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ABSTRACT

Dietary strategies to optimize energy and glucose supply to lactating dairy cows

Ruminants usually absorb only small quantities of glucose directly from the small intestine, because the majority of the glucose, regardless of dietary source, is fermented in the rumen to short chain fatty acids. Thus, the largest part of the glucose for covering the cow's requirement has to be synthesized de novo, i.e. through gluconeogenesis, from precursor molecules. As lactation performance of dairy cows is steadily increasing and similarly, more glucose is required to sustain high milk yields, the question is which feeding strategies might help to adequately supply high-yielding dairy cows with energy and nutrients. In addition to fully utilizing the digestive capacity of the rumen, ruminally undegraded nutrients that can be digested in the small intestine may contribute to an optimized feeding. Starch, which flows into the small intestine and is digested and absorbed as glucose, can contribute to cover the glucose requirements. Often starch sources of low ruminal degradability are also less well digested in the small intestine. Therefore, the challenge is to process grain (starch) such that it is protected from ruminal degradation but digestible postruminally.

The objective of the first experiment was to estimate the ruminal degradation of crude protein (CP) and starch of ground wheat, barley, rye and maize grains as compared to xylose-treated wheat, barley and rye grains. Ruminal degradation was estimated using a standardised *in situ* procedure on ruminally cannulated steers). Data would indicate that the xylose-treatment was effective in reducing the extent of ruminal degradation of CP for the three grains, thereby augmenting the proportion of ruminally undegraded CP (RUP). However, only wheat and barley starches but not rye starch responded to the xylose treatment such that ruminally undegraded starch (RUS) was increased for barley and wheat. All treated grains had lower RUP and RUS values than maize grain. In the second experiment, thirty-six German Holstein dairy cows were assigned to one of two groups who were fed isocaloric and isonitrogenous diets, on dry matter basis, either 16% maize grain and 6.4% soybean meal or 17.8% of a xylose-treated wheat and 4.6% soybean meal. The xylose-treated wheat grain could replace maize grain and part of the soybean meal in a total mixed ration for lactating dairy cows and overall performance was slightly improved. Thus, xylose-treated wheat grain may be an alternative depending on overall ration composition and availability and costs of grain sources.

Finally, a study was conducted to evaluate if intermediary energy metabolism of cows fed with *trans*-10, *cis*-12 conjugated linoleic acid (CLA) was modified such that milk-energy compounds were produced with less intermediary energy expenditure as compared to control cows. Published data on supplemented CLA were assembled. The extent was calculated to which the *trans*-10, *cis*-12 CLA isomer has an impact on glucose and energy conversion in the mammary gland by modifying glucose equivalent supply and energy required for fatty acid and fat synthesis, and if this will eventually lead to an improved glucose and energy status of CLA-supplemented high-yielding dairy cows. A weak to moderate dose-dependent relationship between the amount of CLA administered and the amount of energy in glucose equivalents and energy for the synthesis of milk fat conserved from milk ingredient synthesis became obvious. Abomasal infusion of the *trans*-10, *cis*-12 CLA more consistently conserved energy in glucose equivalents. Milk fat synthesis showed an energy saving with a moderate dose-dependent relationship when CLA was supplemented orally.

In conclusion, feeding a rumen-protected starch source that can be digested in the small intestine appeared more promising in terms of supplying a dairy cow with extra glucose than addressing intermediary glucose metabolism.

ZUSAMMENFASSUNG

Fütterungsstrategien zur Optimierung der Energie- und Glucoseversorgung bei laktierenden Milchkühen

Die umfangreichen mikrobiellen Abbauprozesse in den Vormägen der Wiederkäuer, von denen auch mit dem Futter aufgenommene Stärke betroffen ist, bedingen unter anderem eine nur geringe Glucoseabsorption aus dem Dünndarm. Deshalb muss der größte Teil zur Deckung des Glucosebedarfs von den Tieren neu synthetisiert werden. Mit weiter steigenden Leistungen der Milchkühe und der damit verbundenen zunehmenden Synthese von Lactose und kurzkettigen Fettsäuren sowie der Veresterung von Fettsäuren nimmt auch der Bedarf an Glucose bzw. Glucosevorstufen weiter zu. Hieraus ergibt sich die Frage, mit welchen Fütterungsstrategien eine bedarfsgerechte Versorgung hochleistender Tiere erreicht werden kann. Neben der bestmöglichen Ausnutzung der ruminalen Verdauungskapazität können im Pansen nicht abgebaute (‚beständige') Nährstoffe, welche im Dünndarm verdaut und absorbiert werden, einen Beitrag zur optimierten Fütterung leisten. Stärke, welche in den Dünndarm gelangt und dort als Glucose absorbiert wird, kann zur Bedarfsdeckung beitragen. Häufig eingesetzte Stärkequellen, welche im Vormagen langsam und unvollständig abgebaut werden und somit in größeren Anteilen in den Dünndarm gelangen, zeigen jedoch häufig auch niedrige Verdaulichkeiten im Dünndarm. Damit hochverdauliche Futtermittel einen höheren Beitrag zur direkten Glucoseversorgung der Milchkuh leisten können, ist eine reversible Behandlung nötig.

Das ruminale Abbauverhalten von schnell fermentierbaren Getreiden (Weizen, Gerste und Roggen) in unbehandelter und behandelter Form und Körnermais als Quelle für ein ruminal langsam abbaubares Futtermittel wurde mit standardisierten *in situ*- und in *vitro*-Methoden untersucht. Die Behandlung der drei Getreidevarianten erfolgte mit Xylose in einer wässrigen Calcium-Magnesium-Lignosulfonat-Lösung bei erhöhten Temperaturen. Behandelter Weizen zeigte von den drei Getreidearten die höchsten Gehalte an im Pansen unabgebautem Rohprotein und Stärke, während die Effekte auf den Stärkeabbau bei Gerste weniger stark und bei Roggen nicht nachweisbar waren. Anschließend fand ein Fütterungsversuch mit 36 Kühen der Rasse Deutsche Holstein während der ersten 120 Laktationstage statt. Untersucht wurden die Auswirkungen des Austauschs von Körnermais und eines Teils des Sojaextraktionsschrots mit Xylose behandeltem Weizen auf das Leistungsgeschehen. Die

Ergebnisse belegen die Austauschmöglichkeit ohne negative Auswirkungen auf Leistung oder Gesundheitsgeschehen sowie eine erhöhte Abgabe von Glucose mit der Milch.

Eine weitere Fütterungsstrategie zur Deckung des Bedarfs an Glucose ist die Senkung des Glucoseverbrauchs. Der Einsatz der konjugierten Linolsäure *trans*-10, *cis*-12 führt zu einer Verschiebung der mit der Milch abgegebenen Inhaltsstoffe und der Zusammensetzung des Milchfetts. Die eingesparte Energie – ausgedrückt in Glucoseäquivalenten – bei der Synthese von Milchfett durch eine veränderte Zusammensetzung einerseits und einer erhöhten Glucoseabgabe in Form von Lactose mit gesteigerter Milchleistung andererseits wurde mittels Literaturdaten durch einen biochemisch basierten theoretischen Ansatz überprüft. Es zeigte sich eine potentielle Einsparung an Glucose, allerdings nicht in der Höhe der zusätzlichen Glucoseabgabe mit der Milch bei der Fütterung des behandelten Weizens. Somit stellt die Aufnahme pansenstabiler, im Dünndarm verdaulicher Stärke einen effektiveren Weg zur verbesserten Energie- und Glucoseversorgung der Milchkuh dar als die intermediäre Beeinflussung des Glucoseverbrauchs.

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ABBREVIATIONS

ADF	Acid detergent fibre expressed inclusive of residual ash
ADL	Acid detergent lignin
aNDF	Neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash
ap	ante partum
ASP	Aspartate aminotransferase
ATP	Adenosine triphosphate
BCS	Body condition score
BHB	Beta hydroxybutyrate
BW	Body weight
CF	Crude fibre
CL	Crude lipid
CLA	Conjugated linoleic acid
CM	Cereal grain mix
CON	Control group
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk
ED	*in situ* effective degradability
FA	Fatty acid
FP-CLA	Formaldehyde-protected conjugated linoleic acid
GE	Gross energy

GGT	Gamma glutamyltransferase
GLDH	Glutamate dehydrogenase
GLM	General linear models
IGF-1	Insulin-like growth factor 1
LSM	Least squares means
ME	Metabolizable energy
PMR	Partial mixed ration
pp	post partum
RUP	Ruminally undegraded crude protein
RUS	Ruminally undegraded starch
NEFA	Non-esterified fatty acids
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NEL	Net-energy for lactation
SCFA	Short chain fatty acids
SP	Small particles
SE	Standard error
SEM	Standard error of the mean
St	Starch
Su	Sugar
TMR	Total mixed ration
uCP	utilisable crude protein at the duodenum
WHEAT	Wheat group

CHAPTER 1

General introduction

Lactation performance of dairy cows has steadily increased during the past decades and is also expected to further increase caused by progresses in breeding and nutrition. High milk yields represent difficulties for feeding the lactating cow according to requirements, especially in the first third of lactation. The typical challenge in feeding the early lactating cow is finding the balance between the supply with energy and protein on one side and sufficient physical structure of the ration on the other side. In addition to the total energy requirement the increasing demand for glucose to sustain high milk yields plays an important role. This begins with the methodological challenges of determining the glucose requirement of cows for milk production. As summarized by Matthé et al. (2000) several authors (Kronfeld et al., 1968; Elliot, 1976; Abel 1995; Bergner and Hoffmann, 1997) have conducted assessments of the glucose requirement from the secreted quantity of lactose with milk. The disaccharide lactose consists of glucose and galactose. The secreted lactose is of central importance due to its osmotic effect (Karatzas and Turner, 1997), which is directly linked to performance, i.e. milk yield. Only Elliot (1976) has taken requirements of glucose for maintenance into account but all authors mentioned above have neglected changes in glucose requirements caused by fertility-related events such as pregnancy. Furthermore, glucose is used for the provision of nicotinamide adenine dinucleotide phosphate ($NADPH_2$), which, among other pathways, is required for the synthesis of fatty acids as precursors of milk fat. When the glucose requirement is estimated from the amount of secreted lactose, a dairy cow with a daily milk yield of 50 kg needs 3.6 - 3.8 kg glucose per day (Matthé et al., 2000).

Ruminants usually absorb only small quantities of glucose directly from the small intestine, because the majority of the glucose, regardless of source, is fermented in the rumen to short chain fatty acids (SCFA). Thus, the largest part of the glucose for covering the cow's requirement has to be synthesized de novo, i.e through gluconeogenesis, from precursor molecules. Gluconeogenesis is dependent on the availability of substrates such as propionate, glucogenic amino acids, glycerol and lactate. The cytoplasm of the hepatocyte is the predominant site of the gluconeogenesis and in addition 10 to 15% of total gluconeogenesis may occur in the kidneys (Bergman et al., 1974). However, there can be deviations from

these estimated proportions when animals are fasted. Another challenge for the supply with glucose is the competition between the tricarboxylic acid cycle and gluconeogenesis for oxaloacetate. Activated acetate is formed during fatty acid degradation and it requires oxaloacetate as a donator to be further metabolized. Otherwise, there is an enrichment of the ketone bodies acetoacetate, ß-hydroxybutyrate and acetone. Oxaloacetate is also of central importance for the gluconeogenesis. Propionate is converted to succinate, fumarate and, finally, oxaloacetate before the final steps of the gluconeogenesis. Lactate and several amino acids are used for glucose synthesis via pyruvate and, again, oxaloacetate. Furthermore, glucogenic amino acids are utilized for gluconeogenesis via α-ketoglutarate, succinyl-CoA, fumarate or directly via oxaloacetate.

Due to the high glucose requirement and, at the same time, the low amounts that are being absorbed in ruminants, various strategies have been considered in order to achieve an adequate supply of glucose to high yielding animals. The direct provision of glucose through feedstuffs providing starch of low ruminal degradability or, vice versa, high proportions of ruminally undegraded strarch can be attempted. In rations for dairy cows, high quantities of cereal grains are used, with starch as the major energy source (Huntington, 1997). Starch consists of α-1,4 glycosidic linked amylose and α-1,4 and α-1,6 glycosidic linked amylopectin and is structured in granules having different shapes and sizes (Tester et al., 2004). Due to the different structure of granules and the surrounding protein layer degradation of starch from different feedstuffs occurs at different rates and extents in the rumen (Svihus et al., 2005). Starches of maize and millet, for example, are slowly degraded in the rumen, whereas wheat, barley and rye starches are rapidly degraded (Offner et al., 2003). If the starch is degraded slowly in the rumen and(or) included in large quantities in the ration, more starch will flow to the small intestine, where it can be hydrolysed and the released glucose can be absorbed. There are differences in the animal's capacity to digest starch in the small intestine depending on substrate, i.e. starch source (Ferraretto et al., 2013) but data are equivocal (Matthé et al., 2000; Reynolds, 2006). Starches that are extensively degraded in the rumen are also highly digestible in the small intestine, such as wheat with 675 g/kg of starch entering the small intestine. On the other hand, starch with low ruminal degradability is of low digestibility in the small intestine, e.g. peas with only 341 g/kg of starch entering the small intestine (Moharrery et al., 2014). Thus, an increase of less ruminally degradable starch sources in the ration of dairy cows does not necessarily lead to an increase in glucose absorbed from the small intestine. An increase in rapidly fermentable

carbohydrates leads to an increase in volatile fatty acids, which can subsequently decrease the rumen pH (Emmanuel et al., 2008). Below a certain threshold, this leads to subacute ruminal acidosis and detrimental effects are observed like decreases in dry matter intake (DMI), fibre digestion, milk production and milk fat content (Nocek, 1997).

Various physical and chemical methods have been used to modify site and extent of digestion in ruminats of starch in cereal grains (Offner et al., 2003; Dehghan-Banadaky et al., 2007; Ferraretto et al., 2013). Since whole, unprocessed cereal grains are excreted almost undigested by cows (Barnes and Ørkov, 1982), physical methods such as grinding or rolling the grain are typically used to increase the digestibility of grains. Chemical methods such as application of sodium hydroxide (NaOH) to whole grains are intended to increase the digestibility of whole cereal grains, and on the other hand to reduce the fast degradation of starch in the rumen with the negative consequences outlined above (Lebzien et al., 1996). However, the results in terms of starch digestibility are inconsistent (De Campeneere et al., 2006; Dehghan-Banadaky et al., 2007). The use of harsh chemicals is not widespread due to corrosive properties, associated risks and the costs of the treatments (Iqbal et al., 2012). Thus there is a need for reasonably priced processing methods designed to shifting a part of the starch digestion from the rumen to the small intestine for securing the direct supply of glucose via absorption from the small intestine which also lowers the risk of negative effects on rumen fermentation.

Although the NaOH treatment of barley successfully reduced ruminal starch digestibility, it also lowered the starch digestibility in the small intestine (McNiven et al., 1995). Thus, the changes in the kinetics and extent of ruminal degradation can result in decreased total digestibility of starch, which is clearly undesirable. Treating wheat grains with xylose in aqueous Ca-Mg lignosulphonate solution at elevated temperatures ((WeiPass®; Winowiski et al., 2005) resulted in lower values for ruminal *in situ* starch degradation than untreated wheat grains (Südekum et al., 2004) but it was not reported whether post-ruminal starch digestibility was affected. It appears to determine whether treatments designed to reduce ruminal starch degradation and providing more starch for digestion in the small intestine, have a negative effect on the total digestibility or whether it can contributes to improved glucose supply to the cow. Comparative laboratory analyses and falsification or verification by performance of dairy cows can both help to provide better data.

Another possibility to meet the requirement of glucose is the direct provision of precursors for gluconeogenesis such as glycerol or propylene glycol. The alkanol propylene

glycol has consistently increased the concentration of glucose in plasma and partially also that of insulin, but it decreased the DMI of mixed rations (Nielsen and Ingvartsen, 2004). In a study of Miyoshi et al. (2001) drenching of propylene glycol had a positive effect on energy balance and ovarian function. The drenching of individual animals in big herds is too labour intensive, causes high costs and may also tackle animal welfare issues. Regarding the performance of the lactating cow, however, the biggest problem is the negative impact on the DMI. The use of glycerol as a precursor for gluconeogenesis also lowered DMI prepartum and decreased the yield of energy corrected milk (DeFrain et al., 2004). However, others found less consistent results when glycerol was supplemented (Lomander et al., 2012; Boyd et al., 2013). Supplementation of glycerol tended ($p = 0.13$) to decrease milk fat yield (DeFrain et al., 2004). This may be due to a change in the ratio of acetate to propionate when using glycerol (Schröder and Südekum, 1999). DeFrain et al. (2004) expected a negative impact on cell wall digestibility when adding glycerol to concentrate rich rations as already digestibility values of Südekum and Schröder (2002) have shown. Thus, use of glycerol as additional substrate for gluconeogenesis is limited, due to the impact on rumen fermentation. Glycerol should more likely be seen as a substitute for rapidly fermentable carbohydrates in the rations of ruminants (Schröder and Südekum, 1999).

Another approach to more closely meet the demand for glucose of high-yielding dairy cows would be to reduce the intermediary glucose consumption. Due to the reduced consumption of oxaloacetate this would additionally act as a prevention of ketosis. The most effective saving of glucose would be the reduction of lactose synthesis. However, due to the osmotic effect of lactose which regulates milk yield (Karatzas and Turner, 1997) less lactose synthesis is generally not desirable. Another possibility to spare glucose consumption is to modify the milk fat content. With a lower fat content and thus a lower fat yield less glycerol is needed for fatty acid esterification and less $NADPH_2$ is required for the synthesis of fatty acids. In the past many studies were published where conjugated linoleic acid (CLA), more specifically, the *trans*-10, *cis*-12 isomer has reduced the milk fat content of dairy cows (Harvatine et al., 2009). Studies on the use of CLA with concomitant estimation of the energy balance are equivocal. A positive influence on the energy balance was reported by Castañeda-Gutiérrez et al. (2005) and Liermann et al. (2008), yet other authors found no impact on energy balance (Bernal-Santos et al., 2003; Moallem et al., 2010; Metzger-Petersen, 2013). As potential energy savings are due to the lower milk fat content, this effect can be compensated or even overcompensated by increases in milk yield and, thus, more lactose and

protein synthesis and secretion. There is no information available on glucose consumption based on energy balance as the fatty acid pattern of milk fat has not been considered. Beside lactose, energy for fat synthesis takes an important role in the consumption of glucose for milk production. Cows supplemented with CLA showed a reduction in endogenous glucose production in comparison to the control group (Hötger et al., 2013). Based on the observed increase of plasma glucose concentration and lactose output these authors suggested a lower glucose consumption for the milk fat synthesis. Whether this assumption is true and to what magnitude glucose savings are possible still has to be elucidated. When the *trans*-10, *cis*-12 isomer was fed in a rumen-protected form or directly infused into the abomasum, a shift was observed of the milk fatty acid profile towards more long-chain fatty acids (Perfield et al., 2004; DeVeth et al., 2006; Kay et al., 2007), originating from the mobilization of body fat or from fatty acids directly derived from the ration. Consequently, the concentration of SCFA synthesized de novo decreased. At the same time the consumption of glycerol declines as its demand for the esterification of the same weight of milk fat is lowered, due to the higher molar mass of long-chain fatty acids.

In conclusion, several ways exist to influence or modify the glucose supply of high yielding dairy cows. In addition to aspects of ease of handling, costs and availability, the potential influences on the metabolic situation of the cow have to be considered. To date it appears very difficult – if not impossible – to quantitatively predict ration compositional changes in regard to glucose supply to high-yielding dairy cows

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CHAPTER 2

Scope of the thesis

One peculiarity of ruminants is the absence of appreciable quantities of glucose that are directly absorbed from the small intestine from digested starch. This does particularly apply to dairy cows with an extensive demand for glucose for the production of large volumes of milk. Covering the glucose requirement of dairy cows creates an additional challenge to feeding early lactation dairy cows in addition to the general supply with adequate energy amounts and physical structure of the ration at limited DMI in early lactation. The overall aim of this thesis was to identify different paths for improved glucose supply and thus a metabolic relief of dairy cows. In the past various physical and chemical methods have been used to modify the site and extent of starch digestion in ruminants, with the overall goal to (1) increase the extent of starch digestion of whole cereal grains or (2) to impact on the site of starch digestion such that starch is not fermented in the rumen but can be hydrolyzed and absorbed as glucose in the small intestine. A method to generate ruminally undegraded starch (RUS) is treating wheat grain with xylose in aqueous Ca-Mg lignosulphonate solution at elevated temperatures (denotation WeiPass®). In chapter three of this thesis, this method was applied to cereal grain commodities that are frequently used in feeding of dairy cow. In addition, these treated grains were compared with maize grain as starch source with higher concentrations of RUS due to structural features of tropical (maize) versus temperate (e.g., wheat) cereal grains. Furthermore, standardized *in situ* and *in vitro* methods were compared for estimating the content of RUS and ruminally undegraded crude protein (RUP) in the treated grains.

Chapter four reports on the impact of treated wheat grain on the performance of dairy cows and, thus indirectly addressing glucose supply to the lactating cow. For this purpose, a feeding trial was conducted where maize grain and parts of the protein supplement (solvent-extracted soybean meal) were replaced with WeiPass®.

A possibility of influencing the intermediary glucose consumption is presented in chapter five. Many studies have shown changes in milk fatty acid composition when the *trans*-10, *cis*-12 CLA isomer was supplemented to dairy cow rations. Moreover, a shift was observed from short- and medium-chain fatty acids towards more long-chain (unsaturated) fatty acids. This change might spare glucose. For the calculation of glucose consumption CLA was supplemented, papers with original research on CLA supplementation in dairy cows were

evaluated to calculate the relationship between *trans*-10, *cis*-12 CLA intake and intermediary energy metabolism, especially glucose and fat output by milk secretion. For characterisation of intermediary metabolism, a so called “glucose equivalent supply” and energy required for fatty acid and fat synthesis were calculated.

The main parts, of this thesis (Chapters three, four and five) are manuscripts which are formatted according to the instructions of the journal chosen for submission.

CHAPTER 3

In situ and *in vitro* ruminal degradation of maize grain and untreated or xylose-treated wheat, barley and rye grains

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ABSTRACT

The objective of this study was to estimate the ruminal degradation of dry matter, crude protein (CP) and starch of ground wheat, barley, rye and maize grains as compared to xylose-treated wheat, barley and rye grains. Ruminal degradation was estimated using a standardised *in situ* procedure on three ruminally cannulated mature steers. Data of ruminal degradation of CP and starch was then used to estimate the proportions of ruminally undegraded CP (RUP) and ruminally undegraded starch (RUS) assuming rumen outflow rates of 0.02, 0.05 and 0.08/h. Depending on the assumed rumen outflow rate, treated grains had RUP values (g/kg of CP) which were 204-294 (wheat), 108-231 (barley) and 98-217 (rye) higher than those of the untreated grains. The RUS values (g/kg of starch) of treated wheat were between 110 and 179 higher than the respective values for the wheat. Treatment of barley increased RUS by 48-153 g/kg starch, values which were similar to those observed for RUP. However, the increase in RUS for the treated versus the control rye was small (16-49 g/kg starch) and non-significant. At an assumed rumen outflow rate of 0.08/h, values for RUP (g/kg CP) and RUS (g/kg starch) were 196 and 129 (rye), 413 and 178 (treated rye), 246 and 130 (barley), 477 and 283 (treated barley), 283 and 181 (wheat), 577 and 360 (treated wheat) and 773 and 579 (maize). Our data would indicate that the xylose-treatment was effective in reducing the extent of ruminal degradation of CP for the three grains, thereby augmenting the proportion of RUP. However, only wheat and barley starches but not rye starch responded to the xylose treatment such that RUS was increased for barley and wheat. All treated grains had lower RUP and RUS values than maize grain.

Keywords: Treatment, Grain, Starch, Rumen fermentation, Protected protein

Abbreviations; ADF, acid detergent fibre expressed inclusive of residual ash; aNDF, neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; CP, crude protein; DM, dry matter; ED, *in situ* effective degradability; GLM, general linear models; RUP, ruminally undegraded CP; RUS, ruminally undegraded starch; SP, small particles.

INTRODUCTION

Lactating ruminants require an adequate supply of absorbable amino acids for the synthesis of milk protein from two sources, i.e., from crude protein (CP) synthesized microbially in the rumen and ruminally undegraded feed CP (RUP) that can be digested in the small intestine. Although the supply with microbial CP makes up the majority of duodenal supply, any deficit in requirement must be met by RUP. Because microbial synthesis requires energy from fermented organic matter and energy intake is often limited in particular during early lactation, supply of extra amino acids to the udder for maintaining milk protein synthesis can only be achieved by increased dietary concentrations of RUP sources. Several chemical and physical methods have been identified as being effective in increasing the proportion of RUP of total CP of a feedstuff (Petit et al., 2002; Ljøkjel et al., 2003; Wulf and Südekum, 2005; Lund et al., 2008), yet there is a continuing need for developing and establishing methods which allow estimating the degree of protein protection from ruminal degradation with acceptable expenditure of labour and other costs.

Starch is a unique energy source to ruminants because it can be degraded and fermented either in the rumen and large intestine, yielding short-chain fatty acids as primary metabolites, or it can be digested in the small intestine, where glucose is liberated from starch for absorption which is more energy efficient (Owens et al., 1986) and supplies glucose directly to the ruminant animal. The majority of starch in the diet of high-yielding dairy cows is often from cereal grains, which contain between 570 and 770 g/kg (Huntington, 1997) of starch in dry matter (DM). Starch-rich feedstuffs comprise also pulses, such as peas or field beans, and tapioca.

Physical and chemical treatments of starch sources have resulted in varying effects on ruminal degradation of starch (Offner et al., 2003; Dehghan-Banadaky et al., 2007). Utilizing the *in vitro* gas production profile over time as indication of rate and extent of starch degradation of cereal grains, Südekum (2002) reported that hydrothermal treatments covering a wide range of conditions applied on the feeds, increased the rate of gas production and thus carbohydrate degradation of maize grain considerably and in a favourable direction. The increase in rate of degradation was much lower for the other cereal grains. Moreover, a decrease rather than a further increase in rate of carbohydrate degradation of rapidly degraded starch sources like wheat, rye and barley would have been much more desirable to be able to prevent acidosis when large amounts of rapidly degraded starches are consumed by high-yielding dairy cows. The data presented by Südekum (2002) support earlier observations that

physical treatments in general tend to increase rather than decrease rate and extent of ruminal starch degradation. Thus it appears that chemical methods may be more promising to protect a certain proportion of otherwise rapidly degraded starch from ruminal degradation, thereby increasing starch flow to the duodenum of ruminants.

The aim of the present study was, therefore, to estimate the ruminal degradation of CP and starch of untreated wheat, barley, rye and maize grains as compared to chemically treated wheat, barley and rye grains in order to compare these feeds in terms of potential RUP and ruminally undegraded starch (RUS) delivery to the small intestine in ruminants.

MATERIALS AND METHODS

Feedstuffs

Wheat, barley, rye and maize grains were obtained commercially from Raiffeisen Hauptgenossenschaft Nord AG, Kiel, Germany. All grain commodities were ground through a hammer mill fitted with a sieve with 3-mm pore sizes and, if not being processed further, designated with the grain species name and, where applicable, 'control grain'. Parts of the wheat, rye and barley commodities were further treated with 5% lignin sulphonate (DM basis) and heated by direct addition of steam such that the temperature increased to about 105 °C and moisture content increased to about 200 g/kg. This mixture was held at that temperature for 40 min. The mixture was then returned to ambient temperature by evaporative cooling under a stream of forced air. This cooling process also reduced the moisture content below 150 g/kg (Winowiski et al., 2005). The lignin sulphonate-treated commodities are hereafter designated WeiPass® ('Weizen', German for wheat), GePass ('Gerste', German for barley) and RoPass ('Roggen', German for rye).

In situ procedure

The *in situ* technique basically followed a proposal for a standardized method for concentrate ingredients (Madsen and Hvelplund, 1994). Three steers received a mixed diet consisting of two-thirds of long mixed grass-legume hay and one-third of mixed concentrates which also contained starch. The diet was supplemented with a commercial mineral and vitamin mix. Ruminal DM, CP and starch degradabilities were determined using polyester bags (R510, Ankom Technology, Macedon, NY, USA) with a pore size of 50 (±15) μm.

Quadruplicate samples of each feedstuff were incubated in the rumen of three mature steers. About 1.3 g of feed ground to pass a 2 mm screen was placed in each bag. Each bag was sealed with a commercial cable binder (20 cm length), then bags were clamped to a cylindrical anchor weight (800 g), which was tied to an 80 cm long main line outside the fistula. Prior to incubation, the bags were soaked in warm water (40°C) for 10 min. All bags for all incubation periods were inserted together into the ventral sac of the rumen at 07:00 h immediately before the morning feeding. Incubation periods were 2, 4, 8, 16, 24, and 72 h.

Immediately after removal from the rumen, bags were immersed in ice-water to stop or minimize microbial activity and then washed with cold water in a washing machine for 20 min. Zero time (0 h) disappearance values were obtained by washing pre-soaked, unincubated bags in a similar fashion. Water-soluble material (WS) was estimated by washing quintuple samples through a folded filter paper. Samples (2 g DM) were first soaked in a beaker in 100 ml warm water (40 °C) for 1 min before washed through the folded filter paper (No. $595^{1/2}$; Schleicher & Schuell, Dassel, Germany) using two times 50 ml warm water (40 °C). All washed bags and filter paper residues were freeze-dried. Contents of washed bags were pooled to give one sample per steer and incubation time. Five replicates of filter-paper residues were analysed for each feedstuff. Water-insoluble DM, CP and starch escaping in small particles (SP) from the bags during washing were estimated by subtracting the water-soluble fraction of DM, CP and starch from 0 h values.

In vitro procedure

In vitro gas production was determined using a semi-automated technique according to Mauricio et al. (1999). One gram of the pre-dried and ground (2-mm screen) feed samples was accurately weighed into 125-ml serum flasks. The rumen fluid was from two donor sheep fed twice daily on good quality Italian ryegrass (*Lolium multiflorum*) hay (1100 g fresh weight per day) The gas production was recorded after 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36 and 48 h of incubation.

Chemical analyses

Proximate analyses were done according to the German Handbook of Agricultural Research and Analytic Methods (VDLUFA, 2004) and method numbers are given. All feedstuffs and freeze-dried residues after ruminal incubation were successively ground in

mills with 3 and 1 mm screens and, for starch analysis, with a 0.2 mm screen. The DM of the feedstuffs was estimated by oven-drying a duplicate subsample at 105°C overnight (method 3.1). Crude protein was analyzed by the standard Kjeldahl procedure using Cu^{2+} as a catalyst (method 4.1.1). Crude fat, acid detergent lignin and ash were analyzed using methods 5.1.1, 6.5.3 and 8.1. Free and α-linked glucose (starch) were estimated by an enzymatic method employing a heat-stable α-amylase as described by Brandt et al. (1987). Neutral detergent fibre (aNDF, method 6.5.1; assayed with heat stable amylase and without inclusion of sodium sulphite) and acid detergent fibre (ADF; method 6.5.2) are expressed inclusive of residual ash. Detergent fibre analyses were performed without the use of decalin. Triethylene glycol was used instead of 2-ethoxyethanol in the aNDF procedure

Calculations and statistical analysis

The single values obtained for DM, CP and starch disappearance (DI_i) were corrected (C) for SP by the equation (Weisbjerg et al., 1990):

$$CDI_i = DI_i - SP \cdot (1 - ((DI_i - (SP + WS))/(1 - (SP + WS)))).$$

Degradation of DM, CP and starch (CDEG) was calculated using the equation of McDonald (1981):

$$CDEG = a + b\,(1 - e^{-c\,(t - L)}) \text{ for } t > L,$$

where CDEG = disappearance at time t corrected for SP, a = an intercept representing the proportion of DM, CP and starch solubilised at initiation of incubation (time 0; soluble fraction), b = the fraction of DM, CP and starch insoluble but degradable in the rumen, c = a rate constant of disappearance of fraction b, t = time of incubation, and L = lag phase. The non-linear parameters a, b, c, and L were estimated by a nonlinear regression analysis (PROC NLIN; SAS, 2004). The effective degradability (ED) of DM, CP and starch was calculated using the following equation:

$$ED = a + (bc/(c + k)) \cdot e^{-kL},$$

where k is the estimated rate of outflow from the rumen and a, b, c, and L are the same parameters as described earlier. The equation is a modification to the one published by McDonald (1981), where the term 'e^{-kL}' reads '$e^{-(c+k)L}$'. This suggests that during the lag phase both degradation and passage occur, which is not correct as it is assumed that no degradation takes place during the lag phase (Wulf and Südekum, 2005). The ED of DM, CP

and starch was estimated as ED2, ED5 and ED8 assuming rumen solid outflow rates of 0.02, 0.05, and 0.08/h, which is representative for low, medium, and high feeding amounts (Agricultural Research Council, 1984). The proportions of RUP and RUS (g/kg of CP or starch) were calculated as RUP (RUS) = 1000 - ED.

The *in situ* data were subjected to analysis of variance using the general linear models (GLM) procedure of SAS (2004). The model was:

$Y_i = m + f_i + e_i$,

where Y is the observed response; m the overall mean; f the effect of feedstuff and e is the residual error.

In vitro gas production data were first subjected to PROC MIXED (SAS, 2004). Treatment and time were considered as fixed effects in the model and least squares means were estimated for treatment x time. To describe the dynamics of *in vitro* gas production over time the following Gompertz function (Schofield et al., 1994) was chosen:

$GP = a \cdot \exp(-\exp(1 + ((b \cdot e)/a) \cdot (L - t)))$,

where GP is cumulative gas production (ml), a the theoretical maximum of gas production, b the maximum rate of gas production (ml/h) that occurs at the point of inflection of the curve, L the lag time (h) which is defined as the time-axis intercept of a tangent line at the point of inflection, and t time (h).The parameters a, b and Lag were estimated by nonlinear regression analysis with weighted least squares means (PROC NLIN; SAS 2004), where the least squares means for treatment × time from the above mixed model analysis were used as time series measurements and the standard error of least squares means as weights. Parameters were considered to be significantly different between treatments when the 95% confidence intervals of treatments did not overlap. The effect of treatment on the parameters of gas production were analysed by the GLM procedure of SAS (2004).

RESULTS

In situ degradability

The chemical composition of the seven feedstuffs is presented in Table 1. Table 2 presents data on *in situ* nonlinear parameter estimates and ED values of DM of the seven wheat, barley, rye and maize commodities. The parameter estimates '*a*', '*b*' and '*c*' and the ED values differed ($P<0.05$) between wheat and WeiPass®, whereas the lag phase was zero

for both commodities. The ED at the two faster ruminal passage rates (ED5, ED8) and the rate constant for DM were different between barley and GePass. The same applies to the '*a*' fraction of DM. No difference was observed between rye and RoPass regarding ED values. Interestingly, the xylose-treated feeds (WeiPass®, GePass and RoPass) had a greater water-soluble ('*a*') fraction for DM than the control grains, whereas the opposite was observed for the insoluble but degradable '*b*' fraction. The rate of degradation of this fraction was higher for rye than for barley and wheat ($P<0.10$), and much less for the xylose-treated than the control grains ($P<0.05$). The lowest '*a*' fraction and consequently the lowest ED at all passage rates showed maize. No interactions were observed between grain type and treatment, indicating that the treatment affected ruminal DM degradation of the grain types in a similar manner.

Table 1. Chemical composition of control and xylose-treated wheat, barley, rye and maize grains.

Item	Wheat	WeiPass®	Barley	GePass	Rye	RoPass	Maize
DM (g/kg)	888	886	897	899	894	896	910
DM composition (g/kg)							
Ash	19	25	19	20	14	18	16
Crude protein	134	148	122	115	97	98	89
Crude fat	9	10	29	33	7	12	16
Starch	672	556	573	517	624	580	659
aNDF[a]	142	211	220	288	161	207	123
ADF[b]	32	40	64	95	31	46	31
ADL[c]	14	15	18	33	15	25	9

[a] aNDF, Neutral detergent fibre neutral assayed with a heat stable amylase and expressed inclusive of residual ash.

[b] ADF, acid detergent fibre expressed inclusive of residual ash.

[c] ADL, Acid detergent lignin.

Table 2. *In situ* nonlinear parameter estimates[A] and effective degradability values[B] (ED; g/kg of dry matter) of the dry matter of control (wheat, barley, rye) and xylose-treated (WeiPass®, GePass, RoPass) wheat, barley, rye and maize grains.

Item	Wheat	WeiPass®	Barley	GePass	Rye	RoPass	Maize	SE
a (g/kg)	111^{bc}	286^{f}	92^{b}	134^{cde}	123^{bd}	172^{e}	51^{a}	8.1
b (g/kg)	801^{b}	621^{a}	746^{b}	730^{b}	771^{b}	729^{b}	894^{c}	15.5
c (/h)	0.272^{bc}	0.080^{a}	0.412^{cde}	0.163^{ab}	0.595^{e}	0.307^{bd}	0.051^{a}	0.012
Lag (h)	0^{a}	0^{a}	0^{a}	0^{a}	0.2^{b}	0^{a}	0^{a}	0.03
ED2 (g/kg)	857^{c}	782^{b}	802^{b}	783^{b}	866^{c}	856^{c}	690^{a}	7.0
ED5 (g/kg)	787^{cd}	668^{b}	755^{c}	690^{b}	828^{d}	799^{cd}	500^{a}	9.4
ED8 (g/kg)	729^{c}	596^{b}	713^{c}	621^{b}	792^{d}	750^{cd}	397^{a}	11.2

[A] *a*, the fraction of dry matter solubilized at initiation of incubation; *b*, the fraction of dry matter insoluble but degradable in the rumen; *c*, the rate constant (/h) of disappearance of fraction *b*; Lag, lag phase (h) prior to the commencement of degradation of fraction *b*.

[B] Effective degradability at three ruminal passage rates (i.e., 0.02, 0.05 and 0.08/h).

Table 3. *In situ* nonlinear parameter estimates[A] and effective degradability values[B] (ED; g/kg of crude protein) of the crude protein of control (wheat, barley, rye) and xylose-treated (WeiPass®, GePass, RoPass) wheat, barley, rye and maize grains.

Item	Wheat	WeiPass®	Barley	GePass	Rye	RoPass	Maize	SE
a (g/kg)	111^{ab}	167^{cd}	135^{bc}	84^{a}	370^{e}	149^{bd}	68^{a}	10.4
b (g/kg)	820^{b}	723^{ab}	804^{b}	855^{b}	559^{a}	766^{b}	814^{b}	37.3
c (/h)	0.266^{ad}	0.045^{a}	0.321^{bcd}	0.086^{ab}	0.467^{d}	0.109^{ac}	0.021^{a}	0.001
Lag (h)	0.4	0	0.3	0	0.8	0	0	0.19
ED2 (g/kg)	865^{d}	661^{b}	883^{d}	775^{c}	892^{d}	794^{c}	460^{a}	8.9
ED5 (g/kg)	784^{d}	504^{b}	813^{d}	621^{c}	845^{d}	670^{c}	293^{a}	13.5
ED8 (g/kg)	717^{d}	423^{b}	754^{de}	523^{c}	804^{e}	587^{c}	227^{a}	16.1

[A] *a*, the fraction of crude protein solubilized at initiation of incubation; *b*, the fraction of crude protein insoluble but degradable in the rumen; *c*, the rate constant (/h) of disappearance of fraction *b*; Lag, lag phase (h) prior to the commencement of degradation of fraction *b*

[B] Effective degradability at three ruminal passage rates (i.e., 0.02, 0.05 and 0.08/h).

Table 3 summarizes data on *in situ* nonlinear parameter estimates and ED values of CP of the grain varieties. Control (untreated) wheat, barley and rye had higher values for the '*a*' fraction than their treated counterparts and only rye had a lower value for the '*b*' fraction of the untreated grain ($P<0.05$). Treatment of the grains markedly reduced the rate constant of degradation for rye ($P=0.0064$) and approached a trend for wheat and barley ($P=0.1431$ and $P=0.1053$). Because the magnitude of the difference in rate of degradation was greater than the difference in the size of the '*b*' fraction, the CP from control grains was more extensively degraded ruminally, thus resulting in greater ($P<0.001$) ED values of CP at rumen outflow rates of 0.02, 0.05 and 0.08/h. Irrespective of assumed rumen outflow rate, maize grain had the lowest ED of CP values of all grain commodities ($P<0.0001$).

Table 4 reports *in situ* nonlinear parameter estimates and ED values of starch of the seven commodities. A reduction in the extent of ruminal starch degradation in response to grain treatment was observed for wheat and also the soluble fraction of barley was lower for the treated barley (GePass). The ED of starch of barley and wheat was higher for the untreated than the xylose-treated grains. The smallest '*a*' fraction combined with the greatest '*b*' fraction was observed for maize grain, resulting in the lowest starch ED ($P<0.0001$), followed by WeiPass® ($P<0.003$).

Table 4. *In situ* nonlinear parameter estimates[A] and effective degradability values[B] (ED; g/kg of starch) of the starch of control (wheat, barley, rye) and xylose-treated (WeiPass®, GePass, RoPass) wheat, barley, rye and maize grains.

Item	Wheat	WeiPass®	Barley	GePass	Rye	RoPass	Maize	SE
a (g/kg)	182^{c}	184^{c}	92^{b}	144^{c}	102^{b}	99^{b}	37^{a}	8.6
b (g/kg)	789^{b}	726^{a}	887^{c}	846^{bc}	861^{c}	863^{c}	949^{d}	12.5
c (/h)	0.339	0.136	0.598	0.171	0.348	0.419	0.055	0.001
Lag (h)	0	0	0	0	0.4	0	0.1	0.12
ED2 (g/kg)	926^{ce}	816^{b}	948de	900^{c}	938de	922^{cd}	732^{a}	6.1
ED5 (g/kg)	869^{d}	714^{b}	907^{d}	796^{c}	904^{d}	869^{d}	532^{a}	8.6
ED8 (g/kg)	819^{d}	640^{b}	870^{d}	717^{c}	871^{d}	822^{d}	421^{a}	10.8

[A] *a*, the fraction of starch solubilized at initiation of incubation; *b*, the fraction of starch insoluble but degradable in the rumen; *c*, the rate constant (/h) of disappearance of fraction *b*; Lag, lag phase (h) prior to the commencement of degradation of fraction *b*.

[B] Effective degradability at three ruminal passage rates (i.e., 0.02, 0.05 and 0.08/h).

From the data presented in Table 5 it can be seen that the specific treatment which has been applied to produce the WeiPass®, GePass and RoPass commodities, yielded *in situ* RUP values which were 100-300 g/kg higher than for the control grains. Increases in *in situ* RUS values for WeiPass® compared with wheat, GePass compared with barley and for RoPass compared with rye were also observed. While the increase over control values was marginal for RUS of rye (20-50 g/kg only), considerable increases were observed for barley. Hence, treating rye with an aqueous solution of xylose in sulphite liquor does not seem to be a promising way to improve RUS values. Depending on the assumed rumen outflow rate, RUS values of GePass were up to 150 g/kg higher than those of control barley. The greatest numerical treatment effect was observed for WeiPass® with values up to 180 g/kg higher than those of control wheat.

Table 5. Values for *in situ* ruminally undegraded crude protein (RUP) and starch (RUS) of control (wheat, barley, rye) and xylose-treated (WeiPass®, GePass, RoPass) wheat, barley and rye grains at assumed rumen outflow rates of 0.02, 0.05 and 0.08/h[a].

Outflow rate (/h)	Wheat	WeiPass®	Barley	GePass	Rye	RoPass	Maize
RUP (g/kg of crude protein)							
0.02	135	339	117	225	108	206	540
0.05	216	496	187	379	155	330	707
0.08	283	577	246	477	196	413	773
RUS (g/kg of starch)							
0.02	74	184	52	100	62	78	268
0.05	131	286	93	204	96	131	468
0.08	181	360	130	283	129	178	579

[a] RUP and RUS values were calculated as (1000 - effective degradability) as presented in Tables 3 and 4.

If the increase in *in situ* RUP and RUS for WeiPass® above the control wheat values is expressed relative to the wheat values, it becomes obvious that the proportions of RUP and RUS of WeiPass® had doubled compared with the wheat, i.e., they increased from 280 to 580 g/kg of CP for RUP and from 180 to 360 g/kg of starch for RUS at an assumed rumen outflow rate of 0.08/h. Similarly, the proportions of RUP of GePass had almost doubled compared with the control barley, i.e., it increased from 250 to 480 g/kg of CP (0.08/h rumen

outflow rate). At the same rumen outflow rate, the treatment effect on RUS was even more pronounced, i.e., RUS increased from 130 to 280 g/kg of starch. The greatest values for RUP and RUS were observed for maize grain, inversely to the lowest ED values reported above.

In vitro fermentation

The mean values of cumulative gas production for untreated and treated grains at selected times of incubation and parameters of the Gompertz function are presented in Table 6. There were no differences in maximum volume (*a*) and lag time (Lag) between untreated and treated grains. The rate of gas production (*b*) was greater for the untreated grains ($P<0.05$).

Table 6. Mean values of parameters of gas production estimated with the Gompertz function[A].

Item	Wheat	WeiPass®	Barley	GePass	Rye	RoPass
a (ml)	293 (5.5)	246 (4.2)	272 (5.2)	268 (3.9)	284 (5.4)	282 (3.6)
b[B] (ml/h)	21.7 (1.7)	14.3 (0.8)	18.3 (1.3)	17.1 (0.9)	20.7 (1.6)	15.2 (1.6)
Lag (h)	1.0 (0.5)	1.1 (0.4)	1.1 (0.5)	1.2 (0.4)	0.6 (0.5)	1.1 (0.3)

[A] *a*, theoretical maximum of gas production; *b*, maximum rate of gas production; Lag, lag time; Values are estimates with their SE in parentheses.

[B] Untreated grains greater than treated grains ($P < 0.05$).

DISCUSSION

Chemical composition

The range of values of the chemical composition of the control grains in this study is similar to tabular values (Universität Hohenheim – Dokumentationsstelle, 2007). Xylose addition during the treatment led to a dilution of the analytes in the treated grains. The elevated temperatures seemingly lead to crosslinks of xylose which increased the concentration of fibre fractions, an effect that was also observed when solvent-extracted soybean and cottonseed meals were similarly treated (Can et al., 2011).

In situ degradation

It is generally accepted that high-yielding dairy cows require balanced amounts of ruminally degraded starch and CP, and of RUS and RUP to best utilize the digestive capacity of the rumen and the small intestine. Starchy feedstuffs differ considerably in regard of rate and extent of ruminal starch degradation. As a general observation, maize and in particular sorghum starches have slow rates and low extents of degradation, whereas wheat, barley and rye starches have rapid rates and high extents of degradation in the rumen (Tamminga et al., 1990). Though the general feature of ruminal starch degradation of different starch sources is well accepted, a considerable variation may occur also within starch sources, e.g. between different maize varieties (Philippeau and Michalet-Doreau, 1997; Ramos et al., 2009).

The xylose-treatment at elevated temperatures of cereal grains (Winowiski et al., 2005) induces Maillard reactions between sugar moieties and amino acids (Martins et al., 2001) of the protein matrix of the endosperm which are found in different concentrations depending on the type of grain (McAllister and Cheng, 1996). With decreasing concentration of gluten in the endosperm and an associated diminishing effect of the treatment, both potential and effective degradability of DM and starch decrease to a lesser extent. Thus it appears that the overall success and magnitude of the effect of a xylose-treatment of cereal grains which, in this study, was greatest in wheat and lowest in rye with intermediate values for barley, is related to the appropriate ratio of reactive sugar to free (reactive) amino acid moieties. Ljøkjel et al. (2003) reported that heat treatment but not the addition of glucose reduced ruminal CP degradation of barley. Although both RUP and RUS values were considerably raised by treating wheat with xylose at elevated temperatures, the values of ground maize representing a starch source characterised by a slow and incomplete ruminal degradation were not reached. However, mimicking the ruminal degradation kinetics of maize grain was not the objective of this study. Rather, the results show that the applied treatments may help to create a continuum of RUS and RUP values between cereal grains that are degraded (too) rapidly and extensively in the rumen, such as wheat, and those grains that are often degraded too slowly and incomplete such as maize or sorghum. Therefore, the data presented herein may help to be more flexible in ration planning depending on availability and prices of commodities.

The direct comparison of the results of this study using absolute values, i.e. numbers is only possible to a limited extent. Due to the lack of correction of the soluble fraction for water-insoluble but ruminally degradable small particles in most published work, there is an

underestimation of the "b" fraction and consequently an underestimation of the rate constant of this fraction (Südekum, 2005). In addition to the methods of chemical analysis, also variability within cereal grain species is important. The water solubility of starch of barley (92 versus 57 g/kg) was similar to values reported by Tóthi et al. (2003), who applied the same procedure of separating the 0-h values into a water-soluble fraction and water-insoluble but ruminally degradable small particles. Also further data treatment and estimation of ED values were done using the same procedure as in the present study. In contrast, maize (37 versus 167 g/kg) had a much lower water solubility of starch in this study. However, assuming a fractional rate of passage of 0.05/h, the ED of maize starch was 530 g/kg and that of barley starch 910 g/kg, similar to the relationship of Tóthi et al. (2003) who observed a starch ED of 600 g/kg and 930 g/kg starch for maize and barley grain, respectively.

When the results of this study were compared with effects of other treatments, e.g. sodium hydroxide, formaldehyde or urea, similar results after 12 h of rumen incubation were reported, i.e. CP disappearance was reduced by about 60-160 g/kg (Dehghan-Banadaky et al., 2008). A comparison between barley and barley treated with formaldehyde or glutaraldehyde indicates a lower disappearance of DM, total nitrogen (i.e., CP) and starch (Ortega-Cerrilla et al., 1999). Dehghan-Banadaky et al. (2007) summarized that NaOH treatment reduced ruminal starch degradation and whole-tract starch digestibility of barley compared with physical processing of barley. The degradability of DM, starch and nitrogen (CP) decreased with increasing amounts of formaldehyde in wheat (-240, -330, -310 g/kg) and maize (-50, -70, -110 g/kg) grains in the study of Michalet-Doreau et al. (1997). The relative differences between wheat and maize grains correspond nicely to the present results. Cereal grains treated with a formaldehyde solution showed a lowered ED of starch of about 80 g/kg in barley and about 95 g/kg in wheat, at an assumed outflow rate of 0.08/h (Offner et al., 2003). The same treatment applied to maize grain reduced the ED of starch only by 20 g/kg. In this study, at the same outflow rate of 0.08/h, the xylose treatment caused a reduction of ruminal starch degradation of 150 g/kg in barley and 180 g/kg in wheat. The maize grain in this study showed a lower ED at an outflow rate 0.08/h than the untreated and treated maize grains summarized by Offner et al. (2003). Compared with other chemical methods, the treatment with xylose in an aqueous Ca-Mg lignosulphonate solution at elevated temperatures appears to be an effective method of altering the degradation kinetics of wheat in particular. From data of this study it can be concluded that WeiPass® and GePass can be a valuable source of RUP and RUS in rations of ruminant animals, whereas RoPass would only supply more RUP

but not RUS. However, data presented here would indicate that the treatment can be successfully applied to wheat and barley where the ratio of reactive sugar to reactive amino acid moieties provides protection of both starch and CP from ruminal degradation and this more so for wheat than for barley.

In vitro fermentation

The mean values of cumulative gas production reflect the results of the *in situ* degradability study. Kinetics of gas production of the untreated grains is consistent with earlier reports (Lanzas et al., 2007). Treated grains produced less gas at different times of incubation and, also, the maximum rate of gas production was significantly lower. The protection of the protein matrix and (or) starch from ruminal degradation lowers the fermentation of rye and barley right from the beginning of *in vitro* incubation, whereas wheat shows a reduced fermentation over the entire incubation time and the greatest decline of the maximum rate of gas production.

Gas production of wheat treated with 50 g/kg formaldehyde ceased between 1 and 6 h of incubation but a treatment with 10 g/kg of formaldehyde had no effect (Michalet-Doreau et al., 1997). The gas production of WeiPass® after 6 h of incubation was reduced by about 34%, indicating considerably slower starch fermentation compared with the untreated wheat.

CONCLUSIONS

Treating wheat, barley and rye with xylose in an aqueous Ca-Mg lignosulphonate solution at elevated temperatures increased the proportions of ruminally undegraded protein and starch. It could be demonstrated that WeiPass® and GePass had elevated levels of both RUP and RUS compared with the control grains, whereas only RUP but not RUS was increased when rye underwent the same treatment. Data presented here would hence indicate that the treatment can be successfully applied to wheat and barley with more pronounced effects on wheat than on barley.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CHAPTER 4

Effect of replacing maize grain and soybean meal with a xylose-treated wheat grain on feed intake and performance of dairy cows

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ABSTRACT

This study evaluated wheat grain which was treated with xylose in aqueous Ca-Mg lignosulphonate solution at elevated temperatures (WeiPass®) in order to reduce ruminal degradation of starch and crude protein. Two isocaloric and isonitrogenous diets were formulated with, on dry matter (DM) basis, either 16% maize grain and 6.4% soybean meal (control group, CON) or 17.8% WeiPass® and 4.6% soybean meal (wheat group, WHEAT). Thirty-six German Holstein dairy cows were assigned to one of the two groups according to parity, body weight after calving, and milk yield during the previous lactation. Data collection started at 21 days before the expected calving date until 120 days in milk. The average of DM intake, energy-corrected milk (ECM) yield, and milk fat and protein yields (all given as kg/day) were 18.9, 28.7, 1.25, and 1.02 for CON cows and 19.3, 32.5, 1.36, and 1.11 for WHEAT cows, respectively. Only ECM and milk protein yields were greater ($P < 0.05$) for WHEAT cows. In conclusion, a xylose-treated wheat grain could replace maize grain and part of the soybean meal in a diet for lactating dairy cows and may be an alternative depending on overall ration composition and availability and costs of grain sources.

Keywords: treatment; grain; starch; protected protein; dairy cows

INTRODUCTION

Dairy cows in the first third of lactation require rations with high energy and nutrient, particularly crude protein (CP) concentrations for milk production and at the same time for reproductive performance. With further increases in milk yield it will become more and more challenging to cover energy and nutrient requirements and, at the same time, provide adequate physical structure of the ration. Diets of high yielding lactating cows usually contain cereal grains in order to provide sufficient energy, with starch as the major energy compound (Huntington 1997). A common starch source fed to dairy cows is maize grain, whose starch is slowly and incompletely degraded in the rumen (Offner et al. 2003). Depending on factors like availability, region and market prices also other cereal grain species are utilized such as wheat, barley or rye. These grains represent starch sources which are rapidly and almost completely degraded in the rumen (Offner et al. 2003). Large quantities of rapidly degraded carbohydrates yield vast amounts of short-chain fatty acids in the rumen. Because high grain and, consequently, reduced forage in the ration of dairy cows results in decreased chewing activity by cows and associated low buffering capacity of the ruminal contents, which subsequently lead to a higher risk of subacute ruminal acidosis. Whilst mechanical processing methods increase the ruminal degradability of starch (Nocek and Tamminga 1991), chemical processing methods are used with the aim of a reduction of the rate and extent of starch degradation in the rumen, thereby reducing the risk of acidosis. Furthermore, the degradation of starch to glucose in the small intestine and glucose absorption from the small intestine is more energy- efficient than the degradation of starch in the rumen and its fermentation to short-chain fatty acids which, after absorption from the rumen, requires hepatic gluconeogenesis to supply the animal with glucose (Owens et al. 1986).

A common chemical treatment method is the use of NaOH which however did not lead to consistent results in previous studies (De Campeneere et al. 2006; Dehghan-Banadaky et al. 2007) and also is corrosive and poses health risks to labourers. Another way to lower the ruminal degradability of barley to reduce the risk of subacute ruminal acidosis is applying lactic acid, either at ambient (Iqbal et al. 2009) or elevated temperatures (Iqbal et al. 2012). Alternatively the safe inclusion level of cereal grain in rations for dairy cows can be increased by applying xylose in an aqueous Ca-Mg lignosulphonate solution at elevated temperatures to grains (Winowiski et al. 2005). Wheat grain which was treated in this way (named WeiPass®) showed higher *in situ* values for ruminally undegraded starch (RUS) and CP (RUP) than untreated wheat grain (Benninghoff et al. 2015) which indicates that not only ruminal starch degradation was lowered but at the same time ruminal CP degradation was changed in the

same direction. In the same study (Benninghoff et al. 2015), *in vitro* gas production data supported the view that the xylose treatment may shift partly digestion of starch and CP of wheat grain from the rumen to the small intestine which could help to improve the overall energy and CP (i.e., amino acid) supply to the early lactating dairy cow. To date, no feeding trial was conducted to test or validate if the modification of rumen degradation induced by the xylose treatment affects feed intake and production performance of high-yielding dairy cows.

The objective of this study was to study the effects of replacing maize grain and parts of the soybean meal with WeiPass® on dry matter (DM) intake of a total mixed ration (TMR) and on performance and health indices of lactating dairy cows.

MATERIALS AND METHODS

Design and data collection

The study was conducted with German Holstein cows from the herd of the Lehr- und Versuchsanstalt für Viehhaltung Hofgut Neumühle, Münchweiler/Alsenz, Germany. Thirty-six cows participated in the study and were allotted to one of two treatments according to their body weight, parity and previous lactation performance. Cows were offered a TMR twice a day for *ad libitum* intake. The nutrient composition of the diets was calculated following recommendations of GfE (2001) and the diets were isoenergetic and isonitrogenous. The diets had the same ingredients at an identical inclusion level except the following: The control (CON) diet contained 160 g/kg DM maize grain and 64 g/kg DM soybean meal, whereas the WHEAT diet contained 178 g/kg DM of a xylose-treated wheat grain (WeiPass®; Winowiski et al. 2005) and only 46 g/kg DM soybean meal. The complete substitution of maize grain with WeiPass® was the primary intention of this study whilst the lower inclusion level of soybean meal in the WHEAT diet was necessary to achieve isonitrogenous diets. It as was also assumed that WeiPass® would provide more utilisable crude protein at the duodenum (uCP; Lebzien and Voigt 1999; GfE 2001), a precursor to metabolisable protein, than maize grain. Table 7 provides information on the chemical composition of the TMR ingredients.

All procedures involving dairy cows were conducted according to the regulations of the German animal welfare protection act (Anonymous 2010). The animals were kept in a free-stall barn and feed intake for individual cows – starting 20 days postpartum – was measured daily using the Calan Broadbent feeder door system (American Calan, Northwood, NH, USA). Each visit of a feeder was recorded by a transponder located on a neck collar. The animals were milked twice daily at 05:00 h in the morning and 15:30 h in the afternoon. The

milk yield was automatically recorded and stored in a herd management software (Dairy Planer Version 5.1; Westfalia Surge, Bönen, Germany).

Table 7. Chemical composition of the ingredients of the total mixed rations.

Feedstuff	DM[†]	NEL[‡]	CP[§]	uCP[◊]	CL[*]	CF[$]	ADF[#]	NDF[¶]	Ca[ǁ]	P[&]	St[+]	Su[±]
	[g/kg]	[MJ/kg DM]	[g/kg DM]									
Maize silage	310	7.0	95	142	40	154	218	379	3.2	2.5	358	15
Grass silage	440	5.4	126	120	33	282	364	581	5.5	3.6	0	50
Hay/straw	860	4.5	50	57	25	323	410	646	4.0	2.2	0	60
CM 70:30[°]	880	8.2	121	165	27	57	107	226	0.7	3.9	599	18
Rapeseed meal	900	7.0	390	236	20	130	190	341	6.7	11	0	80
Soybean meal	880	8.6	488	288	15	67	119	242	3.2	6.5	69	108
Maize grain	880	8.4	108	164	45	26	72	177	0.4	2.8	694	19
WeiPass®	900	8.2	148	198	20	30	76	183	0.7	3.9	556	20
Salt	990	-	-	-	-	-	-	-	-	-	-	-
Lime	1000	-	-	-	-	-	-	-	342	-	-	-
Minerals	950	-	-	-	-	-	-	-	60	90	-	-
Urea	100	-	2870	-	-	-	-	-	-	-	-	-

Notes: [†]DM, dry matter; [‡]NEL, Net-energy for lactation; [§]CP, crude protein; [◊]uCP, utilisable crude protein at the duodenum; [*]CL, crude lipid; [$]CF, crude fibre; [#]ADF, acid detergent fibre expressed inclusive of residual ash, estimated as: ADF [g/kg DM] = 1.14 CF [g/kg DM] + 42.2, (r = 0.93) (Kamphues et al. 2004); [¶]NDF, neutral detergent fibre expressed inclusive of residual ash, estimated as: NDF [g/kg DM] = 1.58 CF [g/kg DM] + 135.7, (r = 0.88) (Kamphues et al. 2004); [ǁ]Ca, calcium; [&]P, phosphor; [+]St, starch; [±]Su, sugar; [°]CM cereal grain mix: 700 g/kg barley, 300 g/kg wheat.

The data acquisition for the experimental analysis began 20 days postpartum. This starting point was chosen to avoid possible bias through events around calving. The morning milking was sampled once per week every Monday and served for the determination of selected milk

ingredients. Starting three weeks prepartum, blood samples were collected into tubes to harvest serum from all animals in each group every 7 days during 5 weeks, then at intervals of 14 days, at 12.00 hours at least 5 hours after the morning feeding via coccygeal venepuncture. Every 14 days, starting two weeks prepartum, all cows were weighed and body condition score (BCS) was determined (Edmonson et al. 1989) by the same two individuals. The data acquisition was continued until the cows were 120 days in milk (DIM).

Chemical analyses

All analyses of the chemical composition of the TMR ingredients were done by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Germany. Chemical analyses on feedstuffs were done according to the German Handbook of Agricultural Research and Analytic Methods (VDLUFA, 2004) and method numbers are given. The DM was analyzed by oven-drying at 105°C (3.1). The CP was analyzed by Dumas combustion method (4.1.2), whilst utilisable CP at the duodenum (uCP), which reflects the sum of ruminally synthesized microbial CP and RUP flowing into at the duodenum and thus representing a precursor to metabolizable protein for ruminants, was estimated the near infrared reflectance spectra. Crude fat was analyzed by treating the sample with benzene (method 5.1.1). Crude fibre, sugar and starch were analyzed using methods 6.1.1, 7.1.1 and 7.2.1 respectively. Calcium and phosphorus were analyzed using methods 10.3.1 and 10.6.1.

Analysis of milk composition was conducted at the Landeskontrollverband Rheinland-Pfalz, Thalfang, Germany. Milk fat, protein and urea were determined by an instrumental method employing mid-infrared specific absorption spectroscopy according to the System CombiFoss 6000 (Milkoscan FT 6000 combined with a Fossomatic 5000; Foss Electric, Hillerød, Denmark). The analysis of somatic cells was based on the principle of the Fluoro-Opto-Electronic-Cell-Counting, also using the System CombiFoss 6000.

The blood samples were centrifuged (EBA 12; Hettich, Tuttlingen, Germany) immediately after collection at 3000 · *g* for 10 min at room temperature. The supernatant was stored in 10-ml tubes (Sarstedt, Nümbrecht, Germany) at -18°C until analysis. Aspartate aminotransferase (AST) (kit no. 553-256 G, mti Diagnostics, Idstein, Germany), glutamate dehydrogenase (GLDH) (kit no. 11929992216, Roche Diagnostics, Basel, Switzerland), gamma glutamyltransferase (GGT) (kit no. D95604, DIALAB, Wiener Neudorf, Austria), cholesterin (kit no. 553-124, mti Diagnostics, Idstein, Germany), glucose (kit no. 553-230, mti Diagnostics, Idstein, Germany), non-esterified fatty acids (NEFA) (kit no. 999-75406, Wako

chemicals, Neuss, Germany), beta hydroxybutyrate (BHB) (kit no. RB1008, Randox laboratories, Crumlin, UK), magnesium (kit no. D01243, DIALAB, Wiener Neudorf, Austria) and phosphorus (kit no. D00362, DIALAB, Wiener Neudorf, Austria) were analysed photometrically (ABX Pentra 400, Horiba Medical, Kyoto, Japan). Calcium was analysed colorimetrically (kit no. 553-101, mti Diagnostics, Idstein, Germany) using the Cobas Mira Plus photometric system (Roche, Basel, Switzerland). All analyses were carried out at the Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Germany.

Calculations and statistical analysis

Net energy for lactation (NEL) concentrations of TMR ingredients were estimated according to GfE (1995; 1998):

NEL (MJ) = 0.6 (1 + 0.004 (q – 57)) · ME (MJ), q = ME/GE · 100,

where ME is metabolisable energy and q, the metabolisability of gross energy (GE), is the ratio of ME to GE. The ME values were derived as follows:

Grass silage: ME = + 0.54 + 0.01987 · crude protein + 0.01537 · enzyme soluble organic substance + 0.000706 · crude fat · crude fat – 0.00001262 · enzyme soluble organic substance · crude ash – 0.00003517 · enzyme soluble organic substance · crude protein; other diet ingredients: ME = 14.03 - 0.01386 · crude fibre - 0.01018 · crude ash;

GE (MJ/kg DM) = 0.0239 · crude protein + 0.0398 · crude fat + 0.0201 · crude fibre + 0.0175 · nitrogen free extractives.

The energy corrected milk (ECM) yield was calculated according to GfE (2001):

ECM [kg/d] = Milk yield [kg/d] · (((0.38 · [fat %] + 0.21 · [protein %]) + 1.05) / 3.28).

All data were analysed using the MIXED procedure of SAS (2009) according to the following model:

Y_{ijklm} $\mu + G_i + L_j + C_k + LW_l + (LW_l)^2 + a + e_{ijklm}$

where Y_{ijklm} is the observation for dependent variables, μ the population mean, G_i the group (diet) (i = 1,2), L_j the lactation number (j = 1,2, >3), C_k the month of calving (k = 1-12), LW_l the lactation week (l = 1-17), a the random effect of the animal, and e_{ijklm} represents the residual error. Significance was declared at $p < 0.05$ and trends at $p < 0.10$. Least squares means (LSM) and standard error of the mean (SE) are reported throughout.

RESULTS

The ingredient and chemical compositions of the TMR are presented in Table 8.

Table 8. Ingredient and chemical composition of the total mixed rations.

	CON[†]	WHEAT[‡]
Feedstuffs [g/kg of DM[§]]		
Grass silage	306	306
Maize silage	164	164
Barley:wheat mixture (70:30)	160	160
Grass hay or straw	68	68
Rapeseed meal	64	64
Maize grain	160	
WeiPass®		178
Soybean meal	64	46
Mineral-vitamin mixture	12	12
Urea	2	2
Chemical composition [g/kg of DM unless stated]		
Net-energy for lactation [MJ/kg DM]	6.86	6.82
Crude protein	170	171
Crude fat	33	29
Crude fibre	161	161
ADF[◊]	227	227
NDF[*]	391	391
Starch	256	243
Sugar	40	38

Notes: [†]control group with maize and soybean meal; [‡] group where maize grain and part of the soybean meal were replaced with WeiPass®; [§]DM, dry matter; [◊]ADF, acid detergent fibre expressed inclusive of residual ash, estimated as: ADF [g/kg DM] = 1.14 CF [g/kg DM] + 42.2, (r = 0.93) (Kamphues et al. 2004); [*]NDF, neutral detergent fibre expressed inclusive of residual ash, estimated as: NDF [g/kg DM] = 1.58 CF [g/kg DM] + 135.7, (r = 0.88) (Kamphues et al. 2004).

As intended, the CON and WHEAT diets were isoenergetic and isonitrogenous, and the only difference in chemical composition between the two TMR was that the CON diet contained slightly more starch which was considered to be of little if any biological significance. All observed differences for animal response variables between the diets can thus be assigned to the comparison between the grain types and their nutritional value.

Cows on both diets had the same dry matter intake (Table 9). Similarly, no difference was observed between CON and WHEAT cows for milk yield, body weight and BCS (Table 9). Although the difference of milk yield only approached a trend for being different (p = 0.11) and the concentrations of milk fat and protein were not different, the WHEAT cows yielded more ECM (p = 0.04) and milk protein (p = 0.04) than the CON cows. Milk urea concentrations and somatic cells were not different between the diet treatments.

Table 9. Effects of replacing corn grain and part of the soybean meal with a xylose-treated wheat grain (WeiPass®) in total mixed rations on the performance of dairy cows.

Item	CON†	WHEAT‡	p-Values	SE§
Dry matter intake [kg/d]	18.9	19.3	0.40	0.73
Yield [kg/d]				
Milk	30.3	32.6	0.11	1.45
Energy corrected milk	28.7	32.5	0.04	1.81
Fat	1.25	1.36	0.22	0.08
Protein	1.02	1.11	0.04	0.04
Concentrations				
Fat [%]	4.11	4.16	0.71	0.14
Protein [%]	3.49	3.54	0.63	0.11
Urea [mg/l]	229	227	0.83	9.98
Somatic cells [$\cdot 10^3$/ml]	230	121	0.34	111
Other				
Body weight [kg]	619	626	0.75	21.5
Body condition score	3.17	3.24	0.45	0.09

Notes: †control group with maize and soya; ‡group where maize grain and part of the soybean meal were replaced with WeiPass®; §SE, standard error.

The concentrations of the blood metabolites cholesterin, NEFA, GGT, GLDH, BHB and the minerals calcium, magnesium and phosphorus were not affected by treatment (p > 0.10).

Only the blood glucose concentration was lower (p = 0.02) for cows on the WHEAT diet (Table 10).

Table 10. Effect of replacing corn grain and part of the soybean meal with a xylose-treated wheat grain (WeiPass®) in total mixed rations on blood serum variables of dairy cow.

	CON[†]	WHEAT[‡]	p-Values	SE[§]
GGT[◊] [U/l]	25.5	26.3	0.77	2.73
GLDH[*] [U/l]	15.9	17.9	0.57	3.61
BHB[$] [mmol/l]	0.56	0.62	0.21	0.05
Cholesterin [mmol/l]	3.27	3.33	0.83	0.26
NEFA[#] [µmol/l]	244	224	0.40	23.2
Glucose [mmol/l]	3.80	3.62	0.02	0.08
Calcium [mmol/l]	2.34	2.28	0.13	0.02
Magnesium [mmol/l]	1.05	1.06	0.69	0.02
Phosphate [mmol/l]	1.90	1.93	0.49	0.05

Notes: [†]control group with maize and soya; [‡]group where maize grain and part of the soybean meal were replaced with WeiPass®; [§]SE, standard error; [◊]GGT, γ-glutamyl-transferase; [*]GLDH, glutamate dehydrogenase; [$]BHB, ß-hydroxybutyrate; [#]NEFA, non-esterified fatty acids.

DISCUSSION

In accord with similar ingredient and chemical compositions of the CON and WHEAT diets inclusive of the estimated NEL concentration, the DMI and energy intake were also the same for cows on both diets. Although milk yield was not significantly different between the two diets (p = 0.11) and milk fat and protein concentrations were only marginally different between the two groups of cows, the animals on the WHEAT diet produced considerably more ECM which is the summative result of slight, consistent changes in the aforementioned response variables. Thus, taken together, the results of this study would indicate that more energy and nutrients were available for productive purposes in the WHEAT than the CON cow.

One possible interpretation of the observed difference in ECM yields at the same energy intake is that the xylose-treatment of wheat positively influenced rumen degradation and fermentation of fibre fractions. Although the extent of ruminal starch degradation was greater for WeiPass® than for maize grain (Benninghoff et al. 2015), or, vice versa, the content of

RUS of WeiPass® was less than that of maize grain, the greater amount of ruminally degraded starch that cows consumed with the WHEAT diet did not negatively impact on ruminal fibre degradation but possibly delivered more energy at an even rate. This would ensure a more uniform and stable rumen environment which is typically characterised by less fluctuations of the ruminal pH values, thus promoting activities of cell-wall degrading bacteria. Consequential, this situation would allow for greater roduction of acetate and also microbial CP, the latter resulting in more uCP. These considerations would also support the view that maximising RUS in dairy cow diets is no meaningful objective but optimisation should be sought with the overall goal to best utilise the dairy cow's digestive capacity, which encompasses the rumen and the small and large intesines.

The greater milk protein yield of cows on the WHEAT than the CON diet possibly is due to the combined effect of more uCP (considered above), i.e., more amino acids than can eventually be absorbed from the small intestine, and less gluconeogenesis from amino acids because of the overall improved energy supply to rumen microbes and, hence, the dairy cow.

The xylose treatment of wheat at elevated temperatures has provided evidence that it not only increased RUS and RUP values (Benninghoff et al. 2015) but also improved animal performance (this study). This consistent effect on wheat can be explained by differences among grain species in the distribution of the protein matrix in the endosperm (McAllister et al. 1996), which leads to a better starch protection against microbial attack in the rumen of wheat in comparison to barley. A larger increase in RUS values (g/kg) of wheat than barley in response to the xylose treatment at elevated temperatures was also observed in an *in situ* study in our laboratory (Benninghoff et al. 2015).

Other chemical treatments that were also designed to lower ruminal starch degradation of otherwise rapidly degraded starch sources and improve the performance of ruminants such as the treatment of barley with NaOH, have not provided consistent results (Dehghan-Banadaky et al. 2007). De Campeneere et al. (2006) reported that dairy cows consuming NaOH-treated wheat had a higher yield of fat corrected milk than cows fed rolled wheat. At the same time the ruminal digestibility and the *in vivo* total-tract digestibility of DM, organic matter, starch and CP were decreased. A slightly decreased total-tract digestibility caused by the applied treatment leading to a lowered ruminal degradation of starch and CP with a shift of site of nutrient digestion from the rumen to the small intestine can still lead to improved performance of dairy cows. This pretended contradiction is due to the energetically more efficient use of nutrients which are absorbed from the small intestine causing less energy losses than microbial degradation and fermentation and, subsequently, require less energy expenditure for synthesis

of milk compounds, e.g. lactose (Owens et al. 1986). A shift in site of digestion was also observed when Holstein steers were fed with NaOH- or formaldehyde-treated wheat (Schmidt et al. 2006).

Blood serum variables showed no differences between the CON and WHEAT cows and, compard to reference values (Kraft et al. 1997; Cozzi et al. 2011), no signs of pathological values were observed in either group. Only the glucose concentration was higher in the CON cows. The glucose levels for both groups were within the reference range of glucose for dairy cows (Cozzi et al. 2011), albeit at the upper limit. The slightly lower DMI of the CON group was more than compensated by the greater starch concentration in the CON diet, resulting in a higher starch intake. Matthé et al. (2001) summarized that RUS of wheat which passes into the small intestine is more extensively digested in the small intestine than RUS of maize.

CONCLUSIONS

In conclusion, a xylose-treated wheat grain (WeiPass®) could replace maize grain and part of the soybean meal in a total mixed ration for lactating dairy cows and overall performance might be slightly improved. Thus, xylose-treated wheat grain may be an alternative depending on overall ration composition and availability and costs of grain sources.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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CHAPTER 5

Does *trans*-10, *cis*-12 conjugated linoleic acid affect the intermediary glucose and energy expenditure of dairy cows due to repartitioning of milk component synthesis?

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ABSTRACT

The overall goal of this study was to evaluate if intermediary energy metabolism of cows fed with *trans*-10, *cis*-12 conjugated linoleic acid (CLA) was modified such that milk-energy compounds were produced with less intermediary energy expenditure as compared to control cows. Published data on supplemented CLA were assembled. The extent was calculated to which the *trans*-10, *cis*-12 CLA isomer has an impact on glucose and energy conversion in the mammary gland by modifying glucose equivalent supply and energy required for fatty acid (FA) and fat synthesis, and if this will eventually lead to an improved glucose and energy status of CLA-supplemented high-yielding dairy cows. A possible relationship between CLA supplementation level and milk energy yield response was also studied. Calculations were conducted separately for orally and abomasally administered CLA and based on energy required for supply of glucose equivalents, i.e. lactose, glycerol and $NADPH_2$. Further, modifications of milk FA profile due to CLA supplementation were considered when energy expenditures for FA and fat synthesis were quantified. Differences in yields between control and CLA groups were transformed into glucose energy equivalents. Only abomasal infusion (r^2 = 0·31) but not oral CLA administration (r^2 = 0·11) supplementation to dairy cow diets resulted in less glucose equivalent energy. Modifications of milk FA profiles also saved energy but the relationship with CLA supplementation was weaker for abomasal infusion (r^2 = 0·06) than oral administration (r^2 = 0·38). On average, 10 g/d of abomasally infused *trans*-10, *cis*-12 CLA saved 1·1 to 2·3 MJ net energy expressed as glucose equivalents, whereas both positive and negative values were observed when the *trans*-10, *cis*-12 CLA was fed to the cows.

Keywords: CLA; energy metabolism; glucose turnover; dairy cow.

IMPLICATIONS

This study revealed a weak to moderate dose-dependent relationship between the amount of *trans*-10, *cis*-12 CLA administered and the amount of energy in glucose equivalents and energy for the synthesis of milk fat conserved from milk ingredient synthesis. Because abomasal infusion of the *trans*-10, *cis*-12 CLA more consistently conserved energy in glucose equivalents compared with oral CLA intake, rumen protection of the fed CLA products appears incomplete. Milk fat synthesis showed an energy saving with a weak dose-dependent relationship when CLA was supplemented orally or by abomasal infusion.

INTRODUCTION

Lactation studies on dairy cows have shown that the conjugated linoleic acid (CLA) isomer *trans*-10, *cis*-12 suppresses milk fat synthesis in the mammary gland and lowers milk fat secretion (Gervais et al. 2005; Kay et al. 2007). This isomer acts on de novo synthesis of fatty acids (FA), as well as on the uptake of preformed FA by the mammary gland (Baumgard et al. 2001), thus impacting the pool of precursors involved in fat metabolism and the respective gene expression (Hussein et al. 2013). Although the dairy cow has sufficient energy stored in adipose tissue to overcome a possible energy shortage around parturition, mobilisation and utilisation of this energy is limited by the size of the metabolic glucose pool. Insulin sensitivity was affected by CLA without corresponding changes in tissue variables (Saremi et al. 2014). Moreover, CLA reduced the endogenous glucose production in early lactation, suggesting a glucose sparing effect (Hötger et al. 2013). It is not known, however, if the impact of CLA on milk fat precursors like glucose is strong enough to affect other metabolic pathways, e.g., related to sustaining fertility or health, and if so, how the magnitude of this effect will be.

A positive influence of *trans*-10, *cis*-12 CLA on the energy balance of early lactation dairy cows was reported recently by Liermann et al. (2008). This effect was due to a reduced energy output as a result of a reduced milk fat production and constant milk yields. Others, however, have reported that CLA supplementation had no significant effects on milk yield, milk energy output (calculated from yields of fat, protein and lactose) and energy balance (Bernal-Santos et al. 2003; Moallem et al. 2010; Metzger-Petersen 2013). In all these cases there was a tendency ($P < 0{\cdot}13$, $P < 0{\cdot}001$, and $P < 0{\cdot}08$, respectively) for higher milk yield and higher lactose yield ($P < 0{\cdot}16$, $P < 0{\cdot}20$, and $P < 0{\cdot}14$). Although milk fat content was reduced significantly in the study of Bernal-Santos et al. (2003), slight increases in milk yield and thus, protein and lactose yields compensated for the energy savings from CLA supplementation. Only large quantities (18·35 g/d) of *trans*-10, *cis*-12 CLA reduced milk energy output significantly (Castañeda-Gutiérrez et al. 2005). This increase in milk – and lactose – yield is surprising, since transition dairy cows are notoriously limited in glucose supply.

Milk synthesis requires energy in the form of glucose equivalents, for lactose, glycerol and $NADPH_2$ syntheses. The latter two are required for fat and FA synthesis. Because energetically, fat is the most expensive milk component to synthesize (> 50% of total milk yield; Bauman & Davis 1974), reducing its production and altering its composition can

improve the energy and glucose status of dairy cows. Alterations in milk fat composition may result in a reduced proportion of de novo synthesised FA in milk triglycerides. Due to a higher percentage of preformed, i.e. long-chain, FA that have higher molecular weights, the same amount of milk fat requires less moles of FA, glycerol and $NADPH_2$ which would result in less energy expenditure for milk fat synthesis. As many authors have reported that CLA supplementation increased milk yield with a concomitantly greater lactose production, it should be quantified whether the energy- and glucose-sparing or consuming effect of CLA supplementation prevails. According to the meta-analysis of De Veth et al. (2009) cows on CLA respond with better fertility e.g. reduced days open. This is difficult to explain, however, if CLA would cause an extra challenge to the glucose pool due to the reported higher milk yield.

The overall objective of this study was to evaluate and quantify the modifications of milk and milk component yields in terms of glucose equivalents and in milk fat synthesis based on quantitative dry matter intake (DMI) data that have been found when *trans*-10, *cis*-12 CLA were either orally or abomasally administered to dairy cows. For this purpose available literature data was analysed. Variables considered were milk component yields and milk FA pattern, glucose equivalents, DMI, and amount and route of *trans*-10, *cis*-12 CLA administration.

MATERIALS AND METHODS

Literature data

Studies published between 1999 and 2014 (Tables 11 and 12) were considered to evaluate the effects of *trans*-10, *cis*-12 CLA supplementation on amount of energy recovered in glucose equivalents and energy required for FA and fat synthesis. The *trans*-10, *cis*-12 CLA supplementation ranged from 1·6 to 34·5 g/(d x cow) and CLA was given either by supplementation of the diet, i.e. orally, or by abomasal infusion. Because of presumed differences in transfer efficiency into the mammary gland of *trans*-10, *cis*-12 CLA between dietary intake and abomasal infusion, data of studies with oral intake and abomasal infusion were evaluated separately. Calculations of glucose equivalents required that milk lactose concentrations were reported. Although milk lactose content does not very much, considering its content as being constant would likely have an impact on glucose equivalent estimates given the large amounts of lactose that are synthesised in the mammary gland of lactating dairy cows. The studies varied with respect to duration of supplementation of the CLA

isomer, length of period to study the milk (fat) yield and FA composition and stage of lactation of the cows during CLA supplementation.

Other studies were also considered but not included in the evaluation because they did not satisfy one or more of the criteria:

- Bell & Kennelly (2003), Hutchinson et al. (2012), Moallem et al. (2010) and Piperova et al. (2004) reported milk FA concentrations only for FA of between 14 and 18, 12 and 22, 12 and 20, and 14 and 20 carbon atoms, respectively;

- Loor & Herbein (2003) only infused CLA for 48 h and collected milk samples directly after infusion had stopped;

- Viswanadha et al. (2003) applied CLA intravenously;

- Moore et al. (2005) investigated the effects of CLA on heat-stressed cows;

- Hun et al. (2012) did not report the DMI of the animals and Pappritz et al. (2011) reported pooled milk FA concentrations and DMI which was not recorded simultaneously with milk samples for FA analyses such that milk FA compositon could not be related to DMI.

- Hötger et al. (2013) did not report the total milk fat yield of the animals;

- Huang et al. (2008) did not report how much trans-10, cis-12 CLA was administered to the cows; and

- Medeiros et al. (2010) used crossbred Holstein x Zebu cows, Shingfield et al. (2009) used Finnish Ayrshire cows and Sigl et al. (2010) used Brown Swiss cows instead of Holstein cows and thus, dietary effects on milk component synthesis might be overlaid or masked by inter-breed differences (Stoop et al. 2008).

Table 11. Literature compilation of trials with oral supplementation of trans-10, cis-12 conjugated linoleic acid (CLA)

trans-10, *cis*-12 CLA [g/d]	Type of CLA	Sample size	Period of CLA supplementation	Analysing point of milk fatty acids	Milk components considered	Feeds used	Author
8·8	Ca salt	30	14 d ap§ - 140 d pp¶	week 1, 2, 4, 6, 8, 12, 16, 20 pp	fat, protein, lactose	TMR††, maize silage, grass-legume silage, ground shelled maize, lucerne hay	Bernal-Santos et al. (2003)
9·2 18·3	Ca salt	48	21 d ap - 63 d pp	week 6 pp	fat, protein, lactose	TMR, maize silage, legume silage, high moisture corn	Castañeda-Gutiérrez et al. (2005)
2·4 7·1	Lipid-encapsulated CLA	45	20 d pp - 56 d pp ± 1 d	week 4 and 5of treatment	fat, protein, lactose	TMR, maize silage, lucerne silage, maize meal	Castañeda-Gutiérrez et al. (2007)
10·0 10·0	Ca salt FP-CLA†	3	202 d pp ± 6 d 3 x 3 Latin square; 7 d treatment + 8 d washout	day 6 and 7 of treatment	fat, protein	TMR, chopped lucerne hay, cracked maize	de Veth et al. (2005)
12·0 12·0	Lipid-encapsulated CLA	48	112 d pp ± 5 d 2 x 2 crossover design; 16-d periods	day 12 -16 of treatment	fat, protein, lactose	TMR, maize silage, lucerne haylage, high-moisture shelled maize, soybean meal	de Veth et al. (2006)
4·3 8·6 17·3 34·5	Ca salt	5	93 d pp ± 8 d 5 x 5 Latin square; 5 d treatment + 9 d washout	day 5 d of treatment	fat, protein, lactose	TMR, lucerne hay, steam rolled maize, Barley	Giesy et al. (2002)
6·9	Ca salt	72	1 d pp - 60 d pp	day 28 of treatment	fat, protein, lactose	TMR, grass silage, soja hulls	Hutchinson et al. (2011)

trans-10, *cis*-12 CLA [g/d]	Type of CLA	Sample size	Period of CLA supplementation	Analysing point of milk fatty acids	Milk components considered	Feeds used	Author
3·6 3·6	Lipid-encapsulated CLA	53	1 d pp - 98 d pp	every week	fat, protein, lactose	PMR, maize silage, grass silage, grass hay	Liermann et al. (2008)
4·5	Lipid-encapsulated CLA	14	6 d pp - 126 d pp	day 84 pp	fat, protein, lactose	PMR, grass silage, maize silage, soybean meal	Metzger-Petersen (2013)
4·9 9·9 14·8	Ca salt	19	10 d ap - 21 d pp	1, 7, 15 and 21 d pp	fat, protein, lactose	TMR, lucerne hay, steam-flaked maize	Moore et al. (2004)
9·8 29·3	Lipid-encapsulated CLA	31	9 d ap ± 6 d - 40 d pp; 9 d ap ± 6 d - 10 d pp – 40 d pp	2, 8, and 20 d pp	fat, protein, lactose	TMR, lucerne hay, steam flaked maize	Odens et al. (2007)
8·8	Ca salt	30	227 d pp until dry off	2, 4, 8, 12, 16 and 20 week of treatment	fat, protein, lactose	TMR, maize silage, ground shelled maize, hay crop silage	Perfield et al. (2002)
11·4 12·7	Amid-CLA; Lipid-encapsulated CLA	3	78 d pp ± 13 d 3 x 3 Latin square; 7 d treatment + 7 d washout	day 6 and 7 of treatment	fat, protein	TMR, lucerne hay, cracked maize	Perfield et al. (2004a, b)
9·2 9·2	Ca salt	64	1 d pp - 31 d pp	day 21 pp	fat, protein, lactose	PMR, maize silage, grass silage, wheat, dried sugar beet pulp	Petzold (2014)
2·0 4·0 8·0 16·0	Coated with hydrogenated vegetable fats	5	70 d pp ± 4 d 5 x 5 Latin square; 21-d periods	last 5 d of treatment	fat, protein	TMR, cassava chip, chopped rice straw, soybean meal	Piamphon et al. (2009)

trans-10, *cis*-12 CLA [g/d]	Type of CLA	Sample size	Period of CLA supplementation	Analysing point of milk fatty acids	Milk components considered	Feeds used	Author
11·9‡	Ca salt	38	28 d ap - 49 d pp	2, 4, and 7 weeks pp	fat, protein	TMR, maize silage, maize meal, soybean meal	Selberg et al. (2004)
5·3 10·5 15·8	Ca salt	64	1 d pp - 91 d pp	6 and 12 weeks pp	fat, protein, lactose	PMR maize silage, grass silage, lucerne hay, soybean meal	van Straalen (2004)
6·0 6·0	Ca salt	20	1 d pp - 42 d pp 43 d pp - 105 d pp	every week every week	fat, protein, lactose	PMR, maize silage, grass silage, wheat, barley	Soosten et al. (2011) & Kramer et al. (2013)

† FP-CLA = formaldehyde-protected CLA, intraruminal infusion
‡ weighted average
§ ap = ante partum
¶ pp = post partum
†† TMR = total mixed ration
‡‡PMR = partial mixed ration

Table 12. Literature compilation of trials with abomasal infusion oral of trans-10, cis-12 conjugated linoleic acid (CLA)

trans-10, *cis*-12 CLA [g/d]	Day of lactation	Period of CLA supplementation	Analysing point of milk fat	Design	Milk components considered	Feeds used	Author
10·3	111 ± 12	4 d + 7 d washout	3^{rd} + 4^{th} d of infusion	3 x 3 Latin square *n* = 3	fat, protein	TMR‡, chopped lucerne hay; cracked maize	Baumgard et al. (2000)
3·5 7·0 14·0	228 ± 54	5 d + 7 to 8 d washout	5^{th} d of infusion	4 x 4 Latin square *n* = 4	fat, protein	TMR, chopped lucerne hay, cracked shelled maize	Baumgard et al. (2001)
13·6	286 ± 54	5 d + 14 d washout	$3,5^{th}$ + $5,5^{th}$ d of infusion	2 x 2 crossover *n* = 4	fat, protein	TMR, chopped lucerne hay, cracked shelled maize	Baumgard et al. (2002)
10·8 19·9 31·1	258 ± 43	5 d + 4 d washout	4^{th} + 5^{th} d of infusion	4 x 4 Latin square *n* = 4	fat, protein	TMR, chopped lucerne hay, cracked maize	Chouinard et al. (1999)
4·2† 4·2†	168 ± 49	5 d + 7 d washout	5^{th} d of infusion	3 x 3 Latin square *n* = 3	fat, protein	TMR, chopped lucerne hay, ground maize	de Veth et al. (2004)
10·0	195 ± 16	5 d + 23 d washout	4^{th} + 5^{th} d of infusion	2 x 2 crossover *n* =4	fat, Protein, lactose	TMR, timothy silage, corn silage, rolled barley	Gervais et al. (2009)
9·0 9·0	204 ± 7	10 d + 10 d washout	9^{th} + 10^{th} d of infusion	2 period crossover *n* = 12	fat, protein, lactose	Pasture fed, ad libitum or restricted	Kay et al. (2007)
3·7 7·4 14·8	80 ± 2	4 d + 7 d washout	4^{th} d of infusion	4 x 4 Latin square *n* = 4	fat, protein, lactose	Cut ryegrass / white clover pasture ad libitum	Mackle et al. (2003)

trans-10, *cis*-12 CLA [g/d]	Day of lactation	Period of CLA supplementation	Analysing point of milk fat	Design	Milk components considered	Feeds used	Author
1·9	207 ± 65	14 d + 14 d washout	14th d of infusion	4 x 4 Latin square *n* = 4	fat, protein, lactose	TMR, orchard grass hay, maize pellet	Maxin et al. (2010)
1·6 1·6	72 ± 18	11 d + 7 d washout	last 5 d of infusion	6 x 6 Latin square *n* = 6	fat, protein, lactose	TMR, maize silage	Maxin et al. (2011)
4·0	141 ± 8	5 d + 7 d washout	5th d of infusion	4 x 4 Latin square	fat, protein	TMR, lucerne hay, maize meal	Perfield et al. (2004a, b)
5·0	168 ± 80	4 d + 7 d washout	4th d of infusion	3 x 3 Latin square *n* = 3	fat, protein	TMR, lucerne hay, cracked maize	Perfield et al. (2006)
5·0	149 ± 18	5 d + 7 d washout	4th + 5th d of infusion	4 x 4 Latin square *n* = 4	fat, protein, lactose	TMR, lucerne hay, maize meal	Perfield et al. (2007)
10·0 10·0	128 ± 23	14 d + 7 d washout	12th - 14th d of infusion	4 x 4 Latin square *n* = 4	fat, protein	TMR, maize silage, maize grain, soybean meal	Vyas et al. (2013)

† CLA supplemented as free fatty acid and methyl ester, respectively
‡ TMR = total mixed ration

Calculations

In order to evaluate influences of *trans*-10, *cis*-12 CLA supplementation on energy required for supply of glucose equivalents, i.e. lactose, glycerol and $NADPH_2$, and for FA and fat syntheses, a model was constructed that considered the alterations of *trans*-10, *cis*-12 CLA on amounts of milk FA (C5 to C15 and 60% of C16). Energy requirements for body weight (BW) changes were not considered, because Gruber et al. (2008) have shown that the energy expenditure for retention and mobilization of fat and protein cannot be reliably estimated from BW changes. Moreover, the energy supply was based on the respective average group DMI to take account of differences of glucose supply between diets. Subsequently, these values were used for all further calculations related to the oral intake or abomasal infusion of *trans*-10, *cis*-12 CLA. Energy requirements for synthesis of milk components were integrated into the model as follows:

Lactose

The required energy input for the synthesis of lactose was calculated according to Bergner & Hoffmann (1996, p. 159). The efficiency of conversion of glucose into lactose was assumed to be 97·7%.

Glycerol

The energy requirement for glycerol synthesis was calculated according to Schauff et al. (1992) based on 3 moles of FA per mol of glycerol. Before calculating the amount of glycerol, the mass of two hydrogen moles and one oxygen mole was subtracted from each single FA in order to adjust for removal of H_2O during esterification. Further, the energy required for the esterification with glycerol to yield triacylglycerols (or triglycerides) of the sum of FA was considered. The energy consumption for milk ingredient synthesis was calculated considering ATP yield from glucose and the pathway of converting glucose into glycerol (Bergner & Hoffmann, 1996, p. 135).

$NADPH_2$

The energy cost of supply of the coenzyme $NADPH_2$ for FA synthesis was assumed to be 3 moles of ATP per mol of $NADPH_2$.

Fatty acids

Energy requirement for FA synthesis was calculated based on milk fat yield and milk FA composition. Since FA of milk triglycerides originate from de novo synthesis as well as from preformed FA, milk FA were separated into two classes, i.e. preformed or synthesized de novo. The FA with a chain length greater than 16 C atoms and 40% of the C16 FA (Waghorn & Baldwin, 1984) were classified as preformed FA originating from dietary sources or body reserve mobilization (Bernard *et al.* 2006) and FA with a chain length between C5 and C15 and 60% of C16 (Waghorn & Baldwin, 1984) were assumed to be synthesised de novo. Butyrate was assumed to be available from rumen microbial carbohydrate fermentation and not synthesised de novo. The sources for de novo FA synthesis were acetate (85·4%) and β-hydroxybutyrate (14·6%; Waghorn & Baldwin, 1984).

Finally, calculations were performed after Bergner & Hoffmann (1996, p. 128). The energy required for de novo synthesis of FA was calculated for each FA separately and the result was then translated into the daily FA yield that was calculated from milk fat yield and milk FA composition. Bergner & Hoffmann (1996, p.124) provide details of the energy input of the different steps of the reactions occurring during de novo FA synthesis The assumption was that 3 ATP are generated from one $NADH_2$. Energy for glycerol synthesis was calculated as above.

Statistical analysis

Statistical analysis was performed applying a linear non-weighted regression analysis (SAS, 2004):

$Y = a + b \cdot x$

where Y = response variable, a = change in energy amount (MJ net energy for lactation [NEL] from glucose equivalents as outlined above, i.e. corrected for differences in DMI), b = slope of the line to predict Y, and x = amount of CLA supplemented orally or by abomasal infusion.

RESULTS AND DISCUSSION

This study evaluated the calculated energy expenditure by dairy cows supplemented with *trans*-10, *cis*-12 CLA in a number of studies (Tables 11 and 12). The publications generally

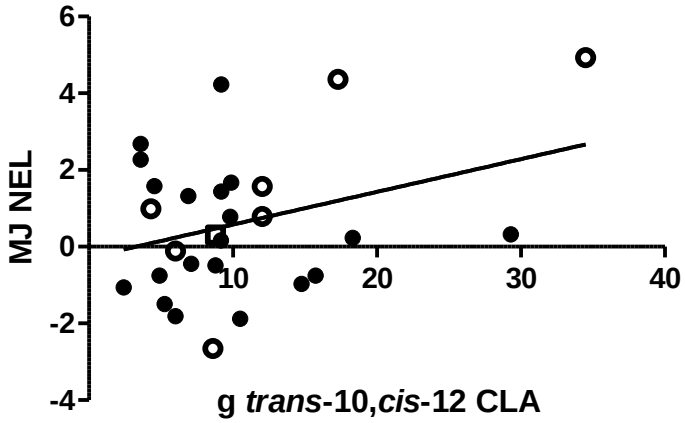
provided detailed information about milk ingredient concentrations and yield and – typically based on a much lower number of observations within experiment – an overview over the FA concentrations in milk fat. Although *trans*-10, *cis*-12 CLA was supplemented via two different routes, for both routes a dose-dependent effect was observed when amount of supplemented *trans*-10, *cis*-12 CLA increased (Figures 1 and 2). Energy savings in the form of glucose equivalents (Figure 1) responded more pronounced when CLA was abomasally infused than when it was fed to the cows. The opposite observation was made regarding the energy supply for milk fat synthesis (Figure 2), which had a greater increase following oral supplementation.

The differences in the strength of the response between oral and abomasal supplementation of the CLA isomer and the greater variability observed with the oral supplementation (Figure 1) indicate that fed CLA products were affected by ruminal events. Obviously, the protection of CLA products against rumen microbial degradation was less than 100%. Another reason for a more variable response when CLA were fed might be that cows in those studies covered a wider range of lactation stages than abomasally infused cows. Cows in early lactation may undergo severe metabolic stress, and are thus less susceptible to CLA. Bernal-Santos et al. (2003) suggested that, at the onset of lactation, the essential cellular signalling systems are weakened, such that *trans*-10, *cis*-12 CLA may be unable to provoke the coordinated reduction in the expression of genes for key lipogenic enzymes. However, Moore et al. (2004) reported a dose-dependent reduction in milk fat concentration starting already at the onset of lactation when cows were fed *trans*-10, *cis*-12 CLA during the transition period until the third week of lactation, which was also the design in the study of Bernal-Santos et al. (2003). It is not known why cows responded so differently in these two experiments.

General differences in the design of the experiments were also related to the length of the CLA supplementation period. Orally supplemented cows were fed the CLA products over several weeks, whereas, the infusion studies never lasted longer than 14 days. In all infusion studies, effects of *trans*-10, *cis*-12 CLA were observed on milk fat concentration and milk FA composition. The effect on milk yield, however, was inconsistent.

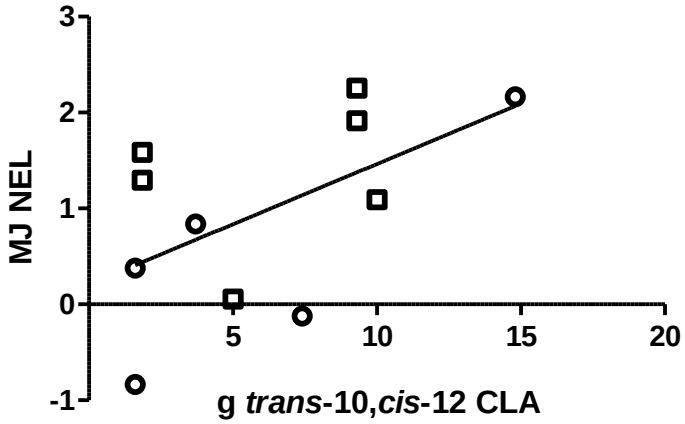
Figure 1. Energy supply for milk component synthesis from glucose equivalents (lactose, glycerol, $NADPH_2$ for synthesis of C5 to C15 fatty acids and 60% of C16) taking into account DM intake as the factor governing overall glucose supply. Symbols display the difference (MJ net energy for lactation [NEL]/d) between control and *trans*-10, *cis*-12 CLA supplemented groups (g/d); Panel (**a**) oral CLA supplementation; (**b**) abomasal CLA infusion. The range of differences (-2·66 to 4·93 MJ NEL) is equivalent to -170 to 314 g of glucose per day. Different symbols indicate time of start of the experiment: filled circles, between 21 d ante partum to 41 d post partum; empty circle, 42 to 120 d post partum; square, 149 to 227 d post partum.

a



[y = -0·2766 (SE = 0·6049; p = 0·6513) + 0·0853 (SE = 0·0476; p = 0·0849) x; r^2 = 0·1098]

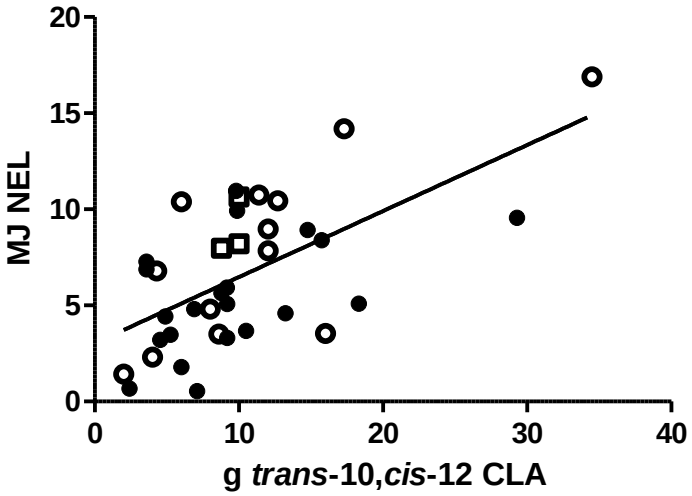
b



[y = 0·2075 (SE = 0·4642; p = 0·6655) + 0·1256 (SE = 0·0630; p = 0·0772) x; r^2 = 0·3065]

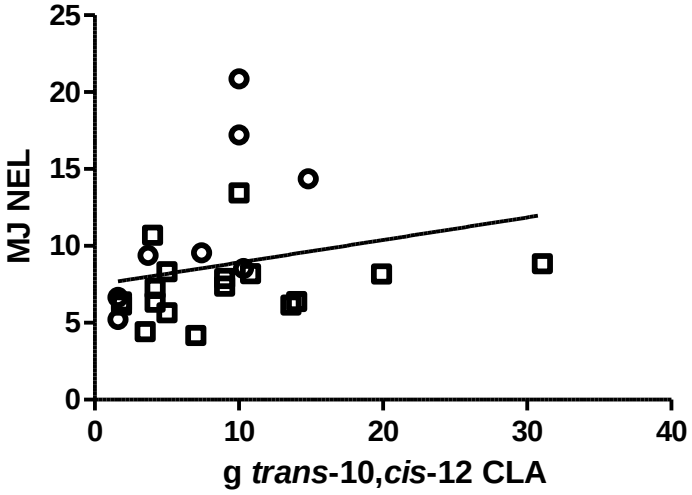
Figure 2. Energy supply (MJ net energy for lactation [NEL]/d) for milk fat synthesis (C5 to C15 fatty acids and 60% of C16) taking into account DM intake as related to supplemental *trans*-10, *cis*-12 CLA (g/d); (**a**) oral CLA supplementation; (**b**) abomasal CLA infusion. Different symbols indicate time of start of the experiment: filled circles, 21 d ante partum to 41 d post partum; empty circle, 42 to 112 d post partum; square, 141 to 286 d post partum.

a



[y = 3·0338 (SE = 0·9092; p = 0·0020) + 0·3437 (SE = 0·0744; p = <·0001) x; r^2 = 0·3787]

b



[y = 7·4553 (SE = 1·2952; p = <·0001) + 0·1466 (SE = 0·1205; p = 0·2362) x; r^2 = 0·0604]

In most studies no effect occurred, yet Mackle et al. (2003) found significant milk yield increases at high CLA supplementation levels but Chouinard et al. (1999) reported lowered milk yields in response to *trans*-10, *cis*-12 CLA supplementation. While infused cows generally were in a positive energy balance, cows from most oral supplementation studies were in the transition period or in early lactation and underwent the typical negative energy balance with the deficiency in glucose. Whilst Harvatine et al. (2009) found a decrease in feed intake for mid-lactation cows with CLA, DM intake by cows during transition and early lactation did not respond to CLA supplementation or showed a slight positive responses (Bernal-Santos et al. 2003; Castañeda-Gutiérrez et al. 2005; Odens et al. 2007; Metzger-Petersen 2013). Milk protein variables were not changed when CLA was supplemented. In most long-term feeding studies the response of milk protein variables was the same as in short-term studies. Only Bernal-Santos et al. (2003) and Metzger-Petersen (2013), who followed the milk and milk component yields far beyond early lactation, observed that average milk yields were elevated by 5·9% and 10·6%, respectively. This would indicate that modified energy expenditure may not be detected during short periods of CLA supplementation and milk energy output and the energy status of the cow may thus be misjudged. This was confirmed in studies of Pappritz et al. (2011), van Soosten et al. (2012) and Hötgers et al. (2013). These authors suggest that CLA leads to an improved energy efficiency.

If less energy is directed to the udder when *trans*-10, *cis*-12 CLA is supplemented, the question is to what extent can the conserved energy be used for other purposes in other organs? Bernal-Santos et al. (2003) observed a trend towards greater ovulation rates and Castañeda-Gutièrrez et al. (2007) found increases in IGF-1 in cows supplemented with 7·1 g *trans*-10, *cis*-12 CLA and a trend was observed for greater values of progesterone during the early luteal phase and of the estradiol to progesterone ratio in follicular fluid. This might affect reproduction through improved ovarian follicular steroidogenesis and increased circulating concentrations of IGF-1. This earlier post partum recovery of IGF-1 with 5 and 10 g/d of *trans*-10, *cis*-12 was also reported by Onnen-Lübben (2009). Castañeda-Gutièrrez et al. (2007) suggested that these benefits to reproductive performance seem to be associated with the *trans*-10, *cis*-12 isomer itself and are independent from energy balance, however it cannot be excluded that the observed improvements in fertility are based on an energy/glucose effect during the transition period.

With the exception of Odens et al. (2007) and Hötgers et al. (2013) plasma glucose concentrations did not respond when more glucose was available to extra-mammary

syntheses. However, substrate and thus also glucose supply to tissues is not only a function of plasma concentration but also of blood flow, which can vary (Davis & Collier, 1985). So in spite of the lack in glucose concentration response, glucose might flow to sites where fertility and health are concerned.

CONCLUSIONS

The literature evaluation revealed a weak to moderate dose-dependent relationship between the amount of CLA administered and the amount of energy in glucose equivalents and energy for the synthesis of milk fat conserved from milk ingredient synthesis. Abomasal infusion of the *trans*-10, *cis*-12 CLA more consistently conserved energy in glucose equivalents which indicates an incomplete rumen protection of the fed CLA products. Milk fat synthesis showed an energy saving with a moderate dose-dependent relationship when CLA was supplemented orally.

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CHAPTER 6

General conclusions

The main focus of this thesis was to study the energy and, more specifically, the glucose supply of dairy cows. Several approaches have been used to relieve the metabolic challenge of high yielding cows during the first third of lactation. Starch sources with varying proportions of RUS are widely used and represent an important component for the supply with glucose via ration ingredients that can be directly absorbed from the small intestine (Chapters 3 and 4). An alternative approach was used in Chapter 5, i.e., reducing of the animals demand for glucose with a supplement, namely *trans*-10, *cis*-12 CLA.

Due to the increased demand for nutrients postpartum and the limited DMI it is generally accepted that RUS can contribute significantly to the glucose supply. The treated wheat (WeiPass®) showed lower ruminal starch degradation than untreated wheat but not as low as maize grain (Chapter 3).

Heat processing is used commercially to reduce the degradation of protein supplements by ruminal microorganisms. This reduction occurs due to Maillard reactions between sugar residues and amino acids (Van Soest, 1982). Factors regulating the rate of the Maillard reaction include type and concentration of reducing sugar (Spark, 1969; Hashiba, 1982; Can and Yilmaz, 2002) and temperature and duration of heating time (Cleale et al., 1987; Can and Yilmaz, 2002). I can be thus assumed that the ration between reactive sugar and amino acid moieties determine the degree of protection of both starch and protein against microbial degradation in the rumen.

The wheat protein matrix was of a size obviously sufficient to pervade the starch structures and by this causing a reduction of the rate and extent of ruminal starch degradation. In barley and rye the same treatment also protected protein and starch from ruminal degradation but to a lesser extent. In the feeding trial on high yielding dairy cows with WeiPass® as a substitute for maize grain and parts of the soybean meal, this data was confirmed (Chapter 4). The treatment of rapidly fermentable starch sources such as wheat may therefore lead to a higher flow of starch to the small intestine and glucose absorption from this site than starch sources that are slowly and incompletely degraded in the rumen such as maize. These results further indicate that a shift of starch digestion to the small

intestine can be achieved without negative effects on total tract starch digestion. One metabolic response of the increased amount of absorbed glucose induced by RUS may be a reduced consumption of glucogenic amino acids (Nocek and Tamminga, 1991), which would also explain that plasma glucose concentrations were not different between the WeiPass® and maize grain diet (Chapter 4).

Another way to impact on the intermediary glucose consumption of dairy cows in early lactation is supplementing rations with the CLA isomer *trans*-10, *cis*-12. The moderate relationship between administered dose of *trans*-10, *cis*-12 CLA and energy expressed as glucose equivalents and energy required for the synthesis of milk fat which was condensed from published studies helps to clarify the often inconsistent results in regard to the energy balance when CLA was fed to dairy cows (Chapter 5). Apart from the different methods of calculating net energy balance, which complicate comparisons across experiments, this study illustrates another problem with interpretation of data. Information concerning the origin of the milk components from either absorbed dietary nutrients mobilization of body reserves or *de novo* synthesis cannot be directly derived from balance data, quantitative comparisons across studies are complicated if not impossible. The two studies reported in Chapters 4 and 5 of this thesis helped to elucidate how glucose supply of dairy cows can be optimized, taking into account the glucose needs of ruminants for different metabolic purposes. WeiPass® turned out to be an effective tool in the optimization of rations for dairy cows. On the other hand, although *trans*-10, *cis*-12 CLA had an influence on intermediary glucose consumption, the observed correlations were only moderate to weak. It has to be considered for discussion and interpretation of the results the available database was small. Several studies could not be included in the database due to missing data regarding DMI or the amounts of the supplemented *trans*-10, *cis*-12 CLA. If the limited size of valuable data is accepted, the following quantitative deduction can be made. When 10 g/d of *trans*-10, *cis*-12 CLA are infused abomasally, an equivalent of 1,100 to 2,300 kJ net energy expressed as glucose equivalents can be saved (Chapter 5) which corresponds to 0.39 to 0.81 moles glucose, equaling 70 to 147 g/d of glucose. Based on a requirement of 3,800 g/d of glucose for a milk yield of 50 kg (based on Matthé et al., 2000) a saving of 1.8 to 3.9% glucose can be achieved.

WeiPass® inclusion at the expense of maize grain and part of the soybean meal increased milk yield by 3.8 kg ECM (Chapter 4). Assuming a typical lactose concentration of 4.8% this would correspond to an increase in lactose yield of 182.4 g/d. Based on an efficiency of lactose synthesis from glucose of 97.7%, 201 g more glucose were produced without

considering protein synthesis. Similar body weights and BCS for the two groups indicate that WeiPass® improved glucose supply and that this improvement was more pronounced than what can be accomplished with *trans*-10, *cis*-12 CLA. In addition to the effects on milk yield and, finally, lactation performance, both feeding strategies can have a positive impact on fertility. Rations rich in starch have had an effect on plasma insulin concentration and thus on the ovarian function, regardless whether starch was fermented in the rumen or digested in the small intestine (Garnsworthy et al., 2009). The feeding of the *trans*-10, *cis*-12 CLA isomer showed a trend towards greater ovulation rates (Bernal-Santos et al., 2003) and a trend for increased progesterone values (Castañeda-Gutièrrez et al., 2007).

The *in vitro* method applied in Chapter 3 is an appropriate way for evaluating the xylose treatment of different starch sources. In contrast to the *in situ* data the cumulative gas production responded to the treatment not only for wheat and barley but also for rye (Table 13). Whether this can simply be explained by the increase of RUP which would reduce gas volume is questionable. Due to the time needed for microbial colonization of feeds in the bags in *in situ* methods, *in vitro* values generally indicate a more extensive ruminal degradation (Nocek, 1988; Foster et al., 2007). This causes differences between methods even at low effective degradation rates. The *in situ* degradation of slowly degradable feedstuffs underestimates the ruminal degradation and fermentation of starch (Offner and Sauvant, 2004; Hindle et al., 2005) such as maize or, in our study, WeiPass®. However, the gas formation has increased with prolonged time of incubation for RoPass and GePass. In the study of Di Marco et al. (2009) both *in situ* and *in vitro* values (method described in Holden, 1999) for different sorghum types overestimated the *in vivo* DM digestibility. A 24-h *in vitro* incubation nicely reflected the *in vivo* data. In our study, gas production at 24 h of incubation (Table 13) still showed a difference between wheat and WeiPass® with lower values for the treated grain, and this continued until the end of incubation period. This would indicate a more consistent and long-lasting protection against ruminal degradation of wheat grain caused by the xylose treatment at elevated temperatures compared with other cereal grains.

Application of 1% formaldehyde to maize and wheat resulted in a reduction of 8 - 16% of *in situ* starch degradation and a reduced *in vitro* gas production. When 5% formaldehyde were added, the *in situ* degradation of starch was lowered by 12 - 34% and stopped gas formation after 2 h (Michalet-Doreau et al., 1997). The *in vitro* data indicated a more pronounced effect on ruminal fermentation than the *in situ* data. Di Marco et al. (2009) concluded that an evaluation at fixed incubation times causes deviations between *in vitro* and

in vivo results which however can be easily overcome by applying multiple or continuous (automatic) readings of the produced gas volume. The cumulative gas production method can therefore be used for developing a detailed picture of ruminal nutrient degradation and can therefore support the more costly and labour-intensive *in situ* methods.

Table 13. Mean values (n = 3) of cumulative gas (ml) produced at different times of incubation for individual feedstuffs

	Incubation time											
Feedstuff	2 h	4 h	6 h	8 h	10 h	12 h	15 h	19 h	24 h	30 h	36 h	48 h
Wheat	29.7	61.9	105	157	197	224	241	258	274	289	299	310
WeiPass®	17.9	41.6	69	100	131	157	182	202	219	235	245	256
Barley	23.8	52.4	85	128	168	198	214	233	250	265	275	286
GePass	20.1	48.9	79	117	153	182	209	229	245	259	269	280
Rye	27.0	66.7	121	156	192	216	235	252	266	280	289	300
RoPass	18.8	45.2	75	107	138	166	198	226	250	267	278	290
SEM	4.1	7.3	13.0	19.8	18.0	17.4	16.4	15.3	14.6	14.3	14.3	14.7

SEM = standard error of the mean

The present work has shown two approaches to influence the metabolic situation of high yielding dairy cows, i.e. treating grain with xylose and providing more RUS (and RUP), and modifying the intermediary glucose consumption through supplementation of the ration with *trans*-10, *cis*-12 CLA. In other studies, the prepartum supplementation of blended sorbitol and mannitol as a glucogenic precursor for dairy cows was ineffective in influencing the metabolic situation (McFadden et al., 2008). The treatment of cereal grains with NaOH has yielded varying results (Dehghan-Banadaky et al., 2007). The NaOH treatment of barley lowered the digestibility of starch in the small intestine to a greater extent than when wheat was treated with NaOH (Moharrery et al., 2014). This again underlines the impact the various protein matrices in cereal grains can have. The starch granules are embedded in a protein matrix which determines the ruminal starch degradability (McAllister et al., 1993) and the success of physical and chemical grain treatments. The oral administration of glucose, i.e. feed intake, cannot contribute to the glucose supply of ruminants as it is completely degraded in the rumen. However, infusion of glucose into the duodenum has increased plasma glucose

concentration but neither milk nor milk lactose yield (Lemosquet et al., 2009). Glucose is utilized through different metabolic pathways and thus contributes to the overall nutrient supply. Infusion of glucose provides information on the metabolic pathways of the animals, but cannot be used as a mode for improved nutrient supply of ruminant animals. In addition to optimizing the rumen fermentation, the use of RUS, particularly in the form of xylose-treated wheat, offers a promising way to a more efficient feeding of high yielding dairy cows.

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