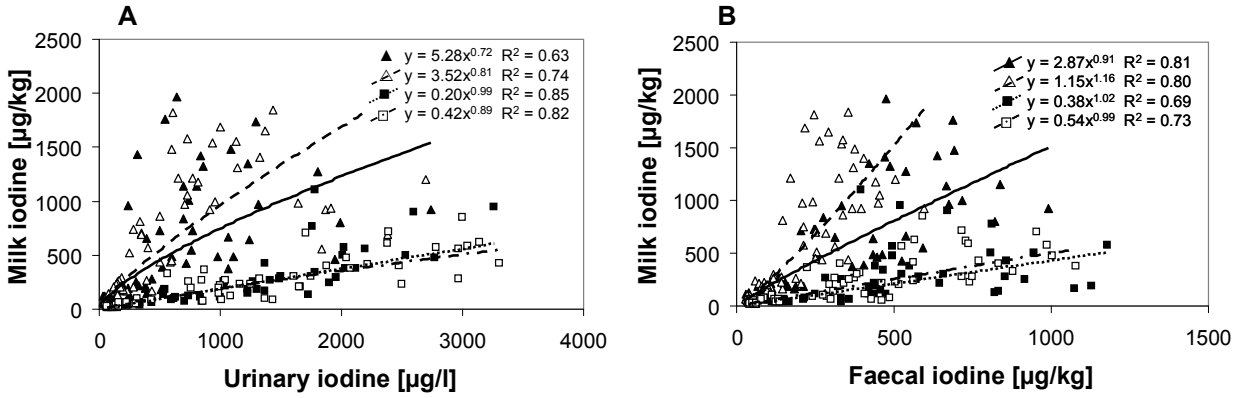

Figure 11: Relation between milk and urinary iodine concentration (A) as well as between the milk and faecal iodine concentration (B) in the experimental groups (▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate)

The T_4 content of blood was shown to be reduced when RSM with a high GSL content is applied (Papas et al., 1979; Vincent et al., 1988; Tripathi et al., 2001) while no impact was seen with low GSL-RSM (Papas et al., 1979; Zech et al., 1993; Ehlers et al., 1994). The T_3 level of blood seems not to be influenced (Papas et al., 1979; Grace and Waghorn, 2005).

4.3 Effect of the iodine species

The International Association of the European Manufacturers of Major, Trace and Specific Feed Mineral Materials (EMFEMA, 2009) compiled studies investigating the bioavailability of different iodine species in farm animals. For ruminants, it was shown that only diiodosalicylic acid (which is not permitted for feed supplementation in Europe) features a low bioavailability and therefore is not recommended for feed supplementation. The compilation as well pointed out that only few and old studies exist, which compare iodide and iodate application.

Table 8 summarizes the results found by different authors. At daily iodine supplementation and some days after single iodine dosing no differences in blood, milk, urinary and faecal iodine concentration between iodide (sodium iodide) and iodate (calcium iodate or sodium iodate) application were described by Miller et al. (1968), Lengemann (1969) and Bretthauer et al. (1972). However, initially lower iodine concentrations were shown in blood, milk, urine and faeces when applying iodate (calcium iodate or sodium iodate) compared to sodium iodide (Lengemann, 1969; Moss and Miller, 1970) which indicates that differences in iodine metabolism occur. It is suggested that the conversion of iodate to iodide before absorption causes a delayed iodine absorption in case of iodate and possibly a shift of absorption to later segments of the gastrointestinal tract (Moss and Miller, 1970; Miller et al., 1975b). In contrast, Leskova (1969) investigated lower serum and milk iodine contents at oral application of potassium iodide compared to potassium iodate.

The results of the present study point out, that no considerable differences occur in milk iodine due to iodide or iodate application when storage conditions prevent great iodine losses from feed (**Paper II**). Slightly higher milk iodine concentrations were reached but could not be ascribed to higher transferred iodine amounts into milk.

Regarding the blood, urinary and faecal iodine contents, differences between iodide and iodate application were only observed for serum and faecal iodine at high iodine supplementations (3-5 or 2-5mg/kg DM) and when feeding diets without goitrogens (**Paper III**). In that case, potassium iodide application led to higher iodine contents in serum and faeces compared to calcium iodate. As a result, the relation between the iodine content of milk and serum, urine and serum as well as milk and faeces differed between iodide and iodate in the DDGS groups (**Paper III, Figures 2A and B and Figure 11B**). Comparable differences were found for the relation between milk and urine (**Figure 11A**). Although the excreted iodine amounts could not be ascertained, the results indicate that the described differences in iodide and iodate absorption (Moss and Miller, 1970; Miller et al., 1975b) may also affect iodine distribution in the body and the proportions of the iodine excreted by the different main routes of iodine excretion of the cow. The reasons for the observed effects cannot be explained from the results of the present study or the current state of knowledge. Further investigation is necessary on the impact of the NIS on the various routes of iodine

transport in the body. Furthermore, it would be of particular importance to investigate if the iodine uptake into NIS containing tissues (like the gastric mucosa or salivary gland) similar to the thyroid is regulated by high iodine dosages, since the alterations just occurred at high iodine supplementations.

The apparently only study which investigated the impact of different iodine species (potassium iodide and EDDI) on the thyroid hormone level of blood ascertained no differences in the T_4 content (Swanson et al., 1990). The present experiment affirmed these results for iodide and iodate application (**Paper III**).

5 Risk assessment of milk iodine for human health

As the implications caused by iodine deficiency but also by iodine excess represent an important public health problem worldwide, they have been extensively investigated. While especially regions which feature a high distance from the ocean are affected by iodine deficiency, iodine excess is predominately observed in regions with high consumption of iodine rich seaweed (0,5-1100mg/100g T) like China or Japan. Although in Europe endemic goiter (caused by iodine deficiency) has almost disappeared, it is stated that still more than 60% of the population of Western and Central Europe live in countries which feature iodine deficiency (Vitty et al., 2009).

Iodine deficiency leads to insufficient thyroid hormone production (hypothyroidism). Chronic hypothyroidism results in an enlargement of the thyroid (goiter) and alterations of the thyroid tissue, possibly causing adenomas. Various iodine deficiency disorders (IDD) are decribed (Delange and Hetzel, 2003) including diminished growth, mental retardation, skeletal deformations and increased incidence of abortions and stillbirths. Furthermore, it is recently suggested that a deficient iodine supply is connected with breast cancer and thyroid cancer (Venturi, 2001; Smyth, 2003; Arroyo-Helguera et al., 2006; Dal Maso et al., 2009). For assessing the iodine status of a population, detecting urinary iodine concentration is the method of choice, since it quite well reflects the iodine intake. For eliminating IDDs the WHO (2001) states that in the target population a median iodine content of 100µg/L has to be achieved. The recommended range for urinary iodine concentration, indicating a sufficient iodine supply is 100-200µg/L. The resulting recommended daily iodine intake is set between 150-200µg/d (D-A-CH, 2000; WHO et al., 2001; DRI, 2001).

The main problem of ensuring a sufficient iodine supply of humans is the small range between deficiency and excess. Regarding the highest stated requirement and the lowest UL (D-A-CH, 2000), the UL is just 2.5 times higher than the recommendation. It is stated that normal adults can tolerate iodine intakes up to 1000µg/d without side effects (WHO, 1994b). However, this Upper Level seems to be much lower in individuals with previous iodine

deficiency. Even if moderate iodine supplementations are applied (150µg/d), an increased incidence of iodine induced hyperthyroidism seems not to be preventable in supplementation programs (Baltisberger et al., 1995). Iodine supplementation programs may lead to chronic high iodine intakes when salt iodization is too high or badly controlled. In Brazil, Algeria, Côte d'Ivoire, Zimbabwe and Uganda median urinary iodine excretions of > 300µg/L, in Chile and Congo of even > 500µg/L were detected (Zimmermann et al., 2005). In iodine-replete areas it was shown, that already small daily administered quantities of iodine may result in alterations of the T_3, T_4 or TSH level of blood (Roti and Vagenakis, 2000). The lowest amount which did not influence thyroid function was 500µg/d (Paul et al., 1988). With regard to the previous deficient supply in Europe, the Tolerable Upper Intake Levels (UL) for adults is set at 500µg/d (D-A-CH, 2000; SCF, 2002) while the WHO (1994b) considers 1000µg/d as safe.

Implications due to iodine excess predominately seem to occur at iodine supplementations after a previous deficiency. An increased frequency of iodine induced hyperthyroidism due to autonomous thyroid nodules as well as higher incidences of Hashimoto thyroiditis and thyroid cancer are decribed (McConahey et al., 1962; Stanbury et al., 1998; Slowinska-Klencka et al., 2002; Azizi et al., 2005). Kahaly (1998) described the developement of thyroid autoantibodies at an iodine supplementation of 500µg/d for 6 months. The occurence of hyperthyroidism seems to be transient disappearing within few years in a population, but it can feature an important health problem including a high risk of tachyarrythmia (Todd et al., 1995).

On the other hand chronic high iodine intakes are seen in regions with common seaweed consumption (Japan, China), with high iodine contents of water (China) or with iodine rich milk when the cows are fed fish products (Iceland). In Japanese students an average iodine intake of 1106µg/d with a maximum of 3840µg/d was observed while in fishermen even intakes up to 10000µg/d were detected (Suzuki et al., 1965; Suzuki and Tamura, 1985; Katamine et al., 1986). In contrast to countries with previous deficiency, iodine intakes up to 1000µg/d do not cause health implications in Japan (BfR, 2001). Compared to the mentioned hyperthyroidism, thyroiditis and thyroid cancer occurring after iodine supplementation in iodine deficient regions, in these regions the development of iodine induced hypothyroidism and goiter was seen, but at far higher average iodine intakes of 8000µg/d (Suzuki et al., 1965; Nagataki, 1974; Konno et al., 1994).

Except for the high iodine intakes by milk in Iceland due to feeding fish products (Sigurdsson and Franzson, 1988), the existing data point out that implications due to excessive iodine intakes by food of animal origin are not very likely.

Due to its contribution to human iodine supply of approximately 40% in Germany, milk was shown to provide a valuable iodine source for humans (Jahreis et al., 2007). For Denmark a

percentage of 44% is stated (Rasmussen et al., 2002). The relative high maximum level of iodine in feed of dairy cows and the high carry over of iodine from feed into milk allows a strong iodine enrichment in milk (**Paper II**, Schöne et al. 2009). It was shown, that a much higher carry over of ingested iodine occurs into milk (30-56% without goitrogens in feed, **Paper II**) compared to eggs (12.5-18.6%, Richter, 1995; 8.9-13.5%, Röttger et al., unpublished data) and meat (0.10-0.24% in pigs, Franke et al. 2008), so that the enrichment of this food of animal origin is much more efficient. Thus, iodine enrichment in milk by feed iodine supplementations, in addition to salt iodization can considerably contribute to improving human iodine supply.

However, different eating habits result in a high variation in the consumed amount of milk. Consequently, for example vegans do not benefit from this measure while children with mostly higher milk consumption (Als et al., 2003) at simultaneously lower requirements (D-A-CH, 2000) and ULs (SCF, 2002) are affected above average.

The latter shows that increasing the iodine content of milk by feed supplementation includes a risk of overdosing in parts of the population. In recent years considerable rises in the iodine content of consumers' milk were seen (Jahreis et al., 2007). Even if the German nutrition table for unskimmed milk still states an iodine concentration of 27µg/L, newer analysis of consumers' milk in Germany detected much higher mean contents in the magnitude of 100-200µg/kg (see **Table 2 of BACKGROUND**). However, the actually supplemented iodine amounts of the stated investigations are unknown, so that a dose response relationship could not be ascertained.

In the course of evaluating the risks of iodine supplementations within the maximum level on human health, a first study was carried out in 2004 with low-yielding cows (Schöne et al., 2009). In this trial mean milk concentrations up to 2762µg/kg were detected leading to a lowering of the maximum level in the EU from 10 to 5mg/kg (EU, 2005). In the magnitude of the new maximum level the study still ascertained a high mean milk iodine content of 1215µg/kg. Due to the low milk yields of the cows (on average 19.8kg/d) it was questionable, if such high concentrations would also occur in higher yielding cows commonly present in milk production.

As mentioned above, the present trial (**Paper II**) with high yielding cows (on average 34.2kg/d) confirmed the high milk iodine concentrations found by Schöne et al. (2009) at the presently permitted maximum iodine supplementation of feed.

In the appropriate period (iodine supplementation of 5mg/kg DM), next to the 6 sampling days (Day 1, 4, 11, 19, 20 and 21) additional milk samples were taken on Days 7 and 15 whereby a plateau of the milk iodine concentration was already reached at the second sampling day (Day 4). For all milk samples after the achievement of the plateau (Day 4-21) the iodine amount in 250mL sample was calculated which approximately corresponds to one

glass of milk. While a glass of milk from cows fed RSM in 80.4% of the samples would cause intakes below 200µg, in case of DDGS 99% of the samples would result in iodine intakes above this highest stated requirement (D-A-CH, 2000; **Figure 12**). Due to the low margin between requirement and UL in human nutrition, in case of DDGS two glasses of milk would already exceed the UL in 91% of the samples.

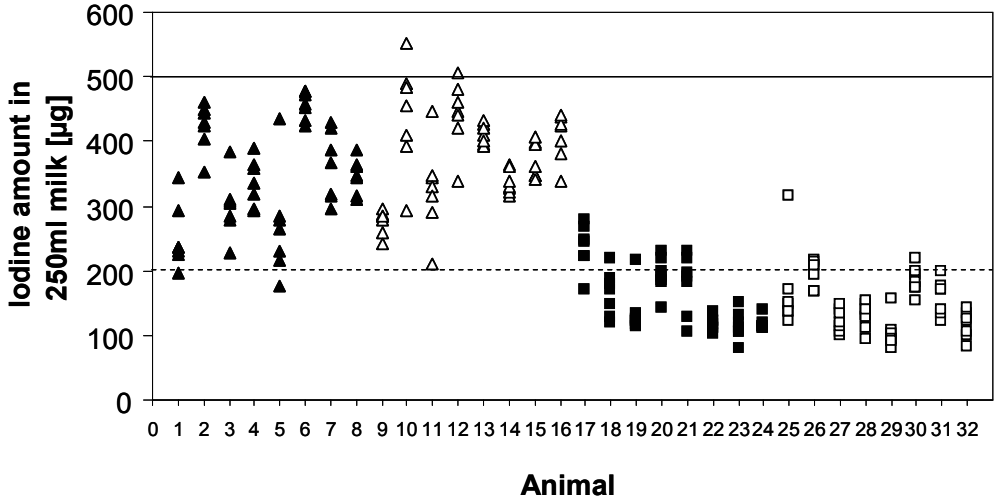

Figure 12: Distribution of the calculated iodine intakes by 250mL milk (7 sampling days of 32 cows) at a feed iodine supplementation of 5mg/kg DM in the experimental groups (▲ DDGS/iodide, △ DDGS/iodate, ■ RSM/iodide, □ RSM/iodate)
--- highest stated requirement by D-A-CH (2000)
— Tolerable Upper Intake Level by D-A-CH (2000)

At the detected average milk iodine concentrations at DDGS feeding (1521µg/kg, mean of iodide and iodate group) and the consumers' per capita consumption of milk in Germany of 62.6kg/year or 172g/d in 2006 (ZMP, 2008), milk produced with the highest presently permitted feed iodine content would cause an iodine intake of approximately 261µg/d. This amount corresponds to 130-174% of the recommendation (150-200µg/d; D-A-CH 2000, WHO 2001) and 26-52% of the UL (500-1000µg/d; WHO 1994b, D-A-CH 2000) for adults. In addition, approximately the same amount of milk products is consumed whereby their iodine content seems to be in the same magnitude like that of milk. Schöne et al. (2003) showed that in cheese production the iodine equilibrates between curd and whey leading to comparable iodine concentrations in cheese like in milk at a simultaneously higher dry matter content. The iodine content of cheese may additionally be increased by using iodized salt in manufacture. Souci et al. (2008) declare iodine concentrations of 30-39µg/kg for camembert cheese (depending on fat content), 40-53µg/kg for Edam, 36µg/kg for Gouda and 51µg/kg for processed cheese. For other milk products like cream (24-29µg/kg) or yoghurt (35-38µg/kg), the nutrition table (Souci et al., 2008) as well states similar iodine contents like in milk. When assuming a milk consumption of 0.5L for 4-6 year old children with a requirement of 120µg/d

(D-A-CH, 2000) and an UL of 250µg/d (SCF, 2002), the calculated iodine intakes at the highest permitted feed iodine supplementation (732 for iodide and 789µg/d for iodate) would correspond to approximately three times the UL.

In addition, it has to be considered that the utilization of iodine containing teat dips may lead to an elevation of the iodine content by approximately 11-150µg/kg (Flachowsky et al., 2007). Thus, the present investigation points out that especially in individuals with high consumption of milk and milk products an exhausting of the present maximum level in feed rapidly may lead to an exceeding of the UL. But even in humans with average milk consumption, the intake of such milk in connection with other native or enriched iodine sources (e.g., eggs, milk products, bread or meat produced with iodized salt, sea fish, oceanic algae and iodized salt) or in combination with iodine supplements could lead to excessive iodine intakes.

Nevertheless, at present the risk of an excessive iodine intake due to milk consumption is quite low, since much lower iodine supplementations than the maximum level are applied in feed manufacture. An investigation of mixed feed in practice in Germany revealed an average amount of 0.79mg/kg DM (Grünewald et al., 2006). At this feed iodine level the milk iodine concentration would amount to 184µg/kg for DDGS feeding and 52µg/kg for RSM application (calculated from the regression equation in **Paper II, Figure 2**). The calculated milk iodine concentrations (especially in the DDGS groups) agree well with the actually found iodine contents in common consumers' milk (see **Table 2 of BACKGROUND**). These concentrations lead to iodine intakes of 32 and 9µg/d, respectively at the present per capita consumption of milk. The corresponding percentage of the iodine intake by milk of the recommended daily intake of adults therefore accounts for 16-22% when applying DDGS and at 5-6% when feeding RSM (**Paper II**). For 4-6 year old children the estimated iodine intake by 0.5L milk (92µg/d for DDGS and 26µg/d for DDGS) still lies below the requirement as well. Thus, the presently applied iodine supplementations seem quite adequate to avoid an exceeding of the UL in humans in most cases. However, the analysis of European milk (Rysava et al., 2007) shows that in some cases much higher milk iodine contents may occur in consumers' milk although milk from different farms is mixed.

The comparison of the iodine intake which would be expected at the highest tested iodine supplementation (261µg/d) with the intake at the presently applied iodine supplementations (32µg/d) shows that the human iodine intake could be increased by approximately 230µg/d through milk at the presently permitted maximum level of iodine in feed. Studies investigating the present iodine intake in Germany estimated intakes of 108 and 136µg in 14-18 year old girls and boys (Remer, 2009) and 111 and 126µg/d in adult women and men (Manz et al., 1998). Thus, the iodine intake would approximately triple. Considering milk, eggs, meat and iodized salt as iodine sources in human nutrition, a worst case iodine intake was assessed

comparable to that of the EFSA (2005). When assuming a daily consumption of 1.5L milk, 100g egg and 9g salt the iodine intake by milk (1521µg I/kg at 5mg/kg DM feed) would amount to 2282µg/d, the intake by eggs to 179µg I/d (1793µg I/kg at 5mg/kg feed; Röttger et al., 2008) and to 180µg I/d by salt (20mg I/kg) while it is suggested that at the most 100µg I/d are ingested by meat (EFSA, 2005). As a result 2741µg iodine would be ingested per day by these comestibles, which is still far lower than the stated 8000µg/d at which adverse effects were seen in populations with chronic high iodine intake (Suzuki et al., 1965; Konno et al., 1994; Nagataki and Yokohama, 1996). However, it is more than five times above the UL of 500µg/d in Europe.

The results show that milk provides an important iodine source in human nutrition at the presently applied iodine supplementations. However, it is not recommended to further enrich milk for improving human iodine supply, since the amount ingested by humans varies widely. It was shown that various factors like rape in feed, different feeding practices, utilization of iodine containing teat dips and variable milk consumption allow no standardized milk iodine concentration and therefore reliable assessment of the iodine intake by milk. As a result the iodine content of milk cannot be declared. With regard to the small span between iodine requirement and UL it seems not possible to state a certain feed iodine supplementation for achieving an optimal supply of the population. It is recommended that iodine is just added to dairy cow's feed according to the demand of the animal avoiding excessive supplementations.

The expectable iodine concentration of milk just can be narrowed down by setting a maximum level which is as low as possible. The aim should be that the iodine content of milk becomes better assessable for the consumer which includes that the values given by the nutrition tables have to be kept up to date.

6 Consequences for the maximum level of iodine in feed of dairy cows

For consumer protection the German Veterinary Medical Society (DVG e.V.) suggests that the iodine content of consumers' milk should not exceed 500µg/kg (Hamann and Heeschen, 1982). Since to the authors' knowledge no newer recommendation for the iodine content of milk is established, the results of the present study (**Paper II**) were evaluated referring to the mentioned concentration. **Table 11** shows that at the present maximum level in case of DDGS no single sample and in case of RSM feeding only 32% of the samples were below this concentration. Regarding this desirable milk iodine concentration, the regression analyzes (**Figure 6B**) suggest, that the daily iodine intake has to be below 40µg (39.5µg in case of iodide and 35.8µg in case of iodate) in cows with milk yields in the magnitude of 34kg/d. From the regression equations for the relation between the milk iodine concentration and the feed iodine concentration (**Figure 6A**) it is indicated that the corresponding

maximum level in feed would have to be set below 2mg/kg DM (1.89 at iodide and 1.67mg/kg DM at iodate application).

On the other hand the maximum level still has to be practicable. Since the present results (**Paper II**) turn out that at RSM application the iodine supplementation at least should amount to 1mg/kg DM, the range between the required supplementation and the maximum level would be too low. Different feeding practices for dairy cows could easily result in an exceeding of the maximum level. Hence, from the present results just a lowering of the maximum level to 3mg/kg could be recommended. At this supplementation level still just 8% of the detected milk iodine concentrations are below 500µg/kg in case of DDGS and 98% in case of RSM (**Table 11**). However, regarding the mean milk iodine concentrations (638µg/kg for iodide and 930µg/kg for iodate, **Paper II, Table 3**) and the per capita consumption of milk in Germany, the proportion of the recommended daily iodine intake (55-106%) and the UL (11-32%) would be considerably lower at a feed supplementation of 3mg I/kg DM compared to 5mg/kg DM. Furthermore, it is to be assumed that the new maximum level will not be exhausted as well.

Table 11: Distribution of the single detected milk iodine concentrations at feed supplementations of 5 and 3mg/kg DM to contents lower and higher than the recommended maximum level of 500µg/kg milk (Hamann and Heeschen, 1982)

Milk iodine concentration	DDGS		RSM	
[µg/kg]	n	[%]	n	[%]
5mg/kg DM				
< 500	0	0	36	32
> 500	112	100	76	68
3mg/kg DM				
< 500	4	8	47	98
> 500	44	92	1	2

7 Applicability of the results to humans

The applicability of the results regarding the impact of the iodine supplementations, RSM and the iodine species on iodine metabolism to humans due to the fundamental differences in the gastrointestinal tract is limited. Furthermore, an iodine transfer into milk only plays a role in lactating women. However, the iodine concentrations of serum and urine seem to be in the same magnitude in humans and cows (despite the great differences in body weigth). In general, excessive iodine intakes due to the missing elimination of iodine via milk may cause higher increases in blood iodine in humans which possibly lead to influences on the thyroid function at comparably lower iodine intakes. For lactating women, Laurberg et al. (2002) showed that the dependence of the milk iodine on the iodine intake in humans is similar to that in dairy cows leading to high milk iodine contents at high iodine supply. Similar relations between milk and urinary iodine concentration were detected in humans (Laurberg et al.,

2002; median: 0.96, 25-75 centile: 0.65-1.96) and dairy cows (**Table 10**, median: 1.01, 25-75 centile: 0.67-1.61) when no goitrogenic substances were applied.

Furthermore, high intakes of SCN or substances generating SCN seem to cause considerably diminished iodine contents of milk as well, whereby in human nutrition other Brassicacea (e.g. cassava, Brussels sprout, cauliflower or mustard) are mainly responsible for the inhibiting effect on iodine metabolism (Laurberg et al., 2002).

Thus, on the one hand high iodine intakes of the mother may also cause implementations in breastfed infants, since they require much less iodine than adults (D-A-CH, 2000). On the other hand high intakes of goitrogens at low iodine supply of lactating women may cause a deficient supply leading to myxedematous cretinism of the child (Laurberg et al., 2002).

For humans a much higher proportion of urinary iodine excretion (more than 90 or 95%) and lower faecal iodine excretion (about 1%) is stated (Cavalieri, 1997; Saller et al., 1998) than in ruminants (40% urine, 30% faeces; Simon, 2008). However, at low iodine intakes it was shown, that up to 37% of the iodine was excreted via faeces and just 63% via urine (Pilz and Anke, 2002). Jahreis (1997) described a lower bioavailability of iodine with increasing parts of organic iodine. Despite the different constitution of the gastrointestinal tract the iodine absorption, secretion and reabsorption seems to show similarities in ruminants and humans. In both species a secretion of hormone degradation products via the bile is decribed (Miller et al., 1975b; Jahreis, 1997). Furthermore, in ruminants for a long time it is known that considerable amounts of iodine reenter the gastrointestinal tract by secretion into the abomasum and that this transfer is diminished by SCN while iodine absorption is minimal in this part of the gastrointestinal tract (Barua et al., 1964; Miller et al., 1975a). Recent studies indicate that similar actions take place in monogastric animals, probably including man (Josefsson, 2009). In rats a considerable secretion of iodine from the bloodstream into the stomach was observed, which was diminished by NIS inhibiting anions while iodine seems not to be absorbed from the gastric content. However, in humans the main absorption of iodine seems to occur in a later part of the gastrointestinal tract compared to the place of endogenous secretion while in ruminants 80-90% of the iodine is described to be absorbed prior to the abomasum. Until now it cannot be assessed to which extent the recently indicated involvement of NIS in iodine absorption in the intestine (Kotani et al., 1998; Nicola et al., 2009) plays a role in humans and ruminants. Thus, the applicability of the results concerning faecal iodine as well as blood and urinary iodine to humans is not completely assessable at the current state of knowledge.

8 Consequences for assessing the iodine supply of dairy cows

While severe iodine deficiency is seen from the occurrence of goiter, moderate or mild deficiency is more difficult to diagnose. In dairy cow herds either urinary, milk or blood iodine is used for assessing the iodine supply (Alderman and Stranks, 1967; Binnerts, 1989; Kroupova et al., 1996; Herzig et al., 1996; Kursa et al., 2004; Launer and Richter, 2005). Like in humans, urinary iodine seems to allow the detection of a sufficient supply in dairy cow herds, but due to the complicated sampling its use as an indicator for the iodine supply is not easily practicable. Urinary iodine plays an important role in non lactating pregnant cows, since particularly the calves are affected by a deficient iodine supply of the cow. While taking blood samples due to time, effort and costs as well seems inappropriate for assessing the iodine status of large herds, milk samples can quite easily be obtained. However, milk iodine features the problem that it is elevated by teat dips so that a deficient supply can just be recognized if no iodine containing teat dips are used.

All these parameters were considerably altered by RSM feeding (**Papers II and III**) and therefore show a high variation in the iodine concentration due to the applied feedstuffs (with or without goitrogens). While in urine and blood, RSM caused increases of the iodine concentration, milk iodine was reduced. It was indicated that in high yielding German Holstein cows on average differences up to ≈1000µg/kg in milk, up to ≈1500µg/L in urine and up to ≈200µg/L in serum may occur within the maximum level for iodine in feed between feed with and without goitrogens (**Papers II and III**). However, at low iodine supply (feed iodine content of 0.5mg/kg and below) in urine and blood only slight or even no changes were observed whereas milk iodine was considerably lower independently from the iodine content of feed.

The present study points out that urinary and blood iodine are increased despite the less amount of available iodine for the thyroid while the mammary gland responds to a GSL exposure in the same way like the thyroid. In this way blood and urinary iodine may pretend a better iodine supply of the animal than existent. Although no significant differences between RSM and DDGS were seen in the magnitude of the iodine requirement and below, urinary and blood iodine do not seem to allow a secure exclusion of a deficient supply when the ration contains rapeseed. Therefore the milk iodine concentration seems to be a better indicator of the iodine supply of dairy cows when RSM is applied and the cows are not treated with iodine containing teat dips.

CONCLUSIONS

The comparison of the performance achieved by the application of DDGS from 90% wheat and 10% barley with that at RSM application points out, that the tested kind of DDGS can replace RSM in rations of dairy cows up to the tested proportion of 16.5% of the diet DM.

It was shown that rising iodine supplementations of feed lead to strong increases of the iodine concentration of serum and of all main routes of iodine elimination (milk, urine and faeces).

RSM application was seen to cause a shift of iodine excretion via milk to increased excretion via both urine and faeces at simultaneous elevation of the serum iodine concentration. It was shown that even 00-RSM strongly diminishes the milk iodine concentration to approximately one third of the concentration of a glucosinolate free ration. Thereby the percentage of reduction seems independent from the applied iodine supplementation. The strongly decreased iodine transfer into milk by RSM application seems to be predominately responsible for the higher faecal iodine which may cause differences in the response of faecal iodine to RSM exposure in lactating and non lactating cattle.

In contrast to the increasing impact of a RSM application on urinary and serum iodine, the mammary gland like the thyroid responds to the glucosinolate exposure with a diminished iodine uptake leading to lower milk iodine concentrations. Therefore at RSM application milk iodine seems to be a better indicator of the iodine supply of the animal than urinary or blood iodine, but only if no iodine containing teat dips are applied.

At the tested storage conditions no considerable iodine losses from feed occur at iodide application which could cause differences in milk iodine compared to iodate application. Differences in metabolism of iodide and iodate are probable, but alterations in milk iodine due to iodide and iodate application seem to be negligible.

The comparison of the present results with a previous trial indicates that higher milk yields do not result in lower milk iodine concentrations at comparable feed iodine concentrations.

It was shown that one third up to one half of the ingested iodine of the cow is transferred into milk (carry over between 30-56%) if the diet contains no GSL, which allows a more efficient enrichment of iodine in milk than in eggs and meat. It was indicated that higher milk yields are connected with a higher carry over of the ingested iodine into milk.

On the other hand, the high milk iodine concentrations at the presently permitted maximum level of iodine in feed when feeding a glucosinolate free ration showed that the UL for iodine in human nutrition would easily be exceeded in humans with a milk consumption above average or in combination with other foods rich in iodine.

However, at the mean iodine concentration which is presently applied in animal feed (0.79mg/kg DM), milk provides a valuable iodine source for human nutrition without being a danger for the society.

An enrichment of food with certain nutrients like already existent for selenium in eggs is not recommendable for the iodine enrichment in milk, since the amount of milk consumed per person varies considerably and therefore leads to an uncontrollable intake of the trace element. In this way further adverse effects are provoked instead of achieving an adequate intake.

The results of the present study strongly suggest that for achieving a lower variation in the iodine concentration of consumers' milk and with regard to food safety, the presently existing maximum level of iodine in dairy cow feed has to be lowered again. Concerning the iodine concentration of consumers' milk, the fulfilment of the animals' demand at RSM feeding and the practicability of the maximum level, the present results support a reduction to at least 3mg/kg.

SUMMARY

In dairy cows a high carry over of feed iodine into milk is described. Since a declaration of the iodine content on food of animal origin seems impossible, excessive iodine intakes caused by milk have to be avoided by setting adequate maximum levels for iodine in animal feed. The existing dose-response studies are insufficient to assess the risk on human health at the presently permitted maximum level (5mg/kg) and to set an appropriate maximum level. Feedstuffs which contain goitrogens (e.g. RSM) cause a considerable reduction of the milk iodine content. However, the existing studies allow no estimation of the extent of reduction at varying supplementation levels with the nowadays used 00-rapeseed. Moreover, the appropriate iodine species for feed supplementation is still discussed. While in table salt the application of iodate is preferred to iodide due to its higher storage stability, data concerning a possibly better suitability of iodide or iodate in feedstuffs are lacking.

Changes in the iodine transfer into milk are expected to be accompanied by alterations in the iodine content of the other important routes of iodine excretion in the cow (urine and faeces) as well as in blood iodine. However, up to now no study investigated the all-embracing influence of the mentioned impact factors on the iodine status (blood iodine, T_3 and T_4) and on all main routes of iodine elimination in the cow (milk, urine, faeces).

DDGS, a by-product of the biofuel manufacture, gains in importance in animal nutrition due to the increasing availability. It is assumed in general that DDGS is equally applicable to other protein feedstuffs in dairy cow feed but the nutrient value still varies considerably due to the utilized raw material and the processing. Since DDGS from wheat and barley is just extensively utilized in animal nutrition for few years, investigations on the impact of different proportions of DDGS in the ration on the performance of dairy cows are rare.

Hence, in the present study the impact of various iodine supplementations up to the permitted maximum level, the impact of RSM feeding compared to a glucosinolate free diet and the influence of iodide compared to iodate application on the iodine content of serum, milk, urine and faeces as well as on the T_3 and T_4 level of blood of dairy cows should be investigated. Special emphasis was placed on the milk iodine content with regard to human iodine supply. Furthermore, the impact of DDGS application (made from 90% wheat and 10% barley) on the performance of the cows was evaluated in comparison to RSM.

For these purposes 32 German Holstein cows (average milk yield: 34.2kg/d) were divided into four groups of eight animals each and received a TMR *ad libitum*. The feed of the different groups differed in the main protein source (DDGS, RSM) and in the applied iodine species (KI, $Ca(IO_3)_2$) resulting in the following groups: DDGS/iodide, DDGS/iodate, RSM/iodide and RSM/iodate. Furthermore, the diets varied in the supplemented iodine amount (0, 0.5, 1, 2, 3, 4 and 5mg/kg DM) applied in seven consecutive periods (of 21 days each). Milk samples were obtained on Days 1, 4, 11, 19, 20 and 21 of each period whereby

morning (5.30 a.m.) and evening milk (15.30 p.m.) were mixed with regard to the milk yield. Blood and faeces samples were taken on Day 21 between 8 and 10 a.m. and samples of spontaneous urine were collected on Day 19 between 8 and 12 a.m.

Since the animals showed similar performances with RSM and DDGS application, it was suggested that the tested DDGS can replace RSM in feed of dairy cows up to a proportion of 16.5% of the diet.

Rising iodine supplementation resulted in linear or almost linear rises of the iodine concentration of serum and of all excretion matrices (milk, urine, faeces). The carry over of ingested iodine into milk amounted to 30-56% for DDGS feeding and to 11-25% for RSM application. The carry over factors were between 0.029-0.267 (DDGS) and 0.065-0.371 (RSM) for serum, between 0.170-0.427 (DDGS) and 0.347-0.745 (RSM) for urine and between 0.069-0.211(DDGS) and 0.143-0.333 (RSM) for faeces. The carry over and the carry over factors showed no constant increase or decrease with rising feed iodine supplementation. Diminished T_4 contents at the highest tested iodine supplementation indicate that the presently existing maximum level of iodine in feed may lead to alterations of the thyroid function of the cows at high feed intakes.

RSM feeding (GSL intake: 11.0-13.7mmol/d) reduced the milk iodine concentration by 51 up to 78%. On the contrary the iodine concentrations of serum, urine and faeces were increased by RSM feeding by up to 130%, 169% and 123% compared to the concentrations in the DDGS groups. Although the totally excreted iodine amounts were not established, it can be concluded from the considerable changes in the iodine concentrations, that the diminished iodine transfer into milk at RSM feeding causes a fortified iodine excretion via urine and faeces at simultaneously increased iodine contents of blood.

It was shown that the milk iodine content similarly to thyroidal iodine is reduced by RSM feeding while the iodine concentrations of urine and blood are increased. Thus, at RSM application the iodine content of milk seems to reflect the iodine supply of the animal best but only if no iodine containing teat dips are applied.

At a storage time of the concentrates of four weeks and the tested storage conditions, no considerable storage losses were observed, so that differences between iodide and iodate application have to be traced back to variations in metabolism. The application of iodide predominately resulted in lower milk iodine concentrations compared to iodate but the comparison of the single groups revealed no significant differences. Higher serum and faecal iodine concentrations were found for iodide application when feeding a GSL free diet with high iodine supplementations (> 3mg/kg DM or > 2mg/kg DM) whereby the differences were significant at the highest tested iodine supplementation (5mg/kg DM) both in serum and faeces. For explaining the detected effects further investigations are required.

Regarding the milk iodine concentration it can be summarized that next to the iodine intake of the animal, goitrogens in feed considerably alter the iodine content while possible changes due to iodide or iodate application are comparably low. It was shown that the milk iodine content rapidly adapts to a changed iodine supply of the animal (plateau reached after 3-11 days). At the presently permitted maximum level of iodine in feed, the mean milk iodine contents in the DDGS groups amounted to 1464µg/kg in the case of iodide and 1578µg/kg in the case of iodate while in the RSM groups concentrations of 718 and 620µg/kg were observed at iodide and iodate application. The comparison of the investigated contents with the results of a previous study pointed out that similar milk iodine concentrations are achieved with low and high yielding cows at comparable feed iodine concentrations, since higher milk yields are accompanied by higher feed intakes and therefore higher iodine intakes which prevent a dilutive effect of the milk yield. Moreover, higher milk yields seem to be connected with a higher carry over of the ingested iodine into milk.

Using the mentioned milk iodine concentrations at DDGS application for calculating the iodine intake by milk (250-270µg/d = 126-180% of recommendation) at the daily per capita consumption (172g/d), visualizes the existing risk of exceeding the UL in human nutrition in Germany (500µg/d) when applying the presently permitted maximum level of iodine in feed of dairy cows. Thus, a re-evaluation of the maximum level is recommended. On the other hand the results of the present study affirm the assumption that the feed iodine supplementation has to be doubled when the ration contains RSM. With regard to the iodine concentration of consumers' milk, the fulfilment of the animals' demand at RSM feeding and the practicability of the maximum level, a lowering to 3mg/kg is supported by the present results. At the mean presently applied feed iodine concentrations (0.79mg/kg DM), however, no implications are expected on human health by milk consumption.

ZUSAMMENFASSUNG

Bei Milchkühen wird ein hoher Transfer von Jod aus dem Futter in die Milch beschrieben. Da eine Deklaration des Jodgehaltes von Lebensmitteln tierischen Ursprungs nicht möglich scheint, muss durch das Festsetzen adäquater Höchstmengen im Futter eine überschüssige Jodaufnahme des Menschen über Milch verhindert werden. Die vorliegenden Dosis-Wirkungs-Studien sind jedoch unzureichend, um das Risiko bei der derzeitigen Höchstmenge (5mg/kg) abschätzen bzw. eine geeignete Höchstmenge festlegen zu können. Glucosinolathaltige Futtermittel (wie Rapsextraktionsschrot, RES) führen zu stark reduzierten Milchjodgehalten. Auch hier lassen die vorhandenen Studien jedoch keine Aussagen über das Ausmaß der Reduzierung durch das heutzutage eingesetzte 00-RES bei verschiedenen Jodzulagen zu. Weiterhin besteht noch immer Unklarheit über die zur Futtermitteljodierung am besten geeignete Jodspezies. Während in Speisesalz aufgrund der höheren Lagerstabilität hauptsächlich Jodat statt Jodid verwendet wird, liegen für Futtermittel keine Daten über eine bessere Eignung einer der beiden Spezies vor.

Bei Änderungen im Übergang von Jod in die Milch ist zugleich von einer Veränderung der Jodgehalte in den beiden anderen bedeutenden Ausscheidungen (Harn und Kot) sowie im Blut auszugehen. Bisher wurde jedoch in keiner Studie der umfassende Einfluss der genannten Faktoren auf den Jodstatus (Blutjod, T_3 und T_4) und auf alle Hauptausscheidungen (Milch, Harn und Kot) der Milchkuh untersucht.

Getrocknete Getreideschlempe, ein Nebenprodukt der Biokraftstoffherstellung, gewinnt aufgrund der zunehmenden Verfügbarkeit an Bedeutung für die Tierernährung. Grundsätzlich ist davon auszugehen, dass diese gleichwertig zu anderen Proteinträgern bei Milchkühen einsetzbar ist, allerdings unterliegt der Futterwert in Abhängigkeit von dem verwendeten Ausgangsmaterial und der Verarbeitung beachtlichen Schwankungen. Da getrocknete Getreideschlempe aus Weizen und Gerste erst seit relativ kurzer Zeit in großem Umfang in der Tierernährung eingesetzt wird, existieren nur wenige Untersuchungen zum Einfluss unterschiedlicher Mengen in der Ration auf die Leistung von Milchkühen.

In der vorliegenden Studie sollte bei Milchkühen der Einfluss verschiedener Jodzulagen bis hin zur erlaubten Höchstmenge sowie der Einfluss von RES im Futter verglichen mit einer glucosinolatfreien Ration und von Jodid- verglichen mit Jodateinsatz auf den Jodgehalt in Blut, Milch, Harn und Kot sowie auf den Gehalt an T_3 und T_4 im Blut untersucht werden. Im Mittelpunkt stand dabei die mit den erreichten Milchjodkonzentrationen verbundene Jodversorgung für den Menschen. Weiterhin wurde der Einfluss von getrockneter Getreideschlempe aus 90% Weizen und 10% Gerste auf die Leistung der Kühe im Vergleich zu RES getestet.

Zu diesem Zweck wurden 32 Milchkühe (Deutsche Holstein, durchschnittliche Milchmenge: 34,2kg/d) in vier Gruppen mit je 8 Tieren aufgeteilt und eine TMR *ad libitum* verabreicht. Das

Futter unterschied sich in der verwendeten Hauptproteinquelle (RES; Schlempe) und in der eingesetzten Jodspezies (Jodid, Jodat), woraus sich die folgenden Versuchsgruppen ergaben: Schlempe/Jodid, Schlempe/Jodat, RES/Jodid und RES/Jodat. In sieben aufeinanderfolgenden Perioden (von jeweils 21 Tagen) variierte das Futter zudem in der zugesetzten Jodmenge (0; 0,5; 1; 2; 3; 4 und 5mg/kg Trockenmasse, T). Die Milchprobenahme erfolgte an den Tagen 1, 4, 11, 19, 20 und 21 jeder Periode, wobei Morgen- (5.30 Uhr) und Abendgemelk (15.30 Uhr) adäquat zur Milchmenge vereinigt wurden. Blut- und Kotproben wurden am 21. Tag jeder Periode zwischen 8 und 10 Uhr morgens genommen, Harnproben am 19. Tag zwischen 8 und 12 Uhr morgens.

Da die Tiere bei RES- und Schlempe-Fütterung ähnliche Leistungen aufwiesen, kann angenommen werden, dass die getestete Getreideschlempe RES im Futter von Milchkühen bis zu einem Anteil von 16,5% der Ration ersetzen kann.

Steigende Jodsupplementationen führten zu linearen bzw. fast linearen Anstiegen der Jodkonzentrationen im Serum sowie in allen Ausscheidungsmatrices (Milch, Harn und Kot). Der Carry over des aufgenommenen Jods in die Milch lag zwischen 30-56% bei Schlempe-Fütterung und zwischen 11-25% bei RES-Einsatz. Die Carry over-Faktoren lagen zwischen 0.029-0.267 (Schlempe) und 0.065-0.371 (RES) im Serum, zwischen 0.170-0.427 (Schlempe) und 0.347-0.745 (RES) im Harn und zwischen 0.069-0.211 (Schlempe) und 0.143-0.333 (RES) im Kot. Carry over und Carry over-Faktoren zeigten keinen konstanten Anstieg oder Abfall mit zunehmender Jodsupplementation. Verminderte T_4-Gehalte bei der höchsten getesteten Jodsupplementation deuten darauf hin, dass die bestehenden Höchstmengen bei hohen Futteraufnahmen bereits zu Veränderungen in der Schilddrüsenaktivität der Kühe führen können.

Die RES-Fütterung (GSL-Aufnahme: 11,0-13,7mmol/d) führte zur Absenkung der Milchjodgehalte um 51-78%. Im Gegensatz dazu wurden die Jodkonzentrationen im Serum durch den RES-Einsatz um bis zu 130%, im Harn um bis zu 169% und im Kot um bis zu 123% gegenüber den Schlempe-Gruppen erhöht. Auch wenn die ausgeschiedenen Jodmengen nicht erfasst wurden, kann aus den deutlichen Veränderungen in den ermittelten Jodkonzentrationen geschlussfolgert werden, dass der verminderte Jodtransfer in die Milch bei RES-Fütterung zu einer verstärkten Jodausscheidung sowohl über Harn als auch über Kot bei gleichzeitig stark erhöhten Blutjodgehalten führt.

Es wurde gezeigt, dass die Milchjodkonzentration auf eine RES-Gabe, ähnlich wie das Schilddrüsenjod, mit einer Verminderung reagiert, während die Jodkonzentrationen in Harn und Blut erhöht werden. Daher scheint der Milchjodgehalt bei RES-Fütterung und Verzicht auf jodhaltige Dippmittel die Verfügbarkeit des Jods für das Tier am besten widerzuspiegeln.

Bei vierwöchiger Lagerung der Kraftfutter wurden bei den vorliegenden Lagerbedingungen keine bedeutenden Jodverluste beobachtet, sodass Unterschiede zwischen Jodid- und

Jodatapplikation auf Differenzen im Metabolismus zurückzuführen sind. Der Einsatz von Jodid führte zu überwiegend niedrigeren Milchjodkonzentrationen, jedoch ließ der Vergleich der einzelnen Gruppenmittelwerte keine signifikanten Unterschiede erkennen. Serum und Kot wiesen bei hohen Jodsupplementationen (> 3mg/kg T bzw. > 2mg/kg T) und glucosinolatfreier Fütterung höhere Jodkonzentrationen für Jodidgabe auf, die in beiden Fällen bei der höchsten getesteten Jodzulage (5mg/kg DM) signifikant waren. Zur Klärung der Ursachen für die gefundenen Unterschiede bedarf es jedoch weiterer Untersuchungen.

Zusammenfassend lässt sich im Hinblick auf die Milchjodkonzentration feststellen, dass diese neben der Jodaufnahme des Tieres sehr stark durch die Fütterung von goitrogenen Substanzen beeinflusst wird, während im Gegensatz dazu mögliche Unterschiede, die aus der Applikation von Jodid oder Jodat resultieren, vernachlässigbar gering sind. Es zeigte sich eine schnelle Anpassung des Milchjodgehaltes an eine veränderte Jodversorgung des Tieres (Plateau nach 3-11 Tagen). Bei der derzeitigen Höchstmenge für Jod im Futter betrug der mittlere Milchjodgehalt in den Schlempe-Gruppen 1464 (Jodid) und 1578µg/kg (Jodat), während in den RES-Gruppen Konzentrationen von 718 (Jodid) und 620µg/kg (Jodat) ermittelt wurden. Der Vergleich der ermittelten Milchjodgehalte mit früheren Ergebnissen zeigte, dass die erreichten Milchjodkonzentrationen bei vergleichbaren Futterjod-konzentrationen in Kühen mit niedriger und hoher Milchleistung ähnlich sind, da bei höheren Milchmengen der Kühe die gleichzeitig höheren Futter- und damit Jodaufnahmen einen Verdünnungseffekt durch die Milchmenge verhindern. Zudem scheinen höhere Milchmengen mit einem höheren Carry over des aufgenommenen Jods in die Milch verbunden zu sein. Die anhand der Konzentrationen bei Schlempefütterung ermittelten Jodaufnahmen über Milch (250-270µg/d = 126-180% der Empfehlung) bei dem durchschnittlichen pro Kopf Verzehr (172g/d) in Deutschland verdeutlichen, dass bei Einsatz der derzeit erlaubten Höchstmenge für Jod im Milchkuhfutter ein Risiko für ein Überschreiten der für Deutschland festgesetzten tolerierbaren Höchstmenge in der Humanernährung (500µg/d) besteht. Daher wird eine Neubewertung der bestehenden Höchstmengen empfohlen. Andererseits bestätigen die Ergebnisse, dass bei Fütterung von RES eine Verdopplung der Jodsupplementation im Futter gegenüber GSL-freier Fütterung zu empfehlen ist. Mit Rücksicht auf den Jodgehalt von Konsummilch, auf die Bedarfsdeckung des Tieres bei RES-Fütterung und auf die Praktikabilität der Höchstmenge kann daher eine Absenkung auf 3mg/kg Futter anhand der ermittelten Ergebnisse befürwortet werden. Bei den derzeit durchschnittlich eingesetzten Futterjodgehalten (0,79mg/kg T) ist durch den Genuss von Milch allerdings keine Beeinträchtigung der Gesundheit zu erwarten.

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EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich an Eides statt, dass die vorliegende Dissertation: „ Effect of various iodine supplementations and species on the iodine transfer into milk and on serum, urinary and faecal iodine of dairy cows fed rations varying in the glucosinolate content" selbständig und nur unter der Verwendung der angegebenen Literatur und Hilfsmittel angefertigt wurde. Die Arbeit lag bisher in gleicher oder ähnlicher Form keiner Prüfungsbehörde vor.

Halle an der Saale, 15.06.2009 .....

Katrin Franke

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