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**Effect of fat in comparison to starch in an isoenergetic diet on the
metabolism of high yielding dairy cows**

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Dedication

To my father soul, my mother, my wife, and my children

'Ayah, Mohamed, and Ramy'

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Abbreviations

AA	Amino acids
ATP	Adenosine triphosphate
BCAA	Branched chain amino acids
BCS	Body condition score
BW	Body weight
CA	Crude ash
CF	Crude fiber
CL	Crude lipid
CLA	Conjugated linoleic acid
CP	Crude protein
DCL	Digestible crude lipid
DM	Dry matter
EAA	Essential amino acids
EB	Energy balance
FA	Long chain fatty acids
ECM	Energy corrected milk
Fig.	Figure
GE	Gross energy
IRMS	Isotope ratio mass spectrometry
ME	Metabolizable energy
N	Number of samples
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NEFA	Non-esterified fatty acids
NEL	Net energy for lactation
NEAA	Non-essential amino acids
NFE	Nitrogen-free extract
NPN	Non-protein nitrogen
p.p.	Post partum
Q	ME/GE · 100
¹² C, ¹³ C, ¹⁴ C	Carbon isotope with atomic weight of 12, 13 or 14, respectively
¹⁴ N, ¹⁵ N	Nitrogen isotope with atomic weight of 14 or 15, respectively

RIA	Radioimmuno assay
SEM	Standard error of mean
TCA	Tricarboxylic acid
TMR	Total mixed ration
VFA	Volatile fatty acids
β -HBA	β -Hydroxybutyrate

1 Introduction

During early lactation, the ability of high producing dairy cows to produce large quantities of milk exceeds their capacity to consume enough feed to meet their energy needs. Thus, the cow must draw upon endogenous energy (adipose tissues) and protein stores. Body fat mobilization during early lactation ranges from 20 to 70 kg, (ANDREW et al. 1994). One kilogram of mobilized reserves provides the cow with about 14.4 to 26.9 MJ net energy for lactation (**NEL**) and 141 to 188 g of crude protein (**CP**) (GfE 2001; NRC 2001). For milk production, 20 MJ NEL are enough for approximately 6 kg milk and 150 g CP are only for approximately 3.5 kg milk. Also, the early lactation period is characterized by high rates of basal or catecholamine-stimulated lipolysis in adipose tissues causing a negative energy balance (**EB**) and sub-optimal milk yields (KRONFELD 1982; McNAMARA and HILLERS 1996). Severe negative EB due to excessive mobilization can reduce lactation persistency and causes a higher incidence of ketosis and related metabolic disorders (KRONFELD 1970), increased incidence of inflammatory diseases such as endometritis (MARKUSFELD 1984) and mastitis (SURIYASATHAPORN et al. 2000), and poor reproductive performance (BUTLER 2000; REIST et al. 2000; STAPLES et al. 1990).

The traditional approach of the animal nutritionists has been to increase the feed intake or the nutrient density of the diet of cows in early lactation to minimize the possibility of nutrient deficiencies (NRC 2001). The feed intake of dairy cows is limited and regulated by many factors (SCHWARZ 1997). Attempts to increase energy density by adjusting the forage to grain ratio to less than about 40: 60 often result in negative effects on ruminal fibre digestion and milk fat percentage without a net gain in energy intake, which leads to a reduction in milk yield (McDONALD et al. 2002). Alternatively, supplementing the diets of lactating dairy cows with protected fat is a way to increase energy intake without the need to increase intake of grain or to decrease the fibre content of the diet (PALMQUIST 1984). Replacement of grains (starch) by protected fat may also allow the incorporation of even higher levels of forage into the diet. However, in contrast to starch, fat supplies no glucogenic components for glucose and lactose synthesis in the liver and mammary gland, respectively. Moreover, dietary fat supplies no energy for ruminal synthesis of microbial protein. On the other hand, enhanced incorporation of food-derived long chain fatty acids (**FA**) into milk fat can reduce the requirements of glucose for nicotinamide adenine dinucleotide phosphate (**NADPH**) synthesis and thereby saves glucose for lactose synthesis (PALMQUIST and JENKINS 1980; BALDWIN and SMITH 1971).

Dietary supplementation with rumen-protected fat as an energy source can improve the

energetic efficiency and milk yield of lactating cows (FIRKINS and EASTRIDGE 1992). However, the milk protein content was decreased in most previous studies (DePETERS and CANT 1992; MOALLEM et al. 1999; ONETTI et al. 2001; WU et al. 1994). CASPER and SCHINGOETHE (1989) attributed the reduced milk protein percentages to a limitation of amino acids (AA) for protein synthesis by the mammary gland. In a review, DePETERS and CANT (1992) accredited the decreased AA concentrations in plasma to increased utilization of AA for gluconeogenesis. This is manifested in increased blood and milk urea levels with the addition of protected fat to diets of lactating dairy cows. However, the effect of dietary fat on urea synthesis in cows has not been measured.

Lactose is considered the primary osmoregulator of milk volume (LINZELL and PEAKER 1971) so that concentration fluctuates little. Consequently, the milk yields depend on the glucose availability for lactose synthesis. For a milk production of 50 kg, a cow needs about 3.6 kg glucose (KRONFELD 1982) and, because of the low amount of absorbed glucose, the glucose has to be synthesized mostly from glucogenic precursors such as propionate or AA. It was demonstrated that in a phase of increased energy demand, carbon atoms from body fat reserves would be transferred into milk lactose (SCHULZE et al. 1992). WEINMAN et al. (1957) discussed that after β -oxidation the carbon atoms of FA are channelled as acetyl-CoA into the tricarboxylic acid cycle (TCA) and substitute the carbon atoms of oxalacetate and are also transferred into the process of gluconeogenesis. The question is whether the substitution of dietary starch by fat results in a transfer of carbon atoms from fat into milk lactose.

The aim of the present study was to investigate some metabolic processes in lactating cows fed on isoenergetic diets differing in starch and fat content. It was of special interest whether, firstly, the substitution of dietary starch by rumen-protected fat results in visible changes in the protein metabolism by increasing the urea flux and decreasing of AA levels in blood plasma as indicators for a change of AA oxidation and, secondly, whether the fat-fed cows use predominantly FA in the processes of milk fat synthesis or as an energy source in oxidation processes.

2 Review of literature

2.1 Fat in rations of lactating dairy cows

Fats are esters of glycerol with long chain FA. Earlier reports indicated that fat, especially unsaturated fat, could not be fed to dairy cows in large quantities because of its negative effects on ruminal fermentation (PALMQUIST and JENKINS 1980). Use of unprotected fat (s) in diets is usually limited to 3% of dietary dry matter (**DM**) due to its serious effects on ruminal fermentation (JENKINS 1993). Protected fats are widely used in the feeding of dairy cows. This is due to two reasons. Firstly, fat possesses the highest energy concentration of the three nutrients (fat, protein, and carbohydrate). Secondly, with dietary fat, it is possible to manipulate the composition of the FA in milk fat, e.g. the content of conjugated linoleic acid (**CLA**). Unsaturated FA are partially transformed, i.e. partially hydrogenated, and the stereo conformation (*cis*, *trans* configuration) is changed. The level of this transformation depends on the total diet, especially the retention time of digesta in the rumen or the ratio of roughage to concentrate (GRIINARI et al. 1998). It is important to know that some FA that are supplied in the diets or synthesized in the rumen, such as CLA *cis* Δ^9 *trans* Δ^{11} and *cis* Δ^{12} -C_{18:1}, significantly decrease the milk fat content of lactating dairy cows (PRECHT et al. 2002).

For high yielding lactating dairy cows, the energy content of dietary fat is of special interest, as mentioned above. The energetic efficiency of long chain FA for adenosine triphosphate (**ATP**) generation in the intermediary metabolism is higher than that of acetate. BERGNER (1996) calculated the energy yield for acetate ($\Delta G^\circ / \Delta H \times 100$) as 36.8% and for palmitate and stearate as 41.6%. In addition, the author stated that the efficiency of utilization of metabolizable energy (**ME**) of digestible fat for milk production in ruminants is about 72%. For comparison, the efficiency of utilization of ME for milk production is about 60%, 72%, 65%, 51%, 56% and 66% for ruminal acetate, absorbed glucose, abomasally infused casein, digestible CP, digestible crude fiber (**CF**), and digestible N-free extract (**NFE**), respectively. Generally, fats are used energetically with more efficiency for milk synthesis than all other digestible nutrients. If the ME of digestible fat is assumed to be 31.2 MJ/kg and ME is converted into NEL with an efficiency of 72% (HOFFMANN 1996), then theoretically, 22.5 MJ NEL/kg fat can be calculated. For comparison, the energy content of digestible starch is about 9.7 MJ NEL/kg. The ME provided by dietary Ca salts of FA in early lactation in most studies could be close to the range 12% to 16% of the total ME, proposed as the most efficient for milk production (BINES et al. 1978). KRONFELD (1976) concluded that 16% of the ME should be in the diets of dairy cows as long chain FA.

2.2 Effect of fat on rumen metabolism and microbial protein

The rumen has a regulatory function on lipid utilization in ruminants because the microflora in the rumen is responsible for the hydrolysis, hydrogenation and isomerisation of dietary lipids and also for the synthesis of microbial lipid from components of the diet (MOATE et al. 2004). Consequently, the FA reaching the small intestine are rich in saturated and monounsaturated FA and bear little resemblance to the composition of unprotected FA provided in the diet. On entering the small intestine, the long chain FA are emulsified by bile salts and are absorbed by the animal (STORRY et al. 1974). The high levels of free FA in the rumen, resulting for example from diets supplemented with fats and oils, can severely impair digestion and depress appetite. Thus, the composition and metabolic activity of the microflora, the breakdown of cellulose and protein, and the production of methane, volatile fatty acids (VFA), and ammonia are some of the processes in the rumen that are known to be affected by the addition of unprotected oils or free FA to the diet (MACHMULLER et al. 2003; STORRY et al. 1974). The extent of these effects depends on the type of such unprotected fat and the method of administration. However, feeding of dairy cows on diets contained Ca salts of FA decreased the total concentration of VFA and acetate to propionate ratio in ruminal contents (BINES et al. 1978; UEDA et al. 2003) depending on the type of basal ration and the amount and FA composition of the lipid (PALMQUIST and JENKINS 1980).

Regarding the synthesis of microbial protein in the rumen, two aspects have to be taken in consideration. 1. Unsaturated fat supplies no energy for microbial growth and inhibits fibre digestion in the rumen (BROUDISCOU et al. 1994). The effect of dietary fat on the amount of organic matter fermented may become sufficient to reduce the microbial protein available to the host animal due to the reduction of the energy availability for microbial protein synthesis (IKWUEGBU and SUTTON 1982; KAYOULI et al. 1986) resulting in an increased loss of ruminal $\text{NH}_3\text{-N}$ and potentially influencing the profile of post-ruminal AA flux/composition (PALMQUIST et al. 1993). 2. The defaunating action of free FA in the rumen (ONETTI et al. 2001) has the potential of improving the efficiency of microbial protein synthesis, expressed as grams of microbial N enter the duodenum per kilogram of organic matter fermented in the rumen (BROUDISCOU et al. 1994; MURPHY et al. (1987). The reason for this is not fully clear. Generally, it is assumed that by the defaunating effect of free FA and the duodenal flow of bacterial N could be increased by decreasing of protozoan predation of ruminal bacteria and competition for substrates between these microorganisms (JOUANY 1995). However, KOENIG et al. (2000) found no reduction of bacterial N recycling in the rumen by defaunation.

KLUSMEYER et al. (1991) reported more efficient microbial protein synthesis in cows fed on 50% forage diets supplemented with protected FA compared to those fed on unsupplemented diets. However, PANTOJA et al. (1995) found unaffected efficiency of microbial protein synthesis and passage of microbial N to the duodenum of lactating cows fed on diets supplemented with Ca salts of FA.

Generally, it can be concluded that protecting of fat from ruminal degradation can reduce the adverse effects of free FA. The effect of protected fat on the different vital processes in the rumen of dairy cows depends on the amount and FA composition of fat supplement, the type of basal ration and the method of administration. The substitution of fermentable carbohydrates by supplemental fat in the diet of lactating dairy cows may reduce or increase the efficiency of microbial protein synthesis.

2.3 Effect of rumen-protected fat

Due to the adverse effects of more free FA in the rations on ruminal fermentation, the diets of ruminants in practice contain only limited amounts of supplementary unprotected oils and fats. Their main function is to aid in pelleting and improve palatability rather than to provide a source of energy. This is in contrast to the higher levels of fats and oils incorporated into diets for non-ruminants for energetic reasons. Techniques for protecting dietary fats and oils from breakdown in the rumen without reducing their subsequent absorption from the small intestine were developed, which led to a greater use of dietary lipids in ruminant nutrition. Oils or fat droplets are encapsulated in a layer of aldehyde treated protein, which because of cross-linking between free amino groups is relatively insoluble and inert to bacterial breakdown in the rumen. Aldehydes other than formaldehyde have also been found to be effective (BRUMBY et al. 1974) and also protecting of whole oil seeds by removal of husks with emulsification, formaldehyde treatment and drying (SCOTT et al. 1972). Recently, the calcium salts of FA (Ca-soaps) were used effectively in feeding of dairy cows (DePETERS and CANT 1992). Ca salts of FA are insoluble in aqueous solution with pH > 4.0. By feeding Ca salts of FA to the ruminant animals, the FA are released due to the effect of acid conditions of the abomasum and become available for enzymatic digestion and absorption in the small intestine.

Feeding of rumen stable fat like Ca salts of FA to lactating dairy cows does not alter fermentation in the rumen because of their insolubility in the rumen (CHALUPA et al. 1986) and maintaining the rumen pH above 6.0 (SCHAUFF and CLARK 1989). Moreover, supplementation of dairy rations with more than 2% (DM) added fat often improves the milk yield (DePETERS and CANT 1992). Dietary FA at 9% may result in reducing their digestibility

(KHORASANI et al. 1992) because of limited biliary and pancreatic secretions into the small intestine (HUBER et al. 1994).

CHRISTENSEN et al. (1998) recorded that feeding of protected fat to lactating dairy cows did not alter the ruminal and post-ruminal digestibility of energy. Diets contained Ca salts of FA had normal acceptable digestibilities of fiber in the rumen and the total gastrointestinal tract in previous studies (JENKINS and PALMQUIST 1984). Also, the digestibility of FA of fat distillates decreased by their hydrogenation, which is probably caused by poor hydrolysis of hydrogenated fat in the rumen, which characterized by a high melting point (JENKINS and JENNY 1989). Maximum efficiency of energy utilization can be achieved theoretically when the diet provides 12 to 16% of ME as Ca salts of long chain FA (BINES et al. 1978). The stability of Ca salts of saturated FA in the rumen provides a good protection against the detrimental effects of free FA on fibre digestion (CHOUINARD et al. 1997). SUKHIJA and PALMQUIST (1990) demonstrated that the relatively saturated Ca salts of palm oil are utilized efficiently in the feeding of lactating dairy cows because the saturated FA are more resistant to dissociation at normal ruminal pH than unsaturated FA. FERLAY et al. (1992) stated that no obvious differences in dairy performance could be observed when Ca salts of FA from palm oil were compared with Ca salts of FA from rapeseed oil. HOLTER and HAYES (1994) reported no benefit from delaying the addition of Ca salts of FA from 1 to 57 d post partum (**p.p.**). SHAVER (1990) highlighted the need for long-term, continuous-design experiments to determine responses of lactating dairy cows to the supplementation of fat for an entire lactation. But, YANG et al. (1978) reported a decrease in the persistency and shortening of lactations in cows fed on an extremely high fat diet (oil seeds protected with formalin) that provided 12% of the dietary DM as FA. Thus, it can be concluded that protected fat, especially the Ca salts of FA, is used efficiently in the feeding of dairy cows at levels of 5% to 6% of the diet DM and provides 12 to 16% of the total ME of the diet.

2.3.1 DM and energy intakes

Dietary supplementation with Ca salts of FA can make a substantial increase in the net energy consumed if DM intakes can be maintained, or nearly so. Thus, COPPOCK and WILKS (1991) considered the addition of a commercial fat product to a corn-soybean meal mixture at 3% (0.75 kg) of a diet, at an equal DM intake of 25 kg, would increase the NEL consumed by 3.43 Mcal (14.4 MJ), which is equal to the energy of about 5 kg of milk with 3.5% fat. The amount of Ca salts of FA required to reach a limitation of intake is probably a function of the energy requirement of the cows and the amount of energy provided by the basal ration and is therefore

dependent on experimental conditions (CHILLIARD et al. 1993; ELMEDDAH et al. 1994). In many studies, the DM intake of lactating dairy cows was not affected by feeding diets supplemented with Ca salts of long chain FA (GARCIA-BOJALIL et al. 1998; KOWALSKI et al. 1999; SCHAUFF and CLARK 1989; SCHROEDER et al. 2003; SKLAN et al. 1994; WU et al. 1993). WEST and HILL (1990) reported unaffected DM and NEL intakes when lactating dairy cows were fed on diets supplemented with 2.5 to 4% Ca salts of FA on DM basis. The same results were also reported by GRUMMER (1988). Conversely, other studies (ANDREW et al. 1991; CHILLIARD et al. 1993; GRUMMER et al. 1990; KLUSMEYER et al. 1991) reported less DM intake of lactating dairy cows fed on Ca salts of long chain FA than of cows not fed on Ca salts of FA. CHOUINARD et al. (1997) found that DM intake decreased linearly as the percentage of Ca salts of canola FA increased from 2 to 4% as a percentage of DM in the diets of lactating cows. CHOUINARD et al. (1998) compared feeding of three types of Ca salts of FA from different sources with a control group of lactating dairy cows. The authors found no differences in DM intake between cows fed the control ration or rations supplemented with Ca salts of FA, however, the intake of DM was decreased quadratically as the insaturation of the dominant FA in the Ca salts increased. Also, GRUMMER et al. (1990) showed that DM intake decreased when cows were fed Ca salts of long chain FA, but the acceptability of this fat supplement was improved when it was presented to cows as a part of the grain mixture. On the other hand, GRUM et al. (1996) found the greatest intakes of DM and digestible energy for cows fed on diet supplemented with prilled saturated FA and soybeans oil with low concentrates content. As a conclusion, the energy intake of lactating dairy cows can increase when the intake of DM is maintained. To minimize the likelihood of reduction in feed intake when fat is introduced into dairy rations, cows should be allowed to adapt to the fat and if possible the fat should be diluted with other feed ingredients.

2.3.2 Milk production performances

Milk yield and efficiency of milk production

Dietary supplementations with rumen-protected fat improve the energetic efficiency and milk yield of early lactating cows, as documented in most published studies. ANDREW et al. (1991) reported that supplemental Ca salts of FA increased energy retention as a percentage of that consumed. GARNSWORTHY (1996) found that replacing of a part of the cereal carbohydrate in the diets of dairy cows with protected fat increased the ME supply and led to an increased milk yield. It is recorded that the largest increases in milk production are obtained by feeding Ca salts of FA to high producing dairy cows during the early stages of lactation (OSTERGAARD et al. 1981). Other experiments (CHALUPA et al. 1986) suggested that the

greatest benefits of feeding of Ca salts of FA are obtained when lactating cows are producing in excess of 35 kg of milk daily. SCHINGOETHE and CASPER (1991) and also RODRIGUEZ et al. (1997a) recorded that feeding of dairy cows during early lactation on high fat diets produced an increase of 3.6% in total milk yield. GARCIA-BOJALIL et al. (1998) and TOMLINSON et al. (1994) stated that the pattern of milk production was unaffected by supplemental Ca salts of FA until approximately 20 d p.p., then milk production was stimulated until the end of their study (first 100 d of lactation). A quadratic response of milk yield to supplemental Ca salts of long chain FA (from 0 to 8 or 9% of dietary DM) has been reported previously in other studies (CHOUINARD et al. 1997, 1998; LUBIS et al. 1990; MADISON-ANDERSON et al. 1997; McNAMARA et al. 2000). SKLAN et al. (1994), and about one third of data in the review of CHILLIARD et al. (1993) reported an increase in milk yield (0.94 kg/d) for lactating cows fed on diets contain Ca salts of FA. HUBER et al. (1994) found that supplementation of a saturated or unsaturated fat source to increase FA content of the diet to 7% of dietary DM increased milk yield. However, there are other reports (McGUFFEY and SCHINGOETHE 1980) of slow responses to supplemented fat in experiments started after peak production. Milk yield has been found to increase linearly when Ca salts of canola oil FA were fed at the rate of 4% of DM to cows that were at 84 d of lactation (CHOUINARD et al. 1997). On the other hand, BAUMGARD et al. (2000) and ENJALBERT et al. (1997) observed that milk yield was only slightly increased or unchanged when dairy cows in the first stage of lactation were fed on diets containing Ca salts of FA from palm oil and also when cows were fed on prilled saturated FA in the study of GRUM et al. (1996).

Dairy cows fed supplemental fat produced nearly 2 kg more fat corrected milk (**FCM**) per day during the later weeks (6 to 14 weeks p.p.) in the trial of JERRED et al. (1990) than cows that were fed no supplemental fat. Milk yield and 4% FCM increased when lactating dairy cows were fed on Ca salts of FA during the early lactation period in most of previous studies (DRACKLEY et al. 1998; MADISON-ANDERSON et al. 1997; MOALLEM et al. 1999). However, the increased milk yield was also recorded with unaffected or decreased 4% FCM (GARCIA-BOJALIL et al. 1998; SCHNEIDER et al. 1990) because the percentage of fat in milk decreased when cows were fed diets contained protected fat. BAUMGARD et al. (2000) and SCHROEDER et al. (2003) reported unchanged milk yield with decreased 4% FCM when lactating dairy cows were fed diets supplemented with Ca salts of FA. HOLTER and HAYES (1994) reported unaffected 4% FCM and milk yield when supplementation of Ca salts of FA was delayed after the early lactation period of dairy cows. In one study, the production of FCM even increased by feeding dairy cows on Ca salts of FA if production of uncorrected milk was not

different (WEST and HILL 1990).

Altered ruminal fermentation patterns and some other factors resulting from a change in forage: concentrate ratio can affect efficiency of milk production. Dietary supplementations with rumen-protected fat improved the energetic efficiency and milk yield of early lactating cows, as documented in most of the previous studies (FIRKINS and EASTRIDGE 1992). However, KOWALSKI et al. (1999) concluded that the efficiency of milk production (kg milk/kg DM intake) was unchanged when dairy cows were fed on diets supplemented with Ca salts of FA and also with prilled saturated FA in the study of GRUM et al. (1996). Feeding of medium to low producing dairy cows that were past the peak of production on Ca salts of FA did not affect the milk production efficiency (SCHAUFF and CLARK 1989). However, GARCIA-BOJALIL et al. (1998) and TOMLINSON et al. (1994) found a stimulated efficiency of milk production of highly producing dairy cows after the 3rd week p.p. Overall, the supplementation of diets with rumen-protected fat increases the milk yield and efficiency of milk production and may increase the FCM production of lactating dairy cows.

Milk composition

Milk fat

There are interactions between milk fat content, kind and amount of supplemental fat, lactation period, milk yield, and EB of dairy cows (GAGLIOSTRO and CHILLIARD 1991a). Fat supplementation can either increase or decrease the percentage of fat in milk, depending on the type of supplemental fat. SUTTON (1989) stated that unsaturated plant oils that inhibit ruminal fibre digestion often cause a reduction in milk fat content. The responses of milk fat secretion to fat supplementation could be lower during mid-lactation than during early lactation. The highest milk fat content responses were observed in late lactating or low yielding goats (CHILLIARD et al. 2003). CHILLIARD et al. (1991) reported in early lactation of dairy cows that endogenous (adipose tissue, mainly C_{16:0}, C_{18:0}, and C_{18:1}) and exogenous FA are included in milk secretion. The endogenous FA are probably sufficiently high to inhibit de novo synthesis of FA in the first week of lactation, which may perhaps decrease by progress of lactation and improving of EB. The authors stated that milk fat output is markedly high during the early lactation period, which may be due to increased milk production. However they recorded that in mid-lactation protected fat increases the daily yield of milk fat as a consequence of increased amounts of unsaturated C₁₈ FA taken up and secreted by the mammary gland. BINES et al. (1978) stated that milk fat increased at low lipid supplementation (661 g/d) due to increased available FA for the mammary gland but reduced at the highest lipid supplementation levels (1100 g/d) due to a severe reduction

in the intra-mammary synthesis of FA. Also, other previous studies (CHOUINARD et al. 1998; DRACKLEY et al. 1998; MOALLEM et al. 2000; SCHROEDRE et al. 2002) found that addition of protected fat to the diet of dairy cows increased milk fat percentage and production as the added fat is able to provide the FA for milk fat synthesis. BANKS et al. (1976) stated that high proportions of long chain FA were transferred directly to milk fat in lactating dairy cows with high fat intake. A summary of 29 comparisons (CHILLIARD et al. 1993) and reports of ENJALBERT et al. (1997), GARCIA-BOJALIL et al. (1998), KHORASANI and KENNELLY (1998) and KOWALSKI et al. (1999) showed that Ca salts of FA of different sources (most of them from palm oil) did not influence the milk fat concentration of dairy cows. On the other hand, JENKINS (1998) found that the supplementation of dairy cows by Ca salts of FA from canola oil reduced the total milk fat concentration. Other studies also indicated that feeding of protected fat (CHOUINARD et al. 1997; 1998) from plant sources (soybean, sunflower, palm, canola and Ca salts of FA) for lactating dairy cows and abomasal infusion (GAYNOR et al. 1994) of vegetable oils to lactating cows decreased the milk fat percentage. All of these oils are high in polyunsaturated FA. GRUMMER (1991) reported that the proportion of FA synthesized de novo decreased linearly as supplemental fat increased from 1 to 5% of dietary DM. JENKINS and JENNY (1989) stated that the more unsaturated sources of added fat (yellow grease) cause more milk fat depression than the more saturated sources (hydrogenated yellow grease). Supplemental Ca salts of FA decreased total VFA and the acetate to propionate ratio in ruminal contents (BINES et al. 1978). Milk fat depression has been seen when the ratio of acetate to propionate is lower than 2.2, the molar percentage of propionate is greater than 25% (DAVIS 1979) and ruminal pH is lower than 6.3 (WOODFORD et al. 1986). As a consequence, the milk fat content could decrease due to a severe reduction in the intramammary synthesis of FA. Also, HERMANSEN (1995) reported that variations in short and medium chain FA in milk fat have a low correlation to dietary addition due to the influence of precursor produced by ruminal digestion.

De novo synthesis of short and medium chain FA from acetate in the mammary gland decreases with high fat intake, thus their levels in milk decrease and transfer of dietary long chain FA into milk increase (CANT et al. 1993; CHILLIARD et al. 1991; SUTTON 1989). A negative effect of dietary fat on the activity of acetyl-CoA carboxylase, the key enzyme for FA synthesis, explains the depression in milk fat content (DELBECCHI et al. 2001). By this mechanism, milk fat depression could occur even in the absence of the alterations of ruminal fermentation that are often observed when highly unsaturated free vegetable oils are fed (SELNER and SCHULTZ 1980). Fat supplemented rations that are high in polyunsaturated FA have been shown to be

related to high contents of *trans* FA in the milk fat. They are the result of incomplete biohydrogenation in the rumen (PRECHT and MOLKENTIN 1995) and the action of Δ -9-desaturase in mammary tissue (PIPEROVA et al. 2000). *Trans* FA are thought to induce milk fat depression in dairy cows. Currently, the main focus of research has been directed towards *trans*-C_{18:1} FA as well as specific CLA isomers to depress milk fat content (PRECHT et al. 2002). It would also be of nutritional interest to enrich milk fat with unsaturated FA without *trans*-binding, i.e. linoleic acid and oleic acid, while concurrently reducing its content of saturated FA such as C₁₂, C₁₄, and C₁₆ (SCHROEDER et al. 2003). CHOUINARD et al. (1998) prepared Ca salts of FA from canola, soybean, and linseed oils, which were selected based on their dominant FA (*cis*- Δ -9-C_{18:1}, C_{18:2}, and C_{18:3}, respectively). Ca salts of FA were added to the ration of dairy cows decreased the proportions of saturated FA that contained C₆ to C₁₆ and increased the proportions of C_{18:0}, *cis*- Δ -9-C_{18:1}, and *trans*- Δ -11-C_{18:1} in milk fat. PRECHT et al. (2001) found that the saturated FA C₁₂, C₁₄ and C₁₆ decreased by 17-19%, stearic acid content of milk fat increased by about 26-28%, and CLA (*cis*- Δ 9, *trans*- Δ 11) increased by 56-97%, which improved the milk quality in cows fed a diet with Ca salts of FA. Butter is rich in lauric, myristic, and palmitic acids, which are assumed to be hypercholesterolemic. The replacement of usual dietary fat by rapeseed oil induces decrease in serum cholesterol. JAHREIS et al. (1996) fed different amounts of full-fat rapeseed or oil-rich rapeseed cake to dairy cows to improve the fatty acid composition of milk fat. Their results demonstrate a decrease of lauric + myristic + palmitic acid percentage by about 18% of whole fatty acids and an increase of stearic and oleic acids in addition to highly increase of *trans* octadecenoic acids.

Therefore, it can be concluded that the supplemental fat in the rations of lactating dairy cows may decrease or increase the milk fat content depending on many factors such as the type, form, amount and composition of supplemental fat, the period of lactation, EB, level of milk production and alterations in *de novo* synthesis of FA in the mammary gland. Positive effects are mostly seen when the FA become more available for mammary gland. The pattern of fatty acids in milk fat is influenced.

Milk protein

Supplementation of the diets of dairy cows with Ca salts of FA (DePETERS and CANT 1992) or abomasal infusion of oils (ROMO et al. 2000) of lactating dairy cows often, though not always, decreases the milk protein content. DePETERS et al. (1989) have shown that the addition of fat to the diets of lactating dairy cows reduces the milk content of total N, casein-N, and whey protein-N compared with isoenergetic diets without supplemental fat, which may be attributed to

the shortage in the supply of glucogenic precursors. RIGOUT et al. (2003) found a strong positive relation between the energy of glucogenic precursors available in both of rumen (propionate) and intestine (glucose), and the milk protein content in dairy cows fed on grass silage-based diets supplemented with glucogenic nutrients either infused in the rumen as VFA or glucose in the duodenum. Milk protein depression resulting from fat supplementation, either protected or unprotected is well documented (CHOUINARD et al. 1997; DRACKLEY et al. 1998; KOWALSKI et al. 1999; MOALLEM et al. 1999; ONETTI et al. 2001; SCHROEDER et al. 2003). The mean depression was 0.07 percentage units (SHAVER 1990). In addition, a review of 82 comparisons (WU and HUBER 1994) showed that, in about one third of them, milk protein decreased when the diets of lactating dairy cows were supplemented with protected fat. This depression was associated with either increased or decreased milk yield. CHOUINARD et al. (1997), and also DePETERS and CANT (1992) recorded that milk protein percentage decreased by about 5% and 10% with feeding of lactating dairy cows on 2% and 4% Ca salts of canola oil (DM basis), respectively. An overall effect of -0.12 percentage unit was reported for 25 comparisons with Ca salts of FA (CHILLIARD et al. 1993) and -0.1 to -0.2 percentage units in the report of DePETERS and CANT (1992). The feeding of supplemental fat to lactating dairy cows decreased the percentage of milk protein but not its output (KLUSMEYER et al. 1991; RODRIGUEZ et al. 1997a; WU et al. 1993, 1994). Increasing of milk yield may be a factor affecting the total protein in milk. About 68% of the change in protein concentration in milk could be accounted for by an increase in milk yield (DePETERS and CANT 1992; WU and HUBER 1994).

SKLAN et al. (1992) observed that such a negative effect of the supplementation of Ca salts of FA on milk protein content was apparent in primiparous cows but may be not in multiparous cows. HOFFMANN et al. (1991) found that milk protein depression of dairy cows supplemented with fat during lactation did not occur until week 8 and was clear till week 22 of lactation period, which appeared to be correlated negatively with milk responses. This was in agreement with the findings of DePETERS and CANT (1992). Some of the literature in the review of WU and HUBER (1994) reported an increased milk protein content with supplemental fat. However, it was not confirmed in other experiments (BAUMGARD et al. 2000; ENJALBERT et al. 1997; MOALLEM et al. 2000). Generally, feeding of lactating dairy cows on diets supplemented with fat often depresses the percentage but not the production of milk protein.

Milk lactose

In dairy cows, the main determinant of the milk yield is the supply of glucose to the

mammary gland (KRONFELD 1982). The glucose requirements of dairy cows are markedly higher during lactation, especially for lactose synthesis (BICKERSTAFFE et al. 1974; DANFAER 1994). In addition, α -glycerol phosphate used for synthesis of triglycerides in milk and adipose tissue is derived from glucose (MADSEN 1983). It is assumed that the most of available glucose (about 70 to 80%) is used for lactose synthesis (BAUMAN and CURRIE 1980; MADISON-ANDERSON et al. 1997). There is a significant correlation between lactose output in milk and glucose input into the mammary gland (FAULKNER and POLLOCK 1989). Furthermore, there is a significant correlation between lactose output in milk and milk glucose concentration; the latter reflects the intracellular glucose concentration that regulates the rate of lactose synthesis (FAULKNER and POLLOCK 1989).

Lactose is considered the primary osmoregulator of milk volume (LINZELL and PEAKER 1971) so that concentration fluctuates little. Lactose concentration in milk is usually not affected or is only slightly altered by dietary manipulation (CASPER et al. 1990). Only a small proportion of literature about supplemental fat feeding to dairy cows focused its studies on milk lactose content and its metabolic relationship to added fat. In many experiments, milk lactose content is not influenced by feeding of lactating dairy cows on diets supplemented with Ca salts of FA (CHOUINARD et al. 1998; KHORASANI and KENNELLY 1998; KOWALSKI et al. 1999; THIVIERGE et al. 1998) or by infusing them by unsaturated FA (WAGNER et al. 1998). However, milk lactose output may be increased by feeding of Ca salts of FA (KOMARAGIRI et al. 1998; MADISON-ANDERSON et al. 1997). MADISON-ANDERSON et al. (1997) and RODRIGUEZ et al. (1997a) reported a higher percentage of milk lactose for lactating dairy cows fed diets containing supplemental fat. In the more recent report of FAHEY et al. (2002), it is stated that milk lactose production ($\text{kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$) was greater from weeks 2 to 12 and from weeks 13 to 20 of lactation in cows fed on Ca salts of FA. CHOUINARD et al. (1997) reported that the lactose level in milk (week 8) and yield (weeks 4 and 8) of lactose were altered in a quadratic pattern when amounts of Ca salts of FA fed to the cows were increased; values were higher for cows that received 2% Ca salts of FA (DM basis) in comparison to the high levels (4%). BINES et al. (1978) and STORRY and BRUMBY (1979) stated that low levels of protected fat to lactating dairy cows increased milk lactose yield, but this yield decreased by high levels of protected fat. This effect could be enhanced when the digested amounts of carbohydrates and protein were lowest. On the other hand, KALSCHEUR et al. (1997) found that lactose content of milk was reduced to approximately 97% of control values by high fat intake in lactating dairy cows, which was a modest decrease, which maintained the osmotic concentration of milk.

Lactose concentration in milk increased in response to abomasally oil infusion and its yield was greater in early lactation than mid-lactation of dairy cows (BENSON et al. 2001). In conclusion, the effect of dietary fat supplementation on milk lactose content or yield in milk is non-existent or not significant.

Milk urea

Urea level in milk has been proportionally associated with the protein to energy ratio of the diet (REFSDAL et al. 1985). The non-protein nitrogenous compounds (NPN) of milk represent approximately 4.9% of the total milk N (CERBULIS and FARRELL 1975). The primary contributor to milk NPN is urea (DePETERS and FERGUSON 1992). In their review, DePETERS and FERGUSON (1992) suggested three sources of urea in milk: end products of protein and NPN digestion (ruminal NH_3), AA catabolism in the liver, and a small part contributed by arginine catabolism in the mammary gland. Sources of variation in milk urea N level include dietary composition, body weight (**BW**), parity, stage of lactation, and diurnal variation (GUSTAFSSON 1993). GUSTAFSSON and PALMQUIST (1993) found that milk urea N was equilibrated with serum urea following a lag of 1 to 2 h when the rate of change of urea N in serum was 3 to 6 mg/dl per hour.

The ruminal ammonia level reaches its peak at about 1 h after feeding followed by a decline to the baseline value by about 6 h. The serum urea level peaks at about 1.5 to 2.0 h after the ruminal ammonia peak. Portal drained viscera release more ammonia with increased liver uptake when dairy cows are infused abomasally with 400 g vegetable oil per day (BENSON et al. 2002). Milk NPN content and yield increased also quadratically with increasing amounts of FA infused in the abomasum in the study of LaCOUNT et al. (1994). This increase in milk NPN and release of ammonia are difficult to explain. Possible explanations include an inhibition of gut motility caused by increased intestinal oil supply (NICHOLSON and OMER 1983), which may have altered the different digestive processes. Alternatively, hormonal and metabolic effects of oil infusion may have altered the metabolism of tissues of portal drained viscera, and thereby ammonia release. In addition, inhibitory effects of any unabsorbed oil on hindgut fermentation and microbial growth, and thus ammonia utilization, may have increased net ammonia absorption. Milk urea N concentration increased in cows fed high fat diets (SOLOMON et al. 2000), especially 2 to 3 h after feeding (RODRIGUEZ et al. 1997b). Also, RODRIGUEZ et al. (1997a) reported in another study that the milk urea N level increased by 4.9 % when lactating dairy cows were fed added fat. The rise in the percentage and yield of milk NPN often appears typical with feeding on diets contained protected fat (CANT et al. 1993; DePETERS and CANT

1992). However, it was also reported that milk NPN was not affected by high fat intake of lactating dairy cows (DRACKLEY et al. 1998; SCHROEDER et al. 2002) or infused abomasally with long chain FA (CHRISTENSEN et al. 1994). Overall, an increase of milk urea content can be expected by high fat intake of lactating dairy cows.

2.3.3 Energy balance, body weight, and subcutaneous fat

In dairy cows, the post partum period is characterized by a variable period of negative EB. Attempts to improve EB during early lactation by feeding energy-dense, fat-supplemented rations have had variable success (JERRED et al. 1990; SKLAN et al. 1991). Even with a negative EB, intake of energy rather than feed capacity may limit DM intake when energy-dense rations are fed to early lactating cows (JERRED et al. 1990). DRACKLEY et al. (1998) reported an improved EB during weeks 4 to 25, with a tendency to increased body condition score (**BCS**) during the same periods for cows fed diets supplemented with fat. Otherwise, BEAM and BUTLER (1998) fed 42 cows on a control diet (4.8% fat of total DM) or a diet supplemented with prilled FA (7% fat), where it was found that the EB was similar between diets. EB was positively correlated with plasma insulin and negatively correlated with NEFA, especially during the early lactation in studies of BEAM and BUTLER (1998) and SPICER et al. (1990). SKLAN et al. (1994) reported an enhanced negative EB when dairy cows were fed on diets supplemented with Ca salts of FA.

In other studies, high fat intake by lactating dairy cows fed on diets supplemented with Ca salts of FA (BINES et al. 1978) or partially hydrogenated oils (SCHROEDER et al. 2002) or infused duodenally with vegetable oils (GAGLIOSTRO and CHILLIARD 1991b) did not reduce mobilization of energy from body reserves. BW and BCS were not affected when lactating dairy cows in the early lactation period were fed on diets supplemented with protected fat in much of the recent literature (DRACKLEY et al. 1998; GARCIA-BOJALIL et al. 1998; KOMARAGIRI et al. 1998). The same results were obtained with feeding lactating dairy cows on diets supplemented with free unsaturated fat (MADISON-ANDERSON et al. 1997) or partially hydrogenated oils (SCHROEDER et al. 2002) or infused abomasally with vegetable oils (ROMO et al. 1996).

The dynamics of changes in subcutaneous fat thickness may be used as an indirect measurement to determine the energy deficit (STAUFENBIEL 1993). However, RASTANI et al. (2001) deduced that the start of a nearly constant course of the ultrasonic measurements of fat depth might not correspond to the time of the end of a negative EB. GRUMMER and CARROLL (1991) suggested that net triglyceride hydrolysis in adipose tissue is increased when additional fat

is added to the diet of dairy cattle. Otherwise, oil infusion into the duodenum of lactating dairy cows did not reduce, or even stimulated adipose tissue lipolysis, *in vivo* and *in vitro* (GAGLIOSTRO and CHILLIARD 1991a, b). However, the perirenal adipocyte volume and the number of adipocytes per gram of tissue DM were not affected. The rump subcutaneous adipocyte diameter decreased by duodenal infusion of lactating dairy cows with vegetable oils. The effect of fat supplementation on lipid metabolism appears to be not only due to increased lipolysis but also due to decreased lipogenesis. STAPLES et al. (1998) collected adipocytes at 60, 120, and 240 d p.p. via biopsy from Holstein cows fed 1) no supplemental fat, 2) whole cottonseeds at 12% of diet DM, or 3) whole cottonseeds plus Ca salts of long chain FA at 2.7% of diet DM. It was found that at all days p.p., adipocytes from cows fed supplemental fat were less lipogenic when incubated with acetate than adipocytes from cows not supplemented with fat. This result was also recorded by McNAMARA et al. (1995). However the latter authors found no difference in the body fat content of cows fed supplemental fat from oil seeds or Ca salts of long chain FA. Other laboratories have reported that supplemental Ca salts of long chain FA to lactating dairy cows resulted in greater BW and BCS loss compared with the lactating cows fed on the control diet (BEAM and BUTLER et al. 1998; HOLTER and HAYES 1994; SKLAN et al. 1991, 1994). As a conclusion, feeding of lactating dairy cows on diets supplemented with protected fat increases the lipolysis and decreases the lipogenic activities of adipose tissues. The feeding of protected fat to lactating dairy cows either does not or only slightly affects the BW and EB of dairy cows.

2.3.4 Metabolic parameters

In early lactation, the energy intake of a cow does not match her high production capacity leading to mobilization of body stores, namely fat and protein. However, during early lactation, the ability of the lactating dairy cow to mobilize body protein is limited, and the proportion of protein in mobilized tissue is low (OLDHAM 1984). The mobilization of body reserves of these cows is mostly from their fat depots. Mobilized body tissue contributes a significant amount of milk energy (BAUMAN and CURRIE 1980), thus, the levels of free FA in blood increases, which increases the uptake of FA by the liver. Consequently, the rate of β -oxidation increases and therefore more acetate has to be oxidized in the Krebs cycle, which needs oxalate (MADSEN 1983). The shortage of oxalacetate increases the ketone bodies level in blood as seen in Fig 1 and increases the incidence of ketosis (BAUMAN and CURRIE 1980). Thus, the different intermediary metabolic parameters are regarded as a mirror for the influences of protected fat on the metabolism of lactating cows.

Plasma glucose

During lactation of cows, glucose turnover increases from 0.45 to 0.65 g·h⁻¹·kg BW^{-0.75}, when plasma glucose level increases only from 2.7 up to 3.3 mmol/l (BERGMAN 1983). Supplemental fat has to save glucose from oxidation and to increase its availability for milk production (GRUMMER and CARROLL 1991; PALMQUIST and JENKINS 1980). If glucose is saved, this effect is not observable from blood glucose levels due to the homeostatic effect. The mean difference in plasma glucose levels between cows fed control and fat supplemented diets in 52 comparisons was 0 ± 3 mg/dl (CHILLIARD 1993). Studies since 1993 have confirmed the lack of a consistent effect of dietary fat on blood glucose (STAPLES et al. 1998). Concentrations of plasma glucose were unaltered in dairy cows fed high amounts of Ca salts of FA (BUCKLEY et al. 2000; FAHEY et al. 2002; KOWALSKI et al. 1999) or vegetable oil (MADISON-ANDERSON et al. 1997; PALMQUIST and CONRAD 1978) or abomasally infused with CLA

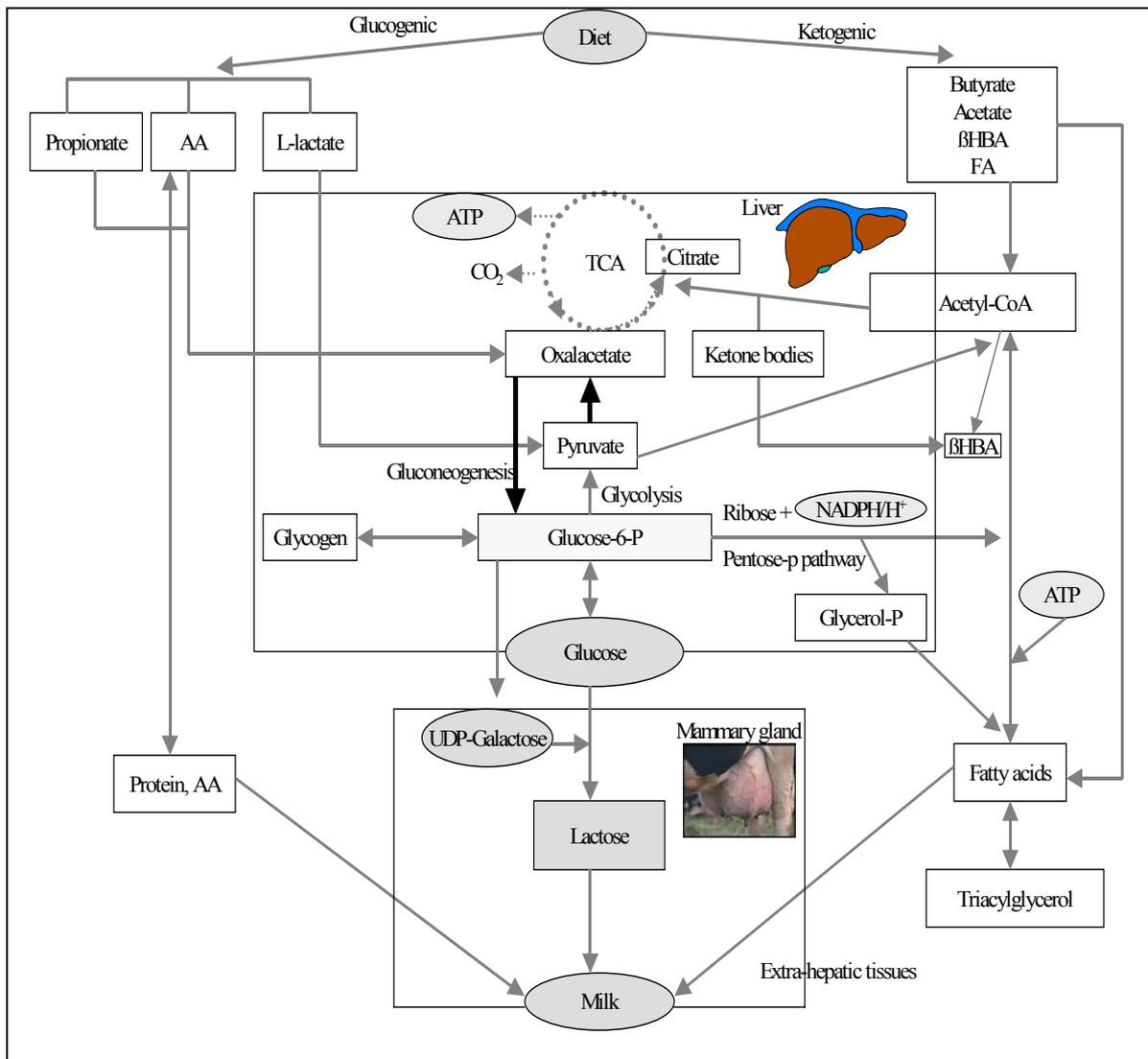


Fig. 1. Simplified scheme of nutrients pathway toward milk production of lactating dairy cows.

(MACKLE et al. 2003) or *cis* and *trans* FA (ROMO et al. 2000). An interaction of fat supplemented diet and time of blood sampling was observed for plasma glucose level (KHORASANI and KENNELLY 1998).

Otherwise, plasma glucose decreased in cows duodenally infused with 0.64 kg/d of rapeseed oil (CHILLIARD and OTTOU 1995). BENSON et al. (2002) reported a decreased liver glucose release and plasma glucose concentration when lactating cows were infused abomasally with 400 g per day of vegetable oil. DRACKLEY et al. (1992) reported a tendency of plasma glucose level to decrease linearly with increasing unsaturation and chain length of abomasally infused long chain FA. SIMAS et al. (1995) found that at week 7 p.p., plasma glucose decreased from 72.7 to 68.7 mg/dl for cows fed Ca salts of long chain FA at 2.5% of diet DM but was unchanged at week 13 p.p. Plasma glucose level was not affected during the first 4 weeks p.p. with feeding of prilled fat to lactating dairy cows in study of BEAM and BUTLER (1998). However, DRACKLEY et al. (1998) stated an increased or tendency to increased level of plasma glucose when supplemental vegetable oil or prilled fat (VAZQUEZ-AÑÓN et al. 1997) or tallow (BETMAN et al. 1996) was fed to lactating dairy cows. Generally, the blood glucose concentrations are usually not appreciably changed or decrease somewhat under circumstances of dietary supplemental fat.

Plasma β -Hydroxybutyrate (β -HBA)

Lactating dairy cows are subjected to a period of negative EB during the early lactation period, increasing the concentrations of ketone bodies in blood. The concentration of β -HBA had a greater peak within the first 3 week of lactation and became somewhat lower after that for the remainder of lactation in the study of DRACKLEY et al. (1998). β -HBA is produced in the ruminal epithelium from butyrate (MADSEN 1983) and as a product of NEFA and n-butyrate metabolism by the liver. Abomasal oil infusion of dairy cows had no effect on β -HBA production by the liver (BENSON et al. 2002) because most of dietary long chain FA by-pass the liver. Plasma level of β -HBA increased or tended to increase in lactating cows fed on diets containing protected fat in many studies (CHILLIARD and OTTOU 1995; JERRED et al. 1990). However, concentrations of β -HBA were also unaffected when fat was included in the diet of lactating cows in other studies (BUCKLEY et al. 2000; DRACKLEY et al. 1998; FAHEY et al. 2002). The same result was recorded when Ca salts of FA (SCHNEIDER et al. 1990) or tallow (RUPPERT et al. 2003) were fed to lactating dairy cows, or when they were infused abomasally with 20 to 80 g of CLA per day (MACKLE et al. 2003). Conversely, BINES et al. (1978) and ERICKSON et al. (1992) reported a reduced blood level of β -HBA in cows fed diets containing Ca salts of FA.

Plasma non-esterified fatty acids (NEFA)

Rates of FA release from adipose tissue depend on numerous factors, including rates of triglycerides hydrolysis, FA oxidation in liver, and reesterification of FA to triglycerides within adipose tissue (VERNON et al. 1985). Subcutaneous adipose tissue was reported to have higher lipolytic activities in vitro in lactating dairy cows during early lactation than in late lactation (PIKE and ROBERTS 1984). Plasma NEFA concentrations were greater immediately p.p. and declined as lactation progressed in the study of GARCIA-BOJALIL et al. (1998). The mammary gland can take up NEFA from plasma (GRUMMER 1991), although chylomicrons and very low-density lipoproteins are the major sources of FA in the mammary gland (MOORE and CHRISTIE 1979). The blood NEFA mainly originate from hydrolysis of adipocyte triglycerides by the activity of lipoprotein lipase, which is activated by supplemental long chain FA (MADSEN 1983). When adipocytes from cows fed supplemental fat were incubated with acetate, they were less lipogenic p.p. than those from cows not supplemented with fat (McNAMARA et al. 1995). Thus, the utilization of supplemental fat reduces lipogenesis with enhancing lipolysis in adipose tissue. This results in an increased concentration of plasma NEFA.

Blood NEFA is negatively correlated with EB of the lactating dairy cows. Thus, large concentrations of plasma NEFA can be expected when cows are in negative EB. Its concentrations were not affected during the first 4 weeks p.p. in the study of BEAM and BUTLER (1998) or reduced during the first 2 weeks p.p. in that of GARCIA-BOJALIL et al. (1998) by feeding lactating cows on diets supplemented with prilled fat in the former study or Ca salts of FA in the latter one. In most other studies, however, concentrations of plasma NEFA are nearly always elevated in cows fed diets supplemented with protected fat (CHOI and PALMQUIST 1996) or canola seed (KHORASANI and KENNELLY 1998) or infused abomasally with vegetable oil (BENSON et al. 2002; LaCOUNT et al. 1994; OLDICK et al. 1997; ROMO et al. 2000). Blood NEFA concentrations tend to increase with abomasal infusion of unsaturated compared with the mostly saturated long chain FA (CHRISTENSEN et al. 1994). However, in other experiments the levels of NEFA were not affected by feeding lactating dairy cows on protected fat (SCHNEIDER et al. 1990) or infused duodenally with vegetable oils (CHILLIARD and OTTOU 1995). ELLIOTT et al. (1994) reported reduced blood levels of NEFA when cows were fed on three different forms of mostly saturated FA. In conclusion, high levels of NEFA in blood plasma of lactating cows are associated with the period of negative EB and are susceptible to increase with high fat intake. However, the effect of dietary fat on the level of NEFA in blood plasma of dairy cows may be variable as also seen for that of β -HBA.

Plasma insulin

Blood plasma insulin concentrations in early lactation are low (HOVE 1978; RONGE et al. 1988) and an increase in plasma insulin concentration as lactation progresses has been widely observed as DM intake increased (LUCY et al. 1991). This is presumably related to an improved tissue EB (BENSON and REYNOLDS 2001). Plasma insulin concentrations usually reflect the energy intake, as the differences in plasma insulin with different diets are due to a difference in the energy status of the animal (STAPLES et al. 1998). GROVUM (1995) has shown a sinusoidal relationship between insulin concentration and intake in ruminants, because higher levels stimulate the DM intake by lowering blood glucose concentration. BEAM and BUTLER (1998) reported a decreased plasma insulin concentration during the first 21 days p.p., which was subsequently positively correlated with the EB during the first 6 weeks p.p. of the dairy cows fed on supplemental fat diet. Propionate plays a predominant stimulatory role in insulin secretion in ruminants (MINEO et al. 1994; OSHIBE et al. 1995). In monogastric species, dietary long chain FA also affects the insulin secretion (OPARA et al. 1994). An increase in dietary long chain FA linearly increased in vitro insulin secretion by the rat pancreas (OPARA et al. 1994). The authors showed that the effect of FA on insulin release is dependent on their chain length and the degree of unsaturation. If the degree of unsaturation has the same effect on insulin secretion in ruminants, a higher insulin response to rumen-protected and unsaturated long chain FA would be expected. The effects of various supplemental fat sources on plasma insulin concentration have been inconsistent (GRUM et al. 1996; MOHAMED et al. 1988). Plasma insulin level was not affected in cows fed on diets contained Ca salts of FA (SCHNEIDER et al. 1990) or infused abomasally with CLA (MACKLE et al. 2003). About 16 of 25 comparisons of control and fat supplemented cows in the report of STAPLES et al. (1998) indicated decreased or a tendency to decreased plasma insulin levels in cows fed on fat supplemented diets. This was confirmed by other studies using rumen-protected FA in the diets of dairy cows (CHOI and PALMQUIST 1996; GARCIA-BOJALIL et al. 1998) or partially hydrogenated fat (SCHROEDER et al. 2002). The lower ruminal production of propionate may be implicated as the reason. BENSON and REYNOLDS (2001) found that the insulin concentration in plasma of lactating dairy cows infused abomasally with vegetable fat was greater in mid lactation than in early lactation. They concluded from their study that the effect of postruminal fat supplementation on insulin concentrations is equivocal and perhaps confounded with the response of feed intake and ultimately body tissue EB.

In non-ruminants (e.g. rat), insulin resistance can be observed with feeding high fat diets (IP et al. 1977). Probably, fat feeding modifies the fat cell so that the available number of binding

sites for insulin on the cell decreases. Lactating cows that were duodenally infused with rapeseed oil (CHILLIARD and OTTOU 1995) or dietary supplemented with fat (PALMQUIST and MOSER 1981) showed no changes in plasma insulin concentration and evidence of insulin resistance was also reported. Insulin resistance was defined as the inability of insulin to stimulate tissue glucose utilization (PALMQUIST and JENKINS 1980). This resistance has been suggested in other studies with cows fed on diets supplemented with fat (PALMQUIST et al. 1992; CHILLIARD and OLLIER 1993). In studies in which fat supplementation improves the energy status of the animal, plasma insulin concentrations may be increased apart from the direct effect of fat (BLUM et al. 1985). CUMMINS and SARTIN (1987) reported that molar ratio of insulin: glucagon in plasma was consistently elevated in dairy cows fed a high fat diet. Over all, the plasma insulin level in lactating dairy cows may be unaffected or sometimes decreased by high fat intake of lactating cows. However, insulin resistance may also develop with high fat intake.

Plasma free amino acids

In a summary of several trials in which high fat diets were fed to dairy cows, CASPER and SCHINGOETHE (1989) reported a reduced concentration of several essential AA (His, Lys, Met, and Thr), nonessential AA (asparagine, aspartic acid, glutamic acid), and total AA in arterial serum in addition to increased mammary gland extraction for Met in lactating cows fed on high fat diets during early lactation. These decreased concentrations might be attributed to enhanced hepatic gluconeogenesis from AA. CANT et al. (1993) found that the concentrations of all arterial essential AA (**EAA**) were reduced when lactating dairy cows were fed on diets supplemented with 4% yellow grease. The δ -amino groups of Arginine, Ornithine, and Citrulline are included in the process of urea formation, which are very important contributors to α -amino N incorporated in the synthesis of nonessential AA (**NEAA**) (ROETS et al. 1979). MOHAMED et al. (1988) reported reduced plasma AA and branched chain AA (**BCAA**) concentrations in extra hepatic circulation of cows fed added dietary fat. The authors have concluded that the reduced concentrations of BCAA (Val, Leu, and Ile) usually reflect reduced absorption of AA from the small intestine as the portal supply of BCAA is metabolised to a lesser extent by the liver than other AA. Therefore, the level of BCAA decreases if the synthesis of microbial protein in the rumen decreases (OLTJEN et al. 1971).

CASPER et al. (1988) assumed that the AA utilized most efficiently are those limiting production. Met, Lys and Thr were the major three limiting AA for lactating cows fed on high fat diet. However, CASPER and SCHINGOETHE (1989) stated these three limiting AA as Met, Lys, and Phe. Conversely, Arg (LANHAM et al. 1992), Thr or Leu (DRACKLEY and

SCHINGOETHE 1986) and His (KORHONEN et al. 2000) have been implicated as among the first three limiting AA for lactating dairy cows. CHRISTENSEN et al. (1994) reported reduced plasma levels of His, Ile, Leu, Thr, and Val with slightly reduced plasma levels of EAA and total AA in lactating dairy cows infused abomasally with long chain FA. PALMQUIST et al. (1993) reported that added fat tended to decrease the intestinal supply of total AA but not EAA (except for Pro). MADISON-ANDERSON et al. (1997) reported no difference between concentrations of AA in arterial and venous plasma except for Ser, which was lower in plasma for cows fed diets supplemented with fat. ERICKSON et al. (1992) recorded that the concentrations of EAA in plasma of arterial blood were not affected except for a tendency to increased Thr in lactating cows fed on diets supplemented with Ca salts of FA during early lactation. PALMQUIST and CONRAD (1978) fed four groups of Holstein cows during early lactation on diets 1) control, 3.2% fat of DM; 2) ground raw soybeans, 5.9% fat 3) hydrolysed fat, 5.7% fat; and 4) hydrolysed fat, 10.8% fat, and concluded that EAA were not affected between groups and the ratio of total EAA to NEAA was below 0.6 for cows fed high fat diets. MAIGA and SCHINGOETHE (1997) reported that the arterial serum level of Thr was lowered in cows fed supplemental fat diets and ratio of total EAA/NEAA was < 0.6. This ratio was > 0.7 in the study of CASPER and SCHINGOETHE (1989). Generally, fat supplementation to diets of lactating cows decreases the levels of some free AA in blood, possibly by using of AA for gluconeogenesis.

Plasma urea

Plasma urea N concentrations were not affected by feeding of lactating dairy cows on diets containing Ca salts of FA (BUCKLEY et al. 2000; FAHEY et al. 2002; KOWALSKI et al. 1999) or tallow (RUPPERT al. 2003) or vegetable oil (DRACKLEY and SCHINGOETHE 1986; MADISON-ANDERSON et al. 1997) or prilled fat and partially hydrogenated fat (JERRED et al. 1990; SCHROEDER et al. 2002) or in dairy cows that were infused abomasally with vegetable oil (BENSON et al. 2002; MACKLE et al. 2003). However, blood urea concentrations increased in other studies with lactating cows fed on diets supplemented with Ca salts of FA (RODRIGUEZ et al. 1997b) or yellow grease (CANT et al. 1993) or hydrogenated fat (PALMQUIST and CONRAD 1978), or infused duodenally with rapeseed oil (CHILLIARD and OTTOU 1995) or abomasally with FA from canola, soybean and sunflower oils (CHRISTENSEN et al. 1994). Conversely, ROMO et al. (2000) recorded lower blood urea concentrations for cows infused abomasally with long chain FA. Any discussion of these different results can only be speculative.

Urea is quantitatively the most important end product of nitrogen metabolism in mammals. In ruminants, an evident amount of the daily-ingested N passes through the urea pool of the animal body. The process of urea formation in the liver involves N from AA oxidation in the liver and ruminally absorbed ammonia. In the rumen, proteins are degraded to peptides, AA and ammonia. Ammonia produced in the rumen is partially used by microbes for protein synthesis (SCHÜLER et al. 1990). Decreasing starch as energy supply results in decreased ammonia fixation by microbes in the rumen as well as an increased AA catabolism in the liver and, consequently, leads to increased urea synthesis (OLTNER and WIKTORSSON 1983). The produced urea is first evenly distributed to all body fluids before its excretion in urine and milk and its diffusion into the gastrointestinal tract (HARMEYER and MARTENS 1980). Urea is also transported from the metabolic pool into the gastrointestinal tract via saliva (HOUPPT 1959; EGAN et al. 1984). In case of reduced N intake, some of the urea is reabsorbed in the tubules of the kidneys (ERGENE and PICKERING 1978). Blood urea concentrations increased in dairy cows fed on diets supplemented with protected fat (RODRIGUEZ et al. 1997a; CANT et al. 1993), which was attributed to elevated hepatic utilization of AA for gluconeogenesis (CANT et al. 1993). Portal drained viscera release more ammonia with increased liver uptake in case of abomasal infusion of vegetable oils to lactating dairy cows (BENSON et al. 2002). The reason needs further examination. No results are available on total body urea production as influenced by dietary fat in lactating dairy cows.

2.4 Transfer of carbon atoms from dietary fatty acids into glucose

The possibility of a conversion of FA into carbohydrates was expressed as early as 1905 by PFLÜGER, who stated unequivocally that fat was the principal source of sugar in diabetes. Although the occurrence of this conversion in the animal body was accepted by a number of workers in the early 1900's, it did not go unchallenged. KREBS et al. (1966) described a contribution of ^{14}C originating from labelled acetoacetate in the glucose-carbon of rat kidney-cortex slices and explained it as a "crossing-over" of carbon atoms in the citric acid cycle and of gluconeogenesis via oxalacetate. STRISOWER et al. (1951) injected an emulsion containing tripalmitin-6- ^{14}C into normal and diabetic rats and determined the ^{14}C content of expired CO_2 and glucose. After 1 hour, about 4% of the injected ^{14}C was recovered in expired CO_2 and at that time blood glucose was also labelled. It was calculated that the observed incorporation of palmitic acid carbons into glucose represented roughly about 4% of the entire glucose turned over in the normal rat and about 8% of that in the diabetic rat.

WILTROUT and SATTER (1972) also showed that carbon transfer from acetate into plasma glucose is about 11% for non-lactating cows and up to 21% during lactation. Therefore, one can assume a higher contribution of carbon from acetate in gluconeogenesis when the demand for glucose increases for lactose synthesis during peak lactation. In peak lactation, when the feed intake of high-yielding dairy cows is insufficient to meet energy requirements, cows may be exposed to a negative EB leading to mobilization of body reserves primarily from depot fat (WILSON et al. 1988), releasing more glycerol and FA into circulation. After β -oxidation of long chain FA, their carbon atoms can be channelled, via acetyl-CoA, into the TCA cycle where they substitute the carbon atoms of oxalacetate and are also transferred into gluconeogenesis (WEINMAN et al. 1957). Three turns of the citric acid cycle are needed to replenish all carbon positions of oxalacetate and CO₂ with carbon atoms originating from acetyl-CoA. SCHULZE et al. (1992) demonstrated by stable isotope analysis that, in a phase of increased energy demand, carbon atoms from body fat reserves will be transferred into milk lactose in fasted or restricted fed cows as a consequence of an increased lipolysis of body fat and the exit of carbon atoms from the Krebs cycle to the gluconeogenesis pathway. The transfer of carbon atoms from dietary fat into lactose has not been studied.

2.5 Conclusion of review

The main determinant of the milk yield is the supply of glucose to the mammary gland. The main determinant of the mammary energetic efficiency is its utilization of long chain FA (KRONFELD 1982). The supply of glucogenic and FA components may be affected by the fat supplementation or replacement of starch by fat in the diet of lactating dairy cows.

Therefore, the objective of the present work was to study whether the demands of mammary gland for glucose and long chain FA can be optimised by substitution of starch by protected fat in the diets of dairy cows. In other words, does fat influence the homeorhetic regulation of nutrients partitioning in milk production? Regarding this question, it was interesting to know whether the dietary fat is available for synthesis in the mammary gland or is predominantly used as an energy source. In addition, it was of interest to know whether in fat-fed cows carbon atoms from FA are incorporated in the processes of lactose synthesis. It was also of interest to know whether the EB of the cow can be improved by dietary fat in early lactation. The later would contribute to an improved reproductive performance, because there exists a positive relation between EB and reproductive functions in dairy cows (ROBINSON et al. 2001).

3 Materials and methods

3.1 Experiment I

3.1.1 Animals

The experiment was carried out on 32 multiparous high yielding dairy cows during the first 100 d of lactation. Cows were assigned on the basis of last lactation yield to two dietary treatments (16 Holstein-Friesian cows in each group). Animals were housed in a free stall barn. They had free-choice access to water and were individually fed. Four weeks p.p., 10 cows were fitted with permanent catheters in the coccygeal vein (5 cows in each group). To avoid stress for the animals, they were catheterised 3 to 4 days before taking of the first blood samples. Fig. 2 shows the anatomic pathway of the catheter to the coccygeal vein (median caudal vein). The cows were sedated with 54.2 mg of acepromazine maleate per animal (4.0 ml Vetranquil® 1%, Albrecht, Germany) and with i.v. injection of 4 mg detomidin hydrochloride per animal (0.4 ml Domosedan®, Farnos, Finland). These were combined with deep epidural anaesthesia using 80 mg Lidocain per animal (0.4 ml Ursocain® 2%, Serum-Werk Bernburg, Germany). A surgical incision about 4 cm long was carried out after disinfection of the skin of the ventral side of the tail between the fourth and fifth vertebrae. About 60-65 cm polyethylene catheter with an internal orifice diameter 0.75 mm and an external orifice diameter 1.3 mm was sited in the caudal vena cava through the coccygeal vein, passed in front of the orifices of the ovary veins (SCHALLENBERGER 1990). Catheters were cared after taking of blood samples two times daily (800 and 1400 h) with heparin-NaCl solution (50 IE heparin per ml 0.9 % NaCl).

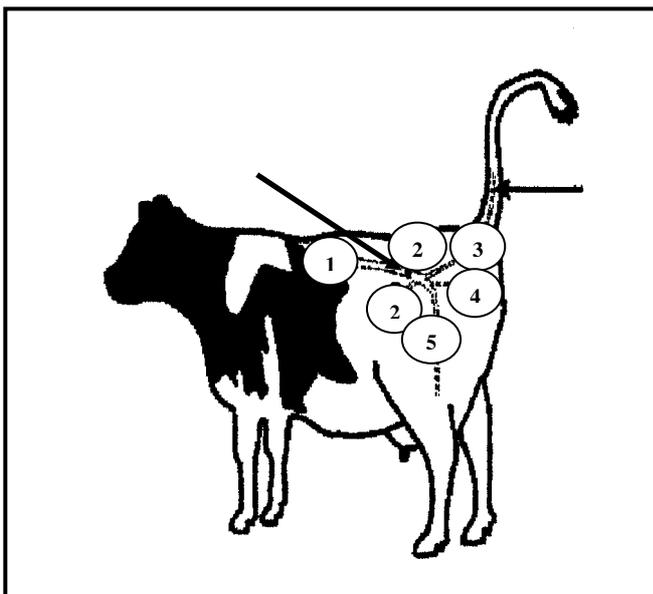


Figure 2 Anatomic pathway of catheterisation of caudal vena cava according to SCHALLENBERGER (1990).

The arrows show the entrance of catheter in the coccygeal vein and its end at the ovary veins orifices.

1. caudal vena cava
2. ovary veins
3. coccygeal vein
4. iliac vein
5. external iliac vein

Animals were handled according to the procedures approved by the ministry of nutrition, agriculture, forest, and fishery of the government of Mecklenburg-Vorpommern, Germany (VI 522a-7221.31-1-024/98). One cow of the control group was excluded due to claw disease.

3.1.2 Feeding

Treatments were arranged as a 2 x 1 factorial design with normal dietary fat content in the diet of control group or inclusion of fat instead of starch in the diet of fat group. Isocaloric and isoproteineous (on the basis of utilizable protein) diets (with regard to the total intake) were formulated. Ingredients composition of diets is presented in Table 1. In the fat group, 2.9 kg tapioca (about 1.9 kg starch) were replaced by about 1 kg of Ca salts of FA and 1 kg of protected soybeans (about 0.8 kg of fat) to reach a total fat content of 2.6% vs. 6.3% (DM basis) in the control and fat group, respectively.

Table 1. Rations used in both groups of Experiment I.

Items	Control group	Fat group
Corn silage, kg DM	5.5	5.5
Grass silage, kg DM	1.9	1.9
Hay, kg DM	2.2	2.2
Dried beet pulp, kg DM	2.7	2.7
Crushed corn grains, kg DM	4.4	4.4
Extracted soybean meal, kg ¹ DM	2.8	2.2
Protected soybeans (38.7% CP), kg ² DM	-	0.88
Cassava (Tapioca), kg DM	2.6	
Ca salts of FA, kg ³ DM		1.00
Urea, kg DM	0.10	0.06
Protected Met, Lys, kg DM	0.04	0.04
Feed lime, kg DM	0.20	
Mineral-vitamin mixture, kg ⁴ DM	0.40	0.40

¹ In fat group, part of it (31%) was protected (Bioprofin[®]-S, Wulfa-Mast GmbH, Dinklage, Germany).

² Bioprofin[®]-V (Wulfa-Mast GmbH, Dinklage, Germany).

³ 80% Nurisol[®] (Nagel KG, Hamburg, Germany), it was prepared from palm oil and 20% Hajenol[®] (Harles and Jentsch GmbH, Uetersen, Germany), it was prepared from soybeans and sunflower oil.

⁴ Commercial Mineral-vitamin mixture (Aktive M, Schaumann KG); constituents pro kg: 140 g calcium, 35 g phosphorus, 100 g sodium, 60 g magnesium, 4500 mg zinc, 2500 mg manganese, 900 mg copper, 360 mg iodine, 40 mg cobalt, 32 mg selenium, 400000 IU vitamin A, 45000 IU vitamin D₃, and 2250 mg vitamin E.

Animals were given controlled diets as total mixed ration (**TMR**) semi-ad libitum, focused on maintaining a maximum energy intake. The amounts of feeds offered and refused were recorded daily to evaluate the nutrients intake. The feed supply was corrected weekly on the basis of individual performance.

Table 2. Chemical composition of the used rations during experiment I.

Items	Control group	Fat group
Organic matter, % of DM	91.7	91.2
Crude fibre, % of DM	13.9	14.5
N-free extract, % of DM	59.2	52.2
Crude protein, % of DM	16.0	16.6
Utilizable protein ¹ , % of DM	16.0	16.0
Ether extract, % of DM	2.6	6.3
Starch, % of DM	23.7	15.6
NEL, MJ/kg DM	7.0	7.4
¹³ C in concentrate mixture, δ-‰	-20.1	-21.0
¹³ C in grounded corn grains, δ-‰	-9.8	-9.8
¹³ C in tapioca, δ-‰	-24.1	
¹³ C in protected fat, δ-‰		-28.1
¹³ C in dietary fat, δ-‰	-23.8	-26.4

¹ Utilizable protein is the sum of ruminal undegradable protein plus ruminal microbial protein at the duodenum. It was calculated on the basis of analysed nutrients and tabulated values of different ingredients of the diets, using the formulas VIc and VIId of the DLG – feed stuffs table for ruminants (DLG 1997).

3.1.3 Milking

Cows were milked three times daily (0400, 1100, and 1900 h), and milk production was recorded at each milking during the first 100 d of lactation.

3.1.4 Body weight and thickness of back subcutaneous fat

Body weight

Body weights were recorded weekly after the first milking during the experimental period (2 to 15 week p.p.) to evaluate the body weight changes during the lactation period.

Back subcutaneous fat thickness

Dorsal fat thickness of the back was estimated using the ultrasonic method described by STAUFENBIEL (1992), which requires experience to differentiate between the different tissue layers under the skin and also to determine the most representative site of back subcutaneous fat.

The site of measurement is just below a line between the dorsal part of the *Tuber ischiadicum* and the high extremity of the *Tuber coxae*, halfway between the points of $\frac{1}{4}$ and $\frac{1}{5}$ the distance between the dorsal part of the *Tuber ischiadicum* and the high extremity of the *Tuber coxae*. The site is located ventrally between the end of *Crista sacralis* and the end of *Os sacrum*. The latter appears by raising the tail upward (Fig. 3). The ultrasonic method used the A and B mode imaging technique with a 5 MHz to 7.5 MHz ultrasonic probe. The probe was placed directly on the skin surface of the measurement site (CIMBAL 1990) to determine distinctly the layer of *Fascia profunda* which has to be differentiated from the other layers. This differentiation is the function of B mode imaging technique, because the fat layers are non-homogeneous as seen in Fig. 4 (STAUFENBIEL 1992).

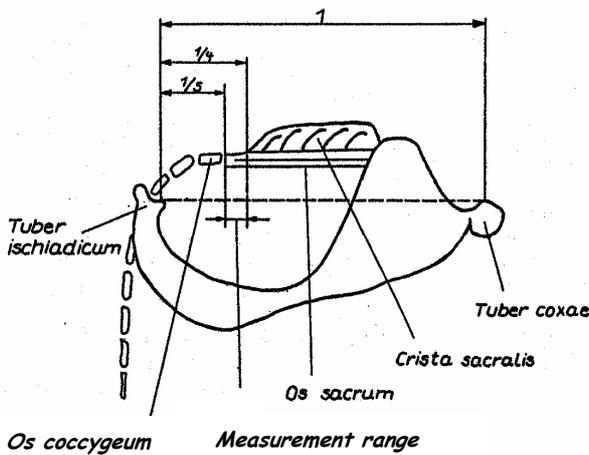


Figure 3. Surgical pathway of the determination of the site of measurement of back fat thickness.

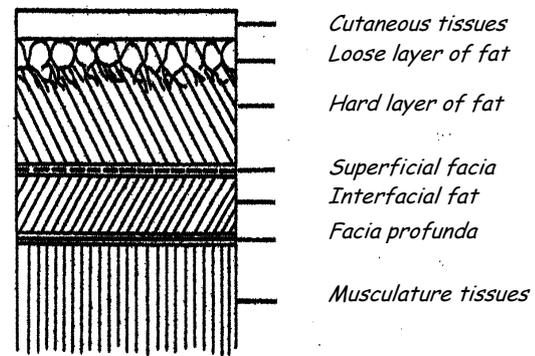


Figure 4. Different anatomical tissues layers of cow back.

3.1.5 Sampling and intravenous infusion of $^{15}\text{N}_2$ -urea

Sampling

Feeds

Dry matter contents of corn and grass silage were estimated daily using a 60°C forced-air oven for 24 h and a 105°C oven for 3 h. Results were used to adjust the ratios of forage and concentrate in the TMR. Corn silage, grass silage, hay, and concentrate samples were collected daily and pooled weekly throughout the experiment, dried for 24 h in a 60°C forced-air oven, grounded to pass a 1-mm Wiley mill screen (Brabender® OHG, Duisburg, Germany), air-equilibrated and analysed for DM, ash, CP, CF, crude fat, starch, and $^{13}\text{C}/^{12}\text{C}$ ratio.

Milk

Milk was sampled weekly from the three consecutive daily milkings and preserved in 20 ml plastic tubes with 0.03 ml of 5% sodium azide (NaN_3 , MERCK, Darmstadt, Germany) and pooled according to milk yield. The pooled samples were stored at 4°C for about 3-4 h after the taking of the last sample till analysis of fat, protein, lactose, urea, and total cells. Unpreserved milk samples were collected and pooled as the preserved samples and stored immediately at -20°C till the analysis of milk allantoin in addition to analysis of $^{13}\text{C}/^{12}\text{C}$ in milk fat, casein and lactose.

Blood

Blood samples were taken weekly from the coccygeal vein by temporary catheters and in some cows by permanent catheters. Three blood samples were collected weekly (2 to 15 weeks p.p.) into 9-ml tubes (Monovete®, SARSTEDT, Nümbrecht, Germany) that contained 1.6 mg potassium-EDTA/ml blood, between 1000 and 1100 h. Tubes were placed into crushed ice. Plasma was isolated from two tubes by centrifugation (1400 g, 15 min, 4°C, Multifuge 3S/3S-R, Heraeus, Osterode, Germany). Samples for each metabolite (glucose, insulin, NEFA, β -HBA, free AA, and urea) were stored separately at -20°C in microcentrifuge tubes (1.5 ml, Eppendorf, Hamburg, Germany). Blood samples were stored in a gas-tight tube at -20°C till analysis of ^{13}C in HCO_3^- . Three cows were excluded from each group during estimation of ^{13}C in HCO_3^- in blood due to technical problems during isotope measurements.

Intravenous infusion of $^{15}\text{N}_2$ -urea and blood sampling for urea kinetic

To estimate the urea pool and flux in the body of cows, the permanently catheterised cows (one cow of the control group was excluded due to infection) were injected with 2 mg $^{15}\text{N}_2$ -urea (95 atom-% ^{15}N , Berlin-Chemie, Berlin, Germany) per kg $\text{BW}^{0.75}$ dissolved in about 15 ml of sterile physiological saline and followed by infusion of about 15 ml of NaCl solution to assure the ^{15}N -urea infusion. Before taking of blood samples, about 2-3 ml blood were taken and discarded, and samples were taken in 9 ml tubes (containing 1.6 mg potassium-EDTA/ml blood) according to the following time scheme: -5, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, 480, 720, 960, and 1440 min after ^{15}N -injection. The samples were placed into crushed ice. The plasma was isolated as previously mentioned and was used for estimation of ^{15}N in urea. The estimation of urea kinetic was carried out in catheterised cows during the period from 6 to 10 weeks of lactation. Each measurement was repeated after 2-4 weeks.

3.1.6 Analytical methods and procedures

Duplicate analyses were carried out for all parameters, as described under 3.1.6.1 to 3.1.6.3.

General information about isotope analyses

Isotope ratios of carbon and nitrogen were assayed by isotope ratio mass spectrometry (IRMS) using an on-line configuration of an elemental analyser with zero-blank autosampler (EA 1108, Fisons Instr., Rodano Milan, Italy), coupled to a continuous flow interface (ConFlo II, Finnigan MAT, Bremen, Germany), which was connected to an isotope ratio mass spectrometer (delta S, Finnigan MAT, Bremen, Germany).

The enrichment of an isotope near natural abundance levels is usually reported as delta, a value given in parts per thousand or per mil [" δ -‰"]. These data values are not absolute isotope abundances (for instance atom-%) but differences between sample readings and one of the commonly used natural abundance standards, which are considered as delta = zero.

Carbon has two stable isotopes – ^{12}C (about 98.8 atom-%) and ^{13}C (about 1.1 atom-%). ^{13}C -abundance was measured against a standard CO_2 , which was calibrated with a reference (IAEA-CH-6, International Atomic Energy Agency, Vienna). The reference material was calibrated against the international PDB (carbonate from a marine fossil of the Pee Dee Belemnite formation in South Carolina) standard for carbon (atom-% $^{13}\text{C} = 1.1112328$). Absolute isotope ratios (^{13}C atom-%: ^{12}C atom-%) are measured for sample and standard. The difference between $^{13}\text{C}/^{12}\text{C}$ ratios of samples and of the PDB-standard was expressed as parts per million as following:

$$^{13}\text{C} [\delta \text{‰}] = \frac{^{13}\text{C}:^{12}\text{C}_{\text{sample}} - ^{13}\text{C}:^{12}\text{C}_{\text{standard}}}{^{13}\text{C}:^{12}\text{C}_{\text{standard}}} \times 1000$$

The PDB standard has a higher $^{13}\text{C}/^{12}\text{C}$ ratio than nearly all other natural carbon-based substances, giving the naturally occurring samples negative delta values. Furthermore, some physical and chemical processes discriminate the heavier atoms resulting in reaction products that are isotopically lighter or heavier than their precursor materials. For instance, carbohydrates and proteins contain more ^{13}C than lipids. The synthesis of FA from carbohydrates includes the decarboxylation of pyruvate to acetyl-CoA. This decarboxylation is implicated in the process of ^{13}C discrimination, resulting in a lower ^{13}C -content in lipids (DeNIRO and EPSTEIN 1977).

Nitrogen exists also in two stable isotopes – ^{14}N (approximately 99.6 atom-%) and ^{15}N (approximately 0.4 atom-%). The ^{15}N -enrichment was measured against a standard gas, which was calibrated against atmospheric air nitrogen (atom-% $^{15}\text{N} = 0.3663033$) with ammonium sulfate as reference (IAEA-N-1).

Results of the study with i.v. infusion of isotopes are expressed in units of atom-% excess or delta-‰ excess. This specifies the level of isotopic abundance above a given background, which is considered zero. The background reading in atom-% or delta-‰ (i.e. before tracer application) is subtracted from the experimental value to give atom-% excess or delta-‰ excess.

3.1.6.1 Feed composition and ^{13}C content

Generally, the Weender Feed Analysis method (NAUMANN and BASLER 1988) was applied for determination of ash, CP, CF, and crude fat content. Crude fat determination was conducted with HCl-hydrolysis according to KUHLA et al. (1983). Starch content was determined according to the method mentioned by ZWIERZ et al. (1981), using starch digestion by a thermostable amylase and anthrone sulfuric acid as indicator reagent. For ^{13}C determination, about 1.5 mg of the finely milled sample was weighed (M5P, Sartorius, Göttingen, Germany) into tin capsules (Code 24005300, FISOONS). The capsules were closed, folded, and then put in the auto sampler of the elemental analyser for analysis. The reproducibility (external precision) of replicate analyses was 0.27 δ -‰.

3.1.6.2 Milk composition and ^{13}C content of its constituents

Milk samples were analysed in the controlled counter association for performance and quality inspection (LKV Mecklenburg-Vorpommern e.V.) for fat, protein, lactose (by infrared spectrophotometric method with Milcoscan 6000 FT system), urea (colorimetrically using a Scalar-Autoanalyzer), and total cells (by a fluorescenceoptic method with a Fossomatic 5000 system; all instruments from Foss Electronic, Hillerod, Denmark).

^{13}C analysis

Milk fat, protein and lactose were separated as follows:
About 50 ml of milk were centrifuged at 900 x g and 4°C for 20 min (Multifuge 3S/3S-R, Heraeus, Osterode, Germany) to separate the fat. The fat layer was taken off by a small spoon, washed with deionised water, and frozen. The pH of skim milk was adjusted at 4.6 by HCl (0.1 mol · L⁻¹) and centrifuged with the same centrifuge at 2800 x g and 4°C for 10 min to separate the casein from the whey. The supernatant was used for lactose isolation. The precipitate was washed with deionised water (pH 4.6) and centrifuged again at 2800 x g and 4°C for 10 min for two times and dried at 90°C. The whey was boiled in a water bath at 100°C for 1h, then cooled at room temperature and centrifuged again at 2800 x g for 20 min at 4°C to separate the albumin from the fluid. The supernatant was evaporated at 80°C till became 3 ml which was centrifuged at 2800 x g for 10 min at room temperature to separate a totally clear fluid, which was put in the refrigerator at 4°C for 2 days. This clear fluid crystallized at 4°C to lactose crystals, which were

separated by decantation and dried at 90°C. The dried casein and lactose crystals were milled using a high-frequency vibration mill (Retsch Mill, MM 200; GmbH, Haan), which ground the crystals to very fine particles. The milk fat, casein, and lactose were assayed for their $^{13}\text{C}/^{12}\text{C}$ ratio as previously mentioned (3.1.6.1) to calculate the ^{13}C -enrichment. For this purpose, about 1.2 mg was weighed in tin capsules for combustion in elemental analyser.

Milk allantoin

The unpreserved stored milk samples of cows were thawed and prepared for analysis of allantoin content by separation of milk fat in 40 ml milk by centrifugation at 900 x g and 4°C for 15 min (Biofuge *Stratus*, Heraeus, Osterode, Germany).

One ml skim milk or 1 ml of a stock solution was mixed with 2 ml trichloroacetic acid (20%, w/v), placed for 15 min in ice bath and then centrifuged for 30 min at 26000 x g at 4 °C (Biofuge 28 RS, Heraeus, Osterode, Germany). The supernatant was filtered (0.22 µm) and used for analysis of allantoin according to the analytical procedure described in detail by CHEN and GOMES (1992) as follows:

One ml of the filtered sample was pipetted into a 15 ml tube, 5 ml of distilled water and 2 ml of 0.5 M NaOH were added. The content of the tube was mixed thoroughly and heated at 100°C for 7 min. After that, 1 ml 0.5 M HCl and 1 ml 0.023 M phenylhydrazine hydrochloride were added and heated again (100 °C, for 7 min). After cooling at 20°C for 7 min, 3 ml HCl (37%) and 1 ml 0.05 M potassium ferricyanide were added. The absorbance was read by the spectrophotometer (GENESIS 20) against water at 522 nm after 15 min at room temperature.

For calibration a stock solution of 632 µmol/l allantoin was prepared by dissolving of 100 mg allantoin (F0123, Sigma) in 10 ml 0.01 M NaOH and diluting to 1000 ml with distilled water. The calibration curve was linearly in the range of 0.1-1.0 OD (optical density).

The daily net amount of microbial protein synthesized in the rumen was calculated according to LEBZIEN et al. (1993). The following equation was used:

$$\text{Microbial-N (g/d)} = \text{allantoin excretion in milk (mmol/d)} \cdot 235 / 9.33$$

3.1.6.3 Blood parameters

^{13}C in blood bicarbonate

Two ml of each blood sample were put into individual exetainers (11 ml, Labco Limited, Buckinghamshire, UK). The exetainers were previously flushed with pure argon (4.6, Linde AG, Germany) and capped. One ml of lactic acid (10%, Merck, Darmstadt, Germany) was injected into each exetainer through a septum. The lactic acid serves to react with bicarbonate of the blood

to release CO₂. The sample was mixed and after 2 h, the gas phase of each exetainer was assayed for ¹³C-enrichment using IRMS. For this purpose, a GasBench II and a IRMS DELTA plus XL (Finnigan MAT, Bremen, Germany) were used as equipment. Enrichment data were calculated as previously mentioned. The used reference CO₂ was calibrated to an international standard. The reproducibility (external precision) of replicate analyses was 0.10 δ-‰.

Plasma glucose, insulin, NEFA, and β-HBA (Received service of analysis)

Glucose was estimated enzymatically by the glucosidase method using an equipment of Bayer (BDE 08306263; 04619399, Bayer, Leverkusen, Germany). Insulin determination was performed by the porcine insulin radio immunoassay (¹²⁵RIA) kit (PI-12K, Linco Research, Inc., St. Charles, MO, USA), which used purified human insulin as standard, a first antibody raised in guinea pigs, and a goat-anti-guinea pig IgG for the bound/free separation. A standard curve was prepared at concentrations from 2 to 200 µl. Cross-reactivity with bovine insulin was 90%. Previous findings have shown that the linco assay exhibited a good linearity for the range of 25 to 100 µl of bovine plasma samples with different insulin concentrations. Though the more available human insulin kit was used for estimation of plasma insulin level in these comparative animal groups. All samples were analysed in duplicate. The sensitivity of the insulin RIA was at 2 µl/ml calculated after measurements by a multi-crystal-gammacounter with a RIA program (LB 2104, Berthold, Bad Wildbad, Germany). Intra- and inter-assay coefficients of variation (precision and reproducibility) were 4.3 and 8.2%, respectively.

NEFA were determined enzymatically using a Randox kit (RB 1007) and β-HBA was estimated spectrometrically using a kit from Randox (RB 1008).

Plasma free amino acids

Plasma samples were thawed and deproteinized to estimate the free AA during weeks 6 and 14 of lactation by automatic ion exchange chromatography using the amino acids analyser Biochrom 20 plus (Biochrom Ltd. Cambridge, UK), equipped with 120 x 4.6 mm ULTROPAC-4 lithium prewash column and 200 x 4.6 mm ULTROPAC-8 lithium high-performance column. Deproteinization of plasma was carried out according to the method 2 of Biochrom Ltd. in the following procedures:

Procedure

- 50 mg of 5-sulphosalicylic acid were put in a small conical centrifuge tube and cooled at 4°C.
- 1 ml of thawed plasma (4°C) was added to the tube and mixed immediately and then was put in the refrigerator at 4°C for 1 h.

- The mixture was centrifuged at high-speed, 17000 x g at 4°C for 15 min (Biofuge *stratus*, Heraeus, Osterode, Germany).
- The clear supernatant was adjusted to pH 2.2 by 0.3 M lithium-hydroxide using pH-meter (pH probe blue line 16, pH meter CG 841, Schott, Mainz, Germany).
- The solution was weighed and stored at -20°C till analysis.
- The clear supernatant was filtered through a 0.2 µm membrane micro-filter before AA analysis in the filtrate.

Plasma urea

Urea was estimated according to the method of VOIGT and STEGER (1967) by aid of microdiffusion as follows:

Solutions and standards:

All chemicals were purchased in high quality from Merck (Darmstadt, Germany).

- Indicator prepared from 0.033 g bromocresol green, 0.066 g methylred in 100 ml ethanol.
- Boric acid solution prepared from 5 g boric acid with 200 ml ethanol and 790 ml deionised water in addition to 10 ml of the indicator.
- Phosphate buffer with pH 6.8 to 6.9 prepared from 448 ml of KH₂PO₄ (0.066 M) and 552 ml of Na HPO₄ (0.066 M).
- Concentrated solution of K₂CO₃ (8.2 M), prepared at 25 °C.
- Standard solution formed from 60 mg urea in 100 ml deionised water.
- 0.01 M HCl
- Urease ≥ 5 U/ mg.

Procedure:

- A double replicate of diffusion vessels (conical flasks with a diffusion cup, which distended from the cover of the flask).
- 5 ml of boric acid solution were put in the conical flask of diffusion vessel.
- 0.5 ml plasma were put in the diffusion cup with 1ml of phosphate buffer and then mixed with traces of urease by thorough rotator movement.
- The flask was left for 3 h at 40°C, and then was cooled to room temperature.
- 1 ml of K₂CO₃ solution was added to the content of the diffusion cup and mixed again with thorough rotator movement.
- Flasks were left at room temperature for 24 h.
- Boric acid solution was titrated with 0.01 M HCl.

- Triplicate of standard and blank samples were prepared with the same method except the plasma sample was replaced by 0.5 ml of standard solution or 0.5 ml deionised H₂O.

Calculation:

Urea content of blood (mmol/l) = the consumed amount of 0.01 M HCl · 10.

¹⁵N-content of plasma urea

The same procedure of determination of plasma urea was also used to isolate the ammonia from urea for determination of ¹⁵N as follows:

In a conical flask, 5 ml from 0.01 M HCl were taken instead of boric acid as absorption solution to accumulate the ammonium. After 24 h at room temperature, the content of flask (5 ml 0.01 M HCl + ammonium) was placed in small closed container and the walls of the flasks were washed with deionised water. Before the analysis of ¹⁵N was done, the solution was evaporated (80°C, air-forced oven, UT 6060, Heraeus, Osterode, Germany) till dryness and then was resolved in 150 µl of purified water. From this solution, 100 µl were put into a tin capsules (Code 24005300, FISON) and evaporated (at 70 °C, air-forced oven, UT 6060, Heraeus, Osterode, Germany). The capsules were closed and carefully folded and the ¹⁵N-excess was measured as previously mentioned (3.1.6). The reproducibility (external precision) of replicate analyses was 0.0008 atom-%.

3.1.7 Calculation and statistical analysis

3.1.7.1 Calculations

Net energy intake

The intake of energy was calculated on the basis of DM intake (kg/d) and the analysed content of nutrients (g/kg DM) as follows (GfE 2001):

Gross energy (GE): (AfB 1998):

$$GE \text{ (MJ/kg DM)} = 0.0239 \cdot CP + 0.0398 \cdot CL + 0.0201 \cdot CF + 0.0175 \cdot NFE.$$

where CP = crude protein; CL = crude lipid; CF = crude fibre; NFE = N-free extract.

Metabolizable energy (ME):

For concentrate (Kuhla 1999):

$$ME \text{ (MJ/kg DM)} = 12.76 - 0.0147 \cdot CA + 0.0165 \cdot CL - 0.0038 \cdot CF + 0.00234 \cdot CP$$

where CA = crude ash.

For corn silage (AfB 1998):

$$ME \text{ (MJ/kg DM)} = 14.03 - 0.01386 \cdot CF - 0.01018 \cdot CA$$

For grass silage (AfB 1998):

$$ME \text{ (MJ/kg DM)} = 13.99 - 0.01193 \cdot CF + 0.00393 \cdot CP - 0.01177 \cdot CA$$

For hay (AfB 1998):

$$\text{ME (MJ/kg DM)} = 13.69 - 0.01624 \cdot \text{CF} + 0.00693 \cdot \text{CP} - 0.0067 \cdot \text{CA}$$

For protected fat (AfB 1995)

$$\text{ME (MJ/kg DM)} = \text{DCL} \cdot 0.0312$$

where DCL = digestible crude lipid in g/kg DM.

The digestibility of Ca salts of FA was estimated on 4 growing bulls (375 ±16 kg BW) in 2 periods (SCHIEMANN 1981). The diet was composed of chopped hay and concentrate mixture (40 : 60) in the first period and the same ration plus Ca salts of FA (350 g/d) was fed in the second period. Faeces were collected in each period of 7 d. The values of digestibility of total fat in the rations was 54.0 ± 0.9% and 76.4 ± 3.2% for the first and second period, respectively. The digestibility of the protected fat was calculated by difference, which was 82.7 ± 3.9%.

Net energy for lactation (NEL):

For all feedstuffs, except the Ca salts of FA (GfE 2001):

$$\text{NEL (MJ)} = 0.6 [1 + 0.004 (q-57)] \cdot \text{ME (MJ)}$$

Where $q = \text{ME/GE} \cdot 100$.

For Ca salts of FA (HOFFMANN 1996):

$$\text{NEL (MJ/kg DM)} = \text{ME (MJ/kg DM)} \cdot 0.72$$

4% Energy-corrected milk

Actual milk production was adjusted for milk composition to calculate energy corrected milk (ECM, 4% fat, 3.15 MJ/kg) as follows:

$$\text{ECM (kg/d)} = \text{actual milk (kg)} \cdot [\text{fat}\% \cdot 0.14 + 0.44]$$

Energy balance

Energy balance was calculated as follows:

$$\text{EB} = \text{NEL intake (MJ/d)} - [\text{maintenance energy requirement (MJ/d)} + \text{milk energy (MJ/d)}]$$

Maintenance energy (MJ/d) was calculated as $0.293 \cdot \text{BW}^{0.75}$ (GfE 2001)

Milk energy (MJ/d) = milk (kg) · [0.95 + 0.38 · fat% + 0.21 · protein %] (GfE 2001)

Urea turnover

For the calculation of ¹⁵N-excess, the corresponding background enrichment (sample at 5 min before injection of ¹⁵N₂-urea) was subtracted from the measured values. The infused ¹⁵N (mg excess) was calculated as follows:

$$^{15}\text{N-excess (mg)} = \frac{\text{N (mg)} \times ^{15}\text{N (atom-\% excess)} \times 15}{14 \times 100}$$

14 and 15 are the atomic weights of nitrogen isotopes.

The dynamics of ^{15}N in blood urea following a two-compartment model was described using the equation of NOLAN and LENG (1974):

$$y(t) = y(0) \cdot (A \cdot e^{-(k \cdot t)} + B \cdot e^{-(l \cdot t)})$$

where,

$$\begin{aligned} y(t) &= {}^{15}\text{N-excess of urea at known time} && [\text{Atom-}\% \text{ }^{15}\text{N-excess}] \\ t &= \text{time after isotope infusion} && [\text{Minutes}] \\ y(0) &= \text{Calculated initial } {}^{15}\text{N-excess of urea} && [\text{Atom-}\% \text{ }^{15}\text{N-excess}] \\ A, B &= \text{fractional intercepts at } t(0) \\ k, l &= \text{Turnover constants} && [\text{min}^{-1}]. \end{aligned}$$

The parameters were calculated using the computer with a kinetic analysis program (SAMM II, Version 1.1.1, Samm Institute, Univ. Washington, USA).

The urea pool and flux were calculated. The urea pool is the total urea N in the body expressed as g N, while the total urea flux is the total amount of N passing through the urea pool during 24 h using the following equations:

$$\begin{aligned} \text{Total urea pool N (g)} &= \text{Infused } {}^{15}\text{N (g)} \cdot 100/y(0) \\ \text{Urea flux N (g/d)} &= \text{Total urea pool N (g)} \cdot (y(0) \cdot A \cdot k + y(0) \cdot B \cdot l) \cdot 60 \cdot 24 \end{aligned}$$

3.1.7.2 Statistical analysis

Data of the first experiment were subjected to independent samples t-test, using a one factorial model procedure of SPSS Version 10.0. Values were considered significantly different if $P < 0.05$. Correlation and linear regression analyses were carried out with the same software package.

3.2 Experiment II

The experiment was carried out in four periods in a cross-over design. In each period two cows were used. In the first subperiod of each period, one cow was fed on the control diet and the other fed on fat diet. The diets were exchanged in the second subperiod (Table 3).

3.2.1 Animals, feeding, and milking

Eight Holstein-Friesian lactating dairy cows in the period from the 6th to 10th weeks of lactation were used. Animals were handled according to the procedures approved by the ministry of nutrition, agriculture, forest, and fishery of the government of Mecklenburg-Vorpommern, Germany (LVL M-V/310-4/7221.3-2.2-001/02). Each cow was housed in metabolic cages for 24 days and subjected to a period of feed adaptation before the two consecutive experimental subperiods (6 days for each) as shown in Fig. 5 and Table 3.

Table 3. Periods and subperiods of experiment II in relation to the used diets.

Period	Cows	Ration of 1 st subperiod	Ration of 2 nd subperiod
1	A	Control diet	Fat diet
	B	Fat diet	Control diet
2	C	Control diet	Fat diet
	D	Fat diet	Control diet
3	E	Control diet	Fat diet
	F	Fat diet	Control diet
4	G	Control diet	Fat diet
	H	Fat diet	Control diet

The animals were catheterised by inserting about 30 cm long permanent flexible catheter (Ø 1.4 x 2.1 mm; Certifix[®]; Mono B 430; BRAUN, Melsungen, Germany) into the jugular vein on the second day of the first subperiod, which remained till the end of the second subperiod. The catheters were serviced for two times daily (0800 and 1400 h) and after taking of blood samples with an infusion of 0.9 % NaCl-solution and 2 ml of heparin-NaCl solution (50 IE heparin per ml 0.9% NaCl).

d1	D2	d3	d4	d5	d6	d7	D8	d9	d10	d11	d12	d13	d14	d15	d16
Adaptation before 1 st -subperiod								Catheter insertion	i.v. Infusion				Adaptation before-		
							Feed exchange								
Milk samples															
D1	d18	d19	d20	d21	d22	d23	d24								
7															
2 nd -subperiod					i.v. Infusion										
Milk samples															

Figure 5. Experimental plan used during experiment II.

The diets were given as TMR from 0600 to 2200 h. The diets were formulated as described in Table 4 and 5. The amounts of feed offered and the residues were recorded daily. The animals were milked two times daily (0600 and 1800 h).

Table 4. Rations used for lactating dairy cows during the two subperiods of experiment II.

Items	Control diet	Fat diet
Corn silage, kg, DM	5.9	5.9
Grass silage, kg, DM	3.2	3.2
Dry forage pellets, kg, DM	1.5	1.5
Dried beet pulp, kg, DM	2.4	2.4
Concentrate mixture, kg ¹ , DM	5.8	5.8
Cassava (Tapioca), kg, DM	1.9	
Ca salts of FA, kg ² , DM		0.8
Protected extracted soybean meal, kg ³ , DM		0.6
Urea, kg, DM	0.1	0.1
Feed lime, kg, DM	0.2	
Mineral-vitamin mixture, kg ⁴ , DM	0.2	0.2

¹ Commercial concentrate mixture (KFM Nord, Güstrow, Germany): 35.8 % soy extracted meal, 23.0% triticale, 20.0% corn meal, 15.3% corn gluten feed, 2.0% rape extracted meal, 2.0% NaHCO₃, 1.4% CaO, 0.25% NaH₂PO₄, 0.15% vit. E mixture, 0.05% NaCl

² Nurisol (Nagel KG, Hamburg, Germany).

³ Bioprofin[®]-S (Wulfa-Mast GmbH, Dinklage, Germany).

⁴ Commercial mineral-vitamin mixture (SALVANA products); constituents per kg: 140 g calcium, 80 g phosphorus, 100 g sodium, 50 g magnesium, 3000 mg zinc, 4000 mg manganese, 1500 mg copper, 10 mg iodine, 15 mg cobalt, 10 mg selenium, 800000 IU vitamin A, 60000 IU vitamin D₃, and 1500 mg vitamin E.

Table 5. Chemical composition of rations used during experiment II.

Items	Control	Fat
Organic matter, % of DM	90.1	90.0
Crude fibre, % of DM	14.5	14.9
N-Free extract, % of DM	56.6	52.0
Crude protein, % of DM	16.6	17.4
Utilizable protein ¹ , g/kgDM	165	168
Ether extract, % of DM	2.5	5.8
Starch, % of DM	22.0	16.8
NEL, MJ/kg DM	6.4	7.0

¹ For calculation see Table 2.

3.2.2 Intravenous infusion of ^{13}C -glucose and sampling

Intravenous infusion of ^{13}C -glucose

The cows were infused intravenously with 3.3 mg of D-[U- $^{13}\text{C}_6$]-Glucose (Cambridge Ltd / 99 atom-%) per kg BW^{0.75}. Glucose was dissolved in 15 ml physiological NaCl solution. The infusion was on the morning of the 3rd day of both experimental subperiods, and this infusion followed by the injection of 15 ml of physiological NaCl solution to assure the infusion.

Sampling

Feeds

DM contents of corn and grass silage were determined daily and samples of corn and grass silage and concentrate mixture were taken daily during both subperiods and handled as in experiment I to analyse the different nutrients content and their content of ^{13}C .

Milk

In each period, milk samples from two consecutive milkings (0600 and 1800 h) were taken and pooled according to milk yield. Samples were preserved in 20 ml plastic tubes containing 0.03 ml of sodium azide at a concentration of 5% (NaN_3 , MERCK, Darmstadt, Germany). Milk samples were stored for 3-4 h at 4°C and analysed for fat, protein, lactose, urea, acetone, and total cells. Unpreserved milk samples were taken daily from each milking for 6 d of each subperiod and stored immediately at -20°C till analysis of $^{13}\text{C}/^{12}\text{C}$ in fat, casein, and lactose.

Blood

Blood samples were taken from the jugular vein by permanent catheters on the 3rd days of the two experimental subperiods at 2, 4, 6, and 8 h after the morning feeding. Whole blood samples were placed in crushed ice, and after centrifugation plasma samples were kept at -20°C till analysis of different blood metabolites as described in experiment I. Other blood samples were taken firstly 10 minutes before the i.v. infusion of D-[U- $^{13}\text{C}_6$]-Glucose, then at 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, and 240 min after infusion to estimate $^{13}\text{C}/^{12}\text{C}$ ratio in blood CO_2 . These samples were placed in crushed ice and stored directly at -20 °C till the analysis.

3.2.3 Analytical methods and procedures

Analyses of diets composition (DM, CP, CF, crude fat, and starch), milk composition (fat, protein, lactose, urea and somatic cells), blood parameters (glucose, insulin, urea and free AA) and estimation of $^{13}\text{C}/^{12}\text{C}$ content of milk (fat, casein and lactose) and blood CO_2 in addition to calculation of utilizable protein and NEL were carried out as in experiment I. Milk acetone content was estimated by the colorimetric method of an auto analyser (Rapid Flow Analyser of Perstop Analytical Corp., Stockholm, Sweden).

3.2.4 Calculations and statistical analysis

Calculations

FCM, milk energy, and efficiency of milk production were calculated as in experiment I.

From the estimated ^{13}C in blood CO_2 , the $\delta^{13}\text{C}$ -excess in blood CO_2 was calculated from the difference between the estimated levels at the considered time and the estimated values before the infusion of D-[U- $^{13}\text{C}_6$]-Glucose.

Statistical analysis

Data were analysed statistically by the paired t-test for dependent variables using the procedure of SPSS version 10.0.

4 Results

4.1 Experiment I

4.1.1 Nutrients intake

Table 6 shows that the replacement of starch by protected fat was reflected by a significant increase in the intake of fat for cows fed on a high-fat diet and of starch for cows fed on control diet. The intakes of DM, CF, metabolizable protein, and NEL were not significantly affected. The intake of protein of cows fed on high-fat diet increased compared with that of control group during the experimental period. The proportion of fat energy in the total energy intake was 6.7% in the control group and 15.8% in the fat group.

Table 6. Nutrients intake of dairy cows from week 2 to 15 of lactation during experiment I.

Items	Control	Fat	SEM	P
	n = 15	n = 16		
DM, kg ·cow ⁻¹ ·d ⁻¹	20.82	20.11	1.55	0.214
Crude fibre, kg ·cow ⁻¹ ·d ⁻¹	2.90	3.04	0.26	0.155
Starch, kg ·cow ⁻¹ ·d ⁻¹	4.94	3.17	0.60	< 0.001
Crude fat, kg ·cow ⁻¹ ·d ⁻¹	0.54	1.27	0.10	< 0.001
Crude protein, kg ·cow ⁻¹ ·d ⁻¹	3.34	3.52	0.25	0.049
Utilizable protein, kg ·cow ⁻¹ ·d ⁻¹	3.30	3.42	0.20	0.189
NEL, MJ ·cow ⁻¹ ·d ⁻¹	145.7	148.6	11.51	0.497

4.1.2 Body weight, subcutaneous fat thickness, and energy balance

There were no significant differences in the averages of body weights and the thickness of subcutaneous fat between both groups of lactating dairy cows (Table 7). The thickness of subcutaneous fat was different at the beginning of the experiment and remained significantly lower ($P < 0.05$) during weeks 2, 4, and 6 of the lactation period in the cows fed on a high-fat diet compared to those fed on the control diet. The difference between the groups disappeared during the last 6 weeks (Fig. 6).

Table 7. Averages of body weight, back subcutaneous fat thickness and energy balance of dairy cows in experiment I during the 2nd to 15th weeks.

Items	Control	Fat	SEM	P
	n = 15	n = 16		
Body weight, kg	630.4	614.1	36.5	0.224
Subcutaneous fat thickness, cm	1.05	0.98	0.11	0.113
Tissues energy balance, MJ/d	-17.4	-19.80	5.2	0.717

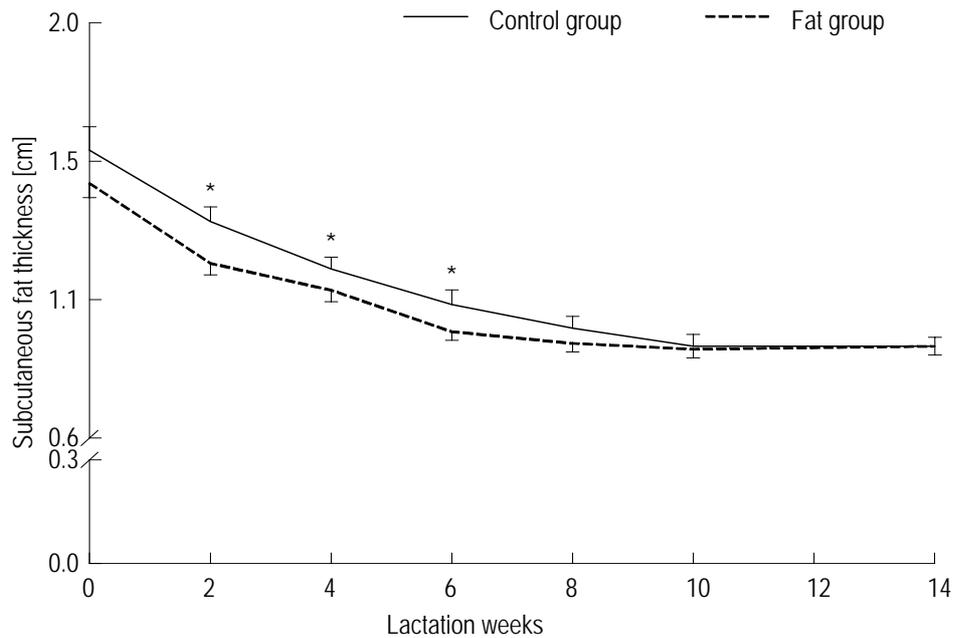


Figure 6. Subcutaneous fat thickness of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference ($P < 0.05$).

The averages of EB of dairy cows were not affected significantly by diets during the experimental period (Table 7). An improvement in EB was seen at the beginning of lactation till the 6th week and was significant at the 4th week as seen in Fig. 7, then the curves of both groups reached the phase of positive EB at the 11th week of lactation.

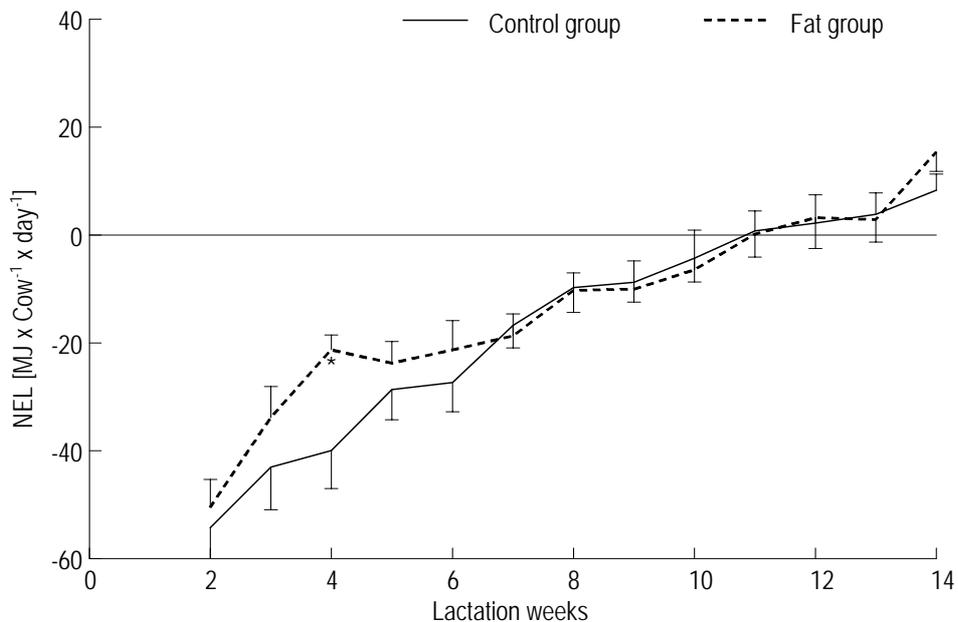


Figure 7. Tissues energy balance of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference ($P < 0.05$).

4.1.3 Milk production

Milk yield and production efficiency

Table 8 shows that the milk yield had a tendency to increase ($P = 0.075$) for the dairy cows fed on a high fat diet compared with the cows of the control group. The milk yield was higher by about $2.7 \text{ kg} \cdot \text{d}^{-1}$ (7 %) than that of the control group. The milk yield curve of the cows of the control group was behind that of cows fed on a high-fat diet from the 6th week till the end of the experimental period, which was significant ($P < 0.05$) during the weeks 10, 11, 12, 13 and 15 of lactation (Fig. 8).

The ECM and efficiency of milk production were not significantly affected by the diets (Table 8).

Table 8. Milk production and composition of dairy cows during experiment I¹.

Items	Control	Fat	SEM	P
	n = 15	n = 16		
Milk, $\text{kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$	38.80	41.53	4.15	0.075
ECM, $\text{kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$	40.49	41.70	4.96	0.503
Fat, %	4.31	4.04	0.59	0.587
Protein, %	3.12	2.91	0.16	0.001
Lactose, %	4.69	4.71	0.13	0.682
Urea, mmol/l	3.57	3.96	0.50	0.040
Fat, $\text{kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$	1.67	1.67	0.28	0.997
Protein, $\text{kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$	1.20	1.21	0.12	0.903
Lactose, $\text{kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$	1.82	1.96	0.20	0.072
Energy, $\text{MJ} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$	125.9	127.7	4.6	0.607
Somatic cells $\times 10^3/\text{ml}$	440	305	471	0.431
Efficiency of milk production ²	0.278	0.281	0.01	0.542

¹ Mean values of week 2 to week 15 of lactation.

² ECM (kg/d) / NEL intake (MJ/d).

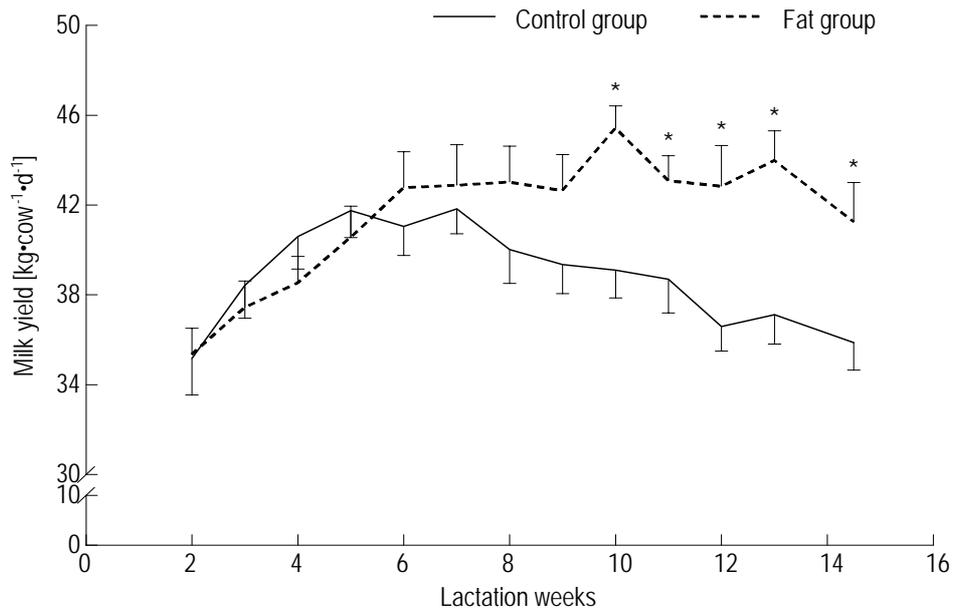


Figure 8. Milk yield of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference ($P < 0.05$).

Milk composition

Milk fat

The means of milk fat percentage and yield were not significantly different between the groups (Table 8). However, the milk fat percentage was depressed relatively in the fat group compared with the control one, which was significant ($P < 0.05$) in weeks 11 and 12 of lactation. The milk fat depression in cows of fat group was parallel with their increased milk yield during the same period (Fig. 8 and 9).

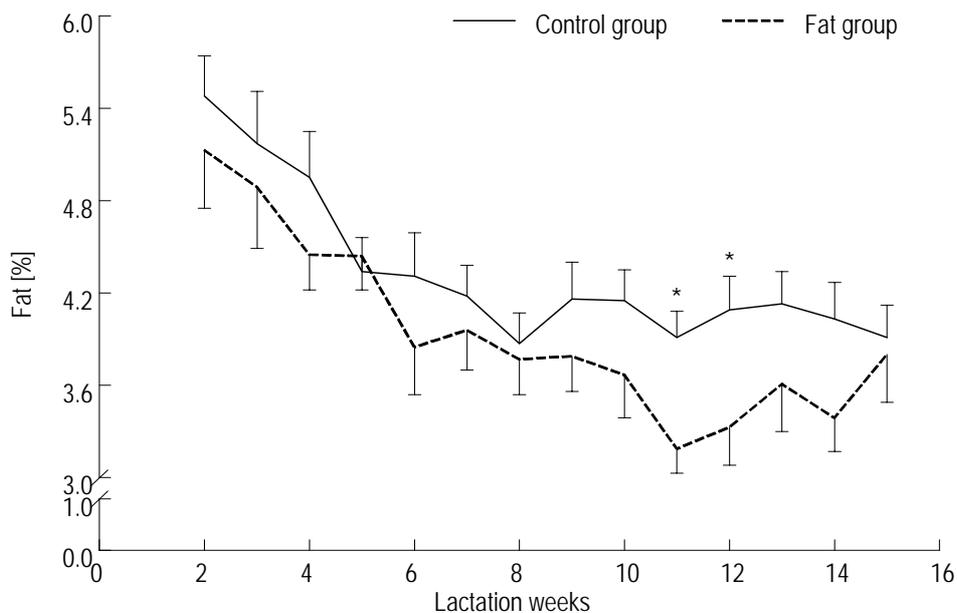


Figure 9. Milk fat content of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference ($P < 0.05$).

Milk protein

Table 8 shows that the milk protein content of the dairy cows fed on the fat diet was significantly depressed ($P = 0.001$) in comparison to the control group, but the milk protein yield was not significantly affected by the diets. The dynamic of the milk protein content in both groups was illustrated in Fig. 10. As seen previously in milk fat content, the depression in milk protein of dairy cows fed on fat diet was parallel with their increased milk yield.

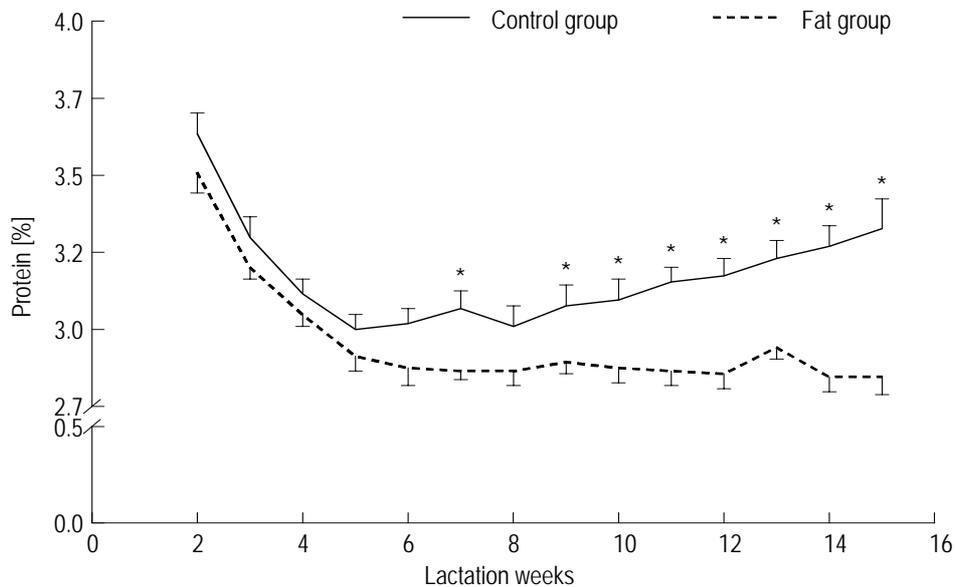


Figure 10. Milk protein content of dairy cows during experiment I. Means of the control ($n = 15$) and fat ($n = 16$) group with SEM. * Significant difference ($P < 0.05$).

Milk lactose

The average yield of milk lactose during the experimental period had a tendency to increase ($P = 0.072$) for cows fed on a high-fat diet compared with those fed on starch diet (Table 8). Milk

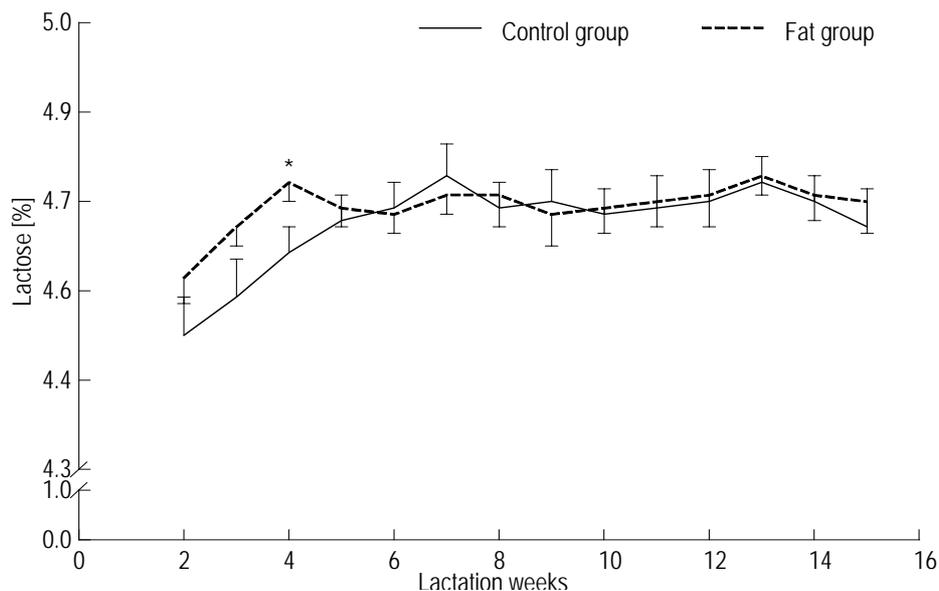


Figure 11. Milk lactose content of dairy cows during experiment I. Means of the control ($n = 15$) and fat ($n = 16$) group with SEM. * Significant difference ($P < 0.05$).

lactose content increased especially at the beginning of lactation in cows fed on a high-fat diet and this increase was significant during the 4th week of lactation (Fig. 11).

Milk urea

The mean of milk urea content was significantly higher ($P = 0.04$) in the fat group than in control one (Table 8). The difference was significant in weeks 8 and 11 of lactation (Fig. 12).

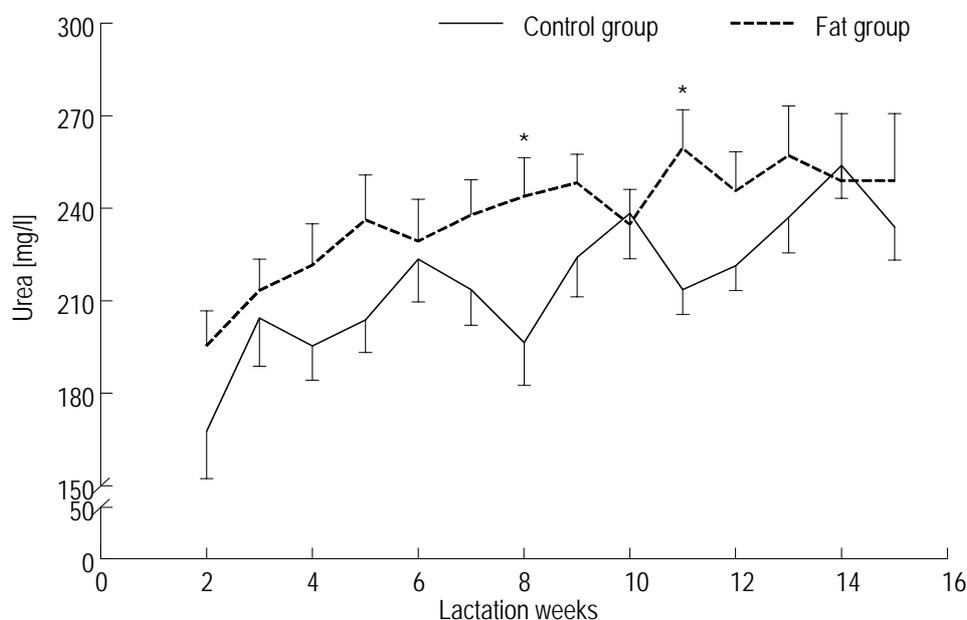


Figure 12. Milk urea content of dairy cows during experiment I. Means of the control ($n = 15$) and fat ($n = 16$) group with SEM. * Significant difference ($P < 0.05$).

Milk energy

As shown in Table 8, the averages of the milk energy yield were not affected by the diets.

4.1.4 Blood parameters

Blood glucose

The means of the levels of glucose in plasma were not affected significantly by replacement of starch by protected fat in diets of cows (Table 9). However, the level was lower in cows of the

Table 9. Glucose, insulin, urea, β -HBA, and NEFA in blood plasma of dairy cows during exp. I¹.

Items	Control n = 15	Fat n = 16	SEM	P
Glucose, mmol/l	3.20	2.83	0.60	0.080
Insulin, μ U/ml	14.76	11.55	3.23	0.010
Blood urea, mmol/l	5.12	5.70	0.93	0.094
β -Hydroxybutyrate, mmol/l	1.16	1.91	0.51	< 0.001
NEFA, μ mol/l	211	307	74.70	0.006

¹ Mean values of week 2 to week 15 of lactation of experiment I.

fat group. As shown in Fig. 13, the glucose curve of cows fed on the fat diet always remains behind that of cows fed on starch diet, which was significant in the 5th and 9th weeks of lactation.

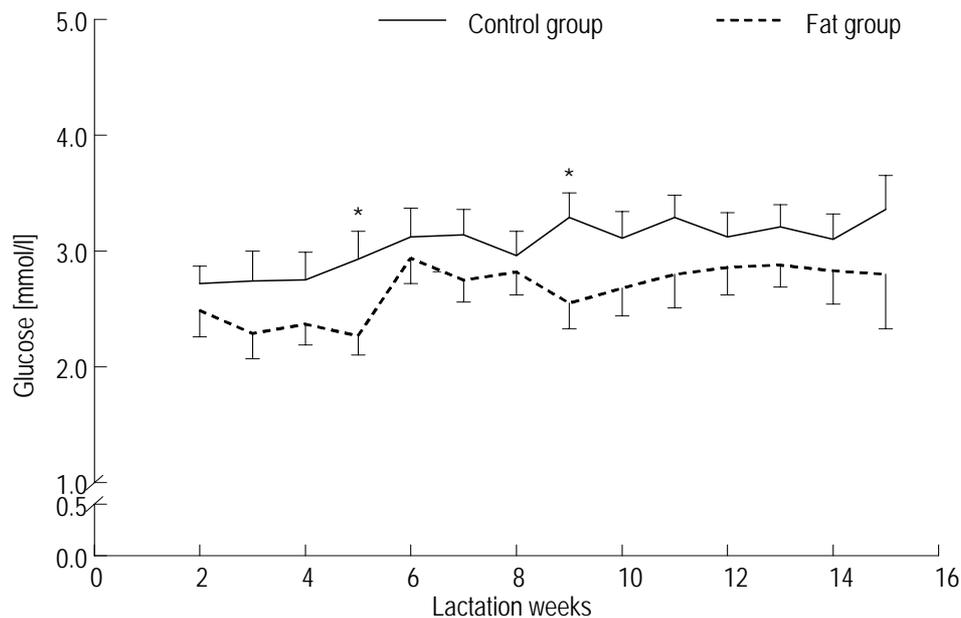


Figure 13. Plasma glucose levels of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference (P < 0.05).

Plasma insulin

In both groups, the insulin concentrations in plasma increased gradually from the beginning of lactation till the 13th week of lactation period. As shown in Table 9 and Fig. 14, the plasma concentrations of insulin were significantly (P = 0.010) decreased in dairy cows fed on the fat diet compared with those fed on the control one.

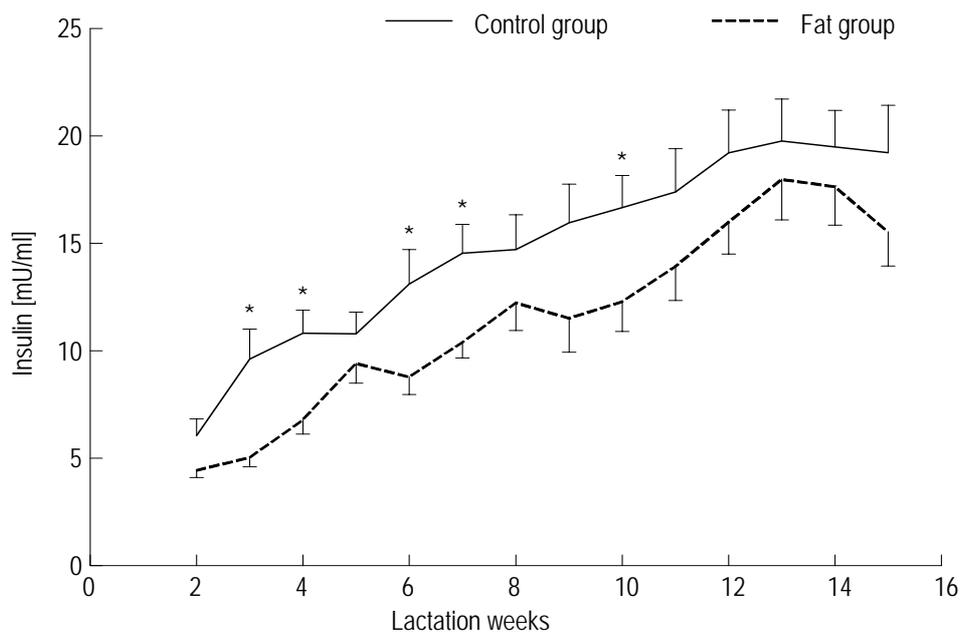


Figure 14. Insulin levels in plasma of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference (P < 0.05).

Plasma β -Hydroxybutyrate (β -HBA)

The mean of β -HBA levels in plasma of cows fed on a high-fat diet increased significantly compared with that of cows fed on the starch diet during the lactation period (Table 9, Fig. 15). The levels of plasma β -HBA in the fat group increased to reach their maximum levels in the 4th and 5th weeks, then decreased gradually till the 14th week of the lactation period (Fig. 15).

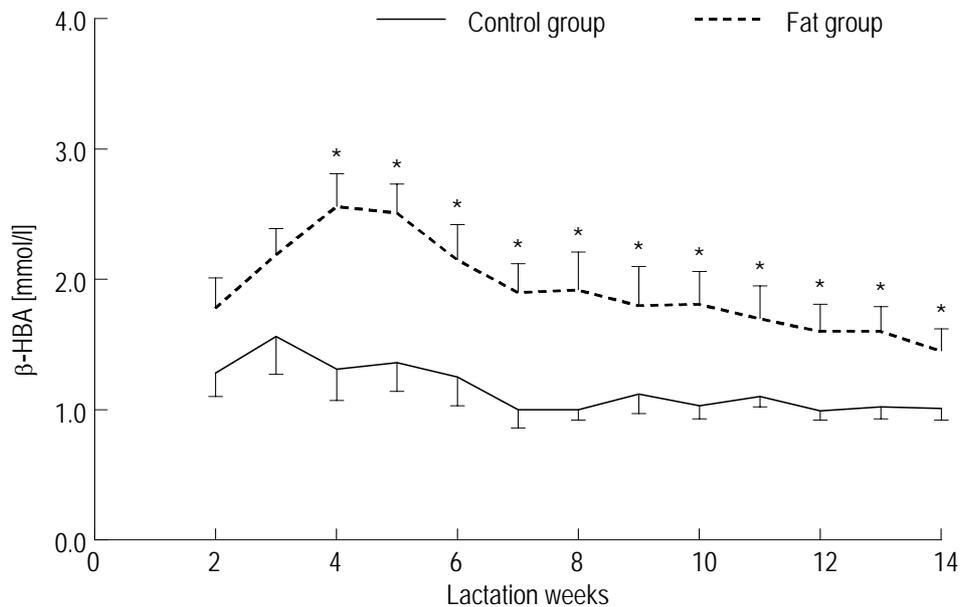


Figure 15. β -HBA levels in blood plasma of cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference (P < 0.05).

Plasma non-esterified fatty acids (NEFA)

The concentrations of NEFA in plasma of both groups of dairy cows increased directly after parturition till the second week of lactation, and then decreased gradually throughout the lactation

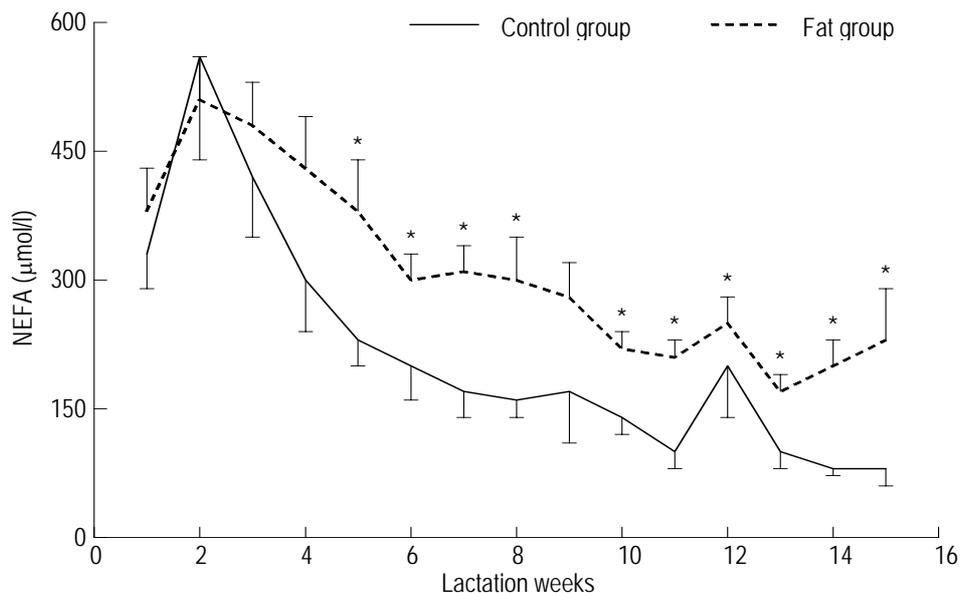


Figure 16. NEFA levels in blood plasma of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference (P < 0.05).

period. The mean of the NEFA levels was significantly ($P = 0.006$) higher for the group of cows fed on a high-fat diet than for those fed on the control diet during the experimental period (Table 9). These increased levels were mostly significant from the 5th week of lactation till the end of the experiment (Fig. 16).

Plasma urea

The plasma urea concentrations of both groups of cows gradually increased from the beginning of lactation till the end of the experiment (Fig. 17). The increased levels of urea in plasma of fat group were mostly higher from the beginning of lactation till the end of the experimental weeks compared with the control group. This increase was significant ($P < 0.05$) during the 7th and 10th weeks of lactation (Fig. 17).

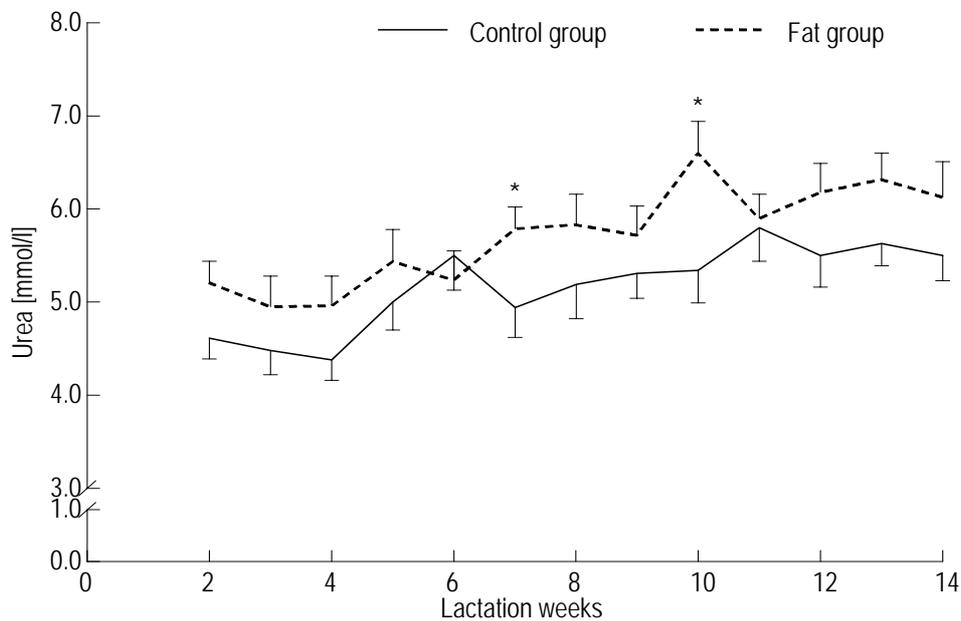


Figure 17. Urea levels in blood plasma of dairy cows during experiment I. Means of the control ($n = 15$) and fat ($n = 16$) group with SEM. * Significant difference ($P < 0.05$).

Plasma free amino acids

The effect of feeding high fat diet to dairy cows on plasma free AA during the experimental period was different according to whether essential or non-essential AA were considered. It is shown in Table 10 that the averages of free essential AA were affected by the diets. The BCAA (Leu, Ile, and Val) and total EAA increased significantly, but Met decreased significantly as well as a tendency for plasma His to decrease during the experimental period. The increased Ile and Leu were significant during both the 6th and 14th weeks of lactation, while only during week 14 for Val. The decrease in Met level was significant during both weeks 6 and 14 of lactation period (Fig. 18).

Table 10. Means of estimated free essential AA (nmol/ml) in blood plasma of dairy cows in the 6th and 14th weeks of lactation during experiment I.

AA	Control	Fat	SEM	P
	n = 15	n = 16		
Methionine	23.3	19.6	4.7	0.040
Threonine	83.1	72.8	19.2	0.151
Isoleucine	98.8	131.7	17.7	< 0.001
Leucine	115.3	147.6	21.9	< 0.001
Valine	184.4	216.8	28.9	0.005
Lysine	89.5	85.3	16.3	0.478
Phenylalanine	64.1	65.5	11.4	0.804
Histidine	44.1	39.4	7.3	0.060
Arginine	68.8	72.9	15.7	0.476
Total EAA	771.4	861.1	94.6	0.015

Table 11. Means of estimated free non-essential AA (nmol/ml) in blood plasma of dairy cows in the 6th and 14th weeks of lactation during experiment I.

AA	Control	Fat	SEM	P
	n = 15	n = 16		
Serine	87.2	73.4	14.3	0.016
Glycine	166.0	175.3	40.6	0.535
Alanine	184.5	180.7	29.7	0.723
1-Methylhistidine	13.2	28.9	7.2	< 0.001
3-Methylhistidine	14.2	15.5	2.7	0.220
Glutamic acid	120.8	130.5	29.4	0.379
Glutamine	130.5	119.7	32.5	0.371
Aspartic acid	24.3	27.6	7.9	0.270
Tyrosine	44.8	41.8	8.5	0.345
Ornithine	63.9	62.6	9.4	0.719
Hydroxyproline	11.0	11.8	2.9	0.442
Proline	68.4	71.1	9.3	0.428
Cysteine	ND ¹	ND ¹		
Total NEAA	928.7	938.7	115.4	0.815
Total AA	1700	1786	322	0.297
EAA/NEAA	0.81	0.92	0.12	0.013

¹ Concentrations were not detectable.

The total essential AA increased significantly for the cows fed on a high-fat diet compared to those fed on the control diet during the experimental period (Table 10). The averages of the levels of single NEAA were not affected significantly except for significant increased concentrations of 1-Methylhistidine and significant decreased concentrations of Ser, which was clearly observable for both amino acids during both 6 and 14 weeks of lactation (Fig. 18). The total non-essential AA were not affected significantly by the fat intake of dairy cows (Table 11).

The average ratio of the total EAA to total NEAA increased significantly in the dairy cows fed on the fat diet in comparison to those fed on the control diet during the experimental period (0.81 vs. 0.92 for control and fat groups, respectively) as shown in Table 11.

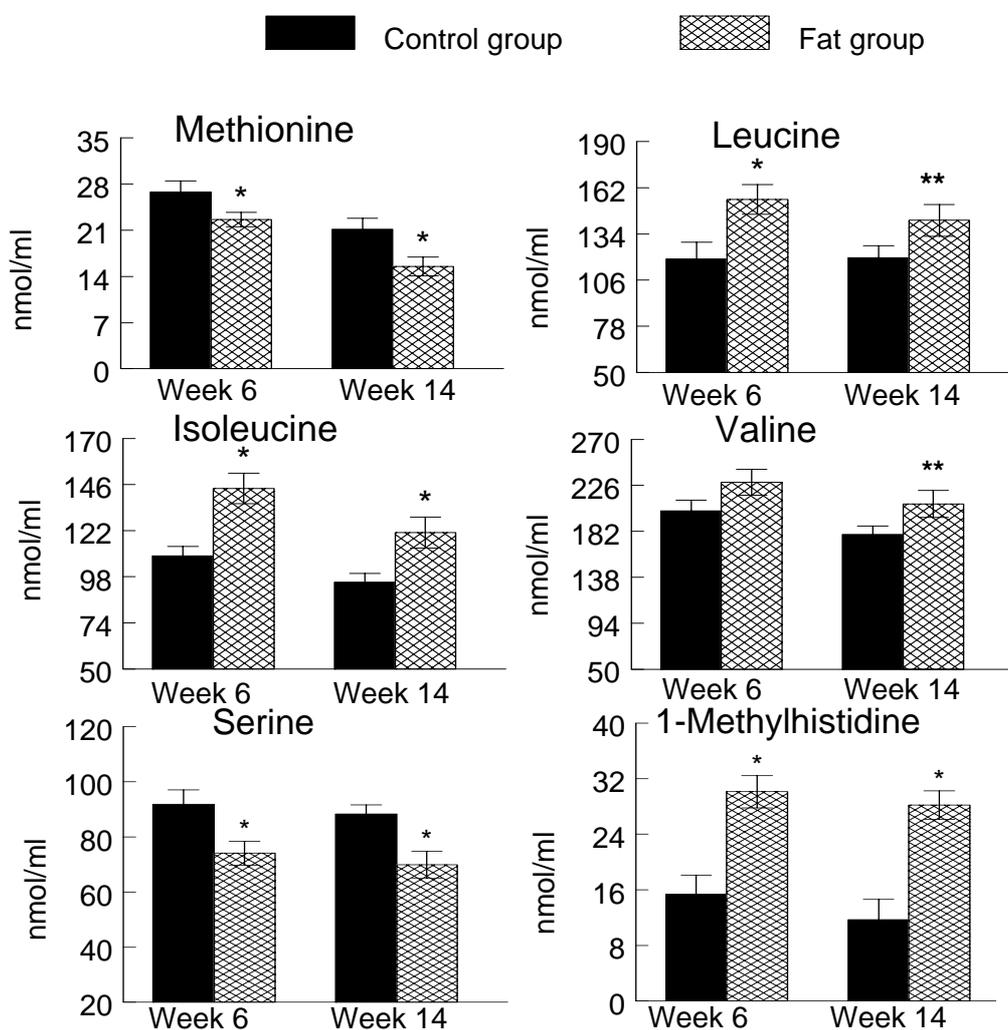


Figure 18. Levels of some free AA in blood plasma of dairy cows during experiment I. Means of the control (n = 15) and fat (n = 16) group with SEM. * Significant difference ($P < 0.05$), ** Significant difference ($P < 0.01$).

4.1.5 Microbial protein in the rumen

Table 12 shows that the average of milk allantoin content and consequently the calculated ruminal microbial N tended to decrease ($P = 0.086$) for the cows fed on a high-fat diet in comparison to cows in the control group. The decrease was significant during the 10th week of lactation ($P = 0.027$) and with tendency to be significant ($P = 0.107$) in the 6th week of lactation as seen in Fig. 19.

Table 12. Milk allantoin, microbial N, total urea pool, and urea flux in whole body of cows during experiment I.

Items	Control	Fat	SEM	P
Milk allantoin ¹ , mmol · d ⁻¹	9.33	8.30	1.60	0.086
Microbial N, g · d ⁻¹	235.1	209.1	40.1	0.086
Total urea pool ² , mg N · kg BW ^{-0.75}	169.0	178.0	34.40	0.685
Urea-flux ² , g N · kg BW ^{-0.75} · d ⁻¹	1.69	2.08	0.21	0.031

¹ n = 15 in both groups, values of one cow of fat group were excluded.

² n = 4 (control group), 5 (fat group).

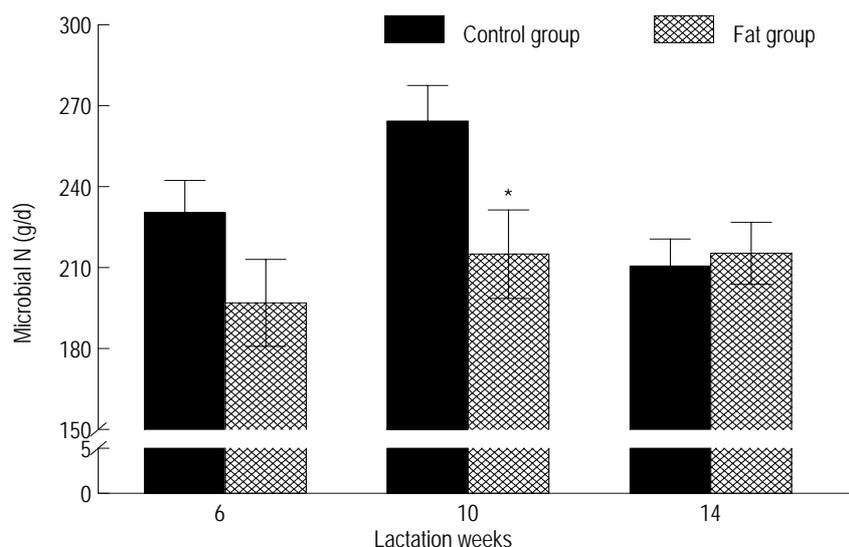


Figure 19. Net synthesis of microbial-N in the rumen, calculated by allantoin excretion in milk of dairy cows during experiment I. Means of the control and fat group (n = 15) with SEM. * Significant difference ($P < 0.05$).

4.1.6 Urea kinetics

The substitution of starch in the diets of dairy cows by protected fat led to a significant increase in the total urea flux but did not significantly affect the mean of the total urea pool of the body of cows (Table 12). The infused ¹⁵N₂-urea concentrations disappeared in the blood in two phases, which was seen in the kinetic curves as two urea compartments; during the first phase the isotope decreased sharply then slowly with time in the second kinetic phase (Fig. 20).

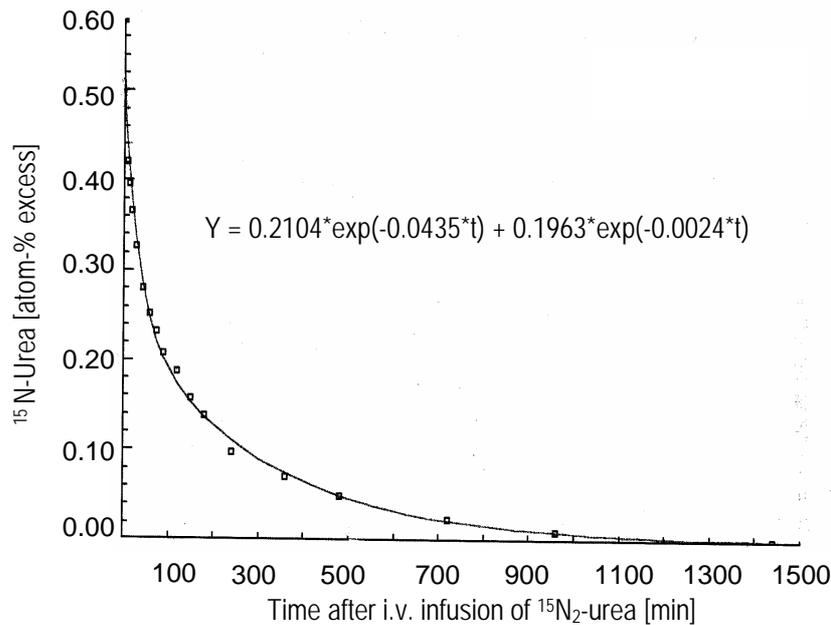


Figure 20. An example of ^{15}N -kinetics curves of blood urea in cows of control group after intravenous infusion of $^{15}\text{N}_2$ -urea in experiment I.

4.1.7 Correlation coefficient between milk and blood parameters

Table 13 shows that milk yield correlated negatively with the protein content of milk ($r = -0.58$, $P < 0.05$) and milk somatic cells number ($r = -0.70$, $P < 0.01$) in cows of the control group but positively correlated with plasma insulin level ($r = 0.69$, $P < 0.01$) in cows of the fat group. Milk fat content correlated positively with milk protein content ($r = 0.51$, $P < 0.05$) and plasma glucose level ($r = 0.73$, $P < 0.01$) in cows of the fat group. Somatic cells number of milk correlated negatively with milk lactose ($r = -0.75$; $P < 0.01$) and protein content ($r = -0.50$, $P < 0.05$) and also with plasma level of β -HBA ($r = -0.52$, $P < 0.05$) in the cows of fat group.

In cows of the fat group, plasma glucose concentration correlated negatively with milk urea content ($r = -0.70$, $P < 0.01$) and plasma urea level ($r = -0.80$, $P < 0.01$) and positively with milk fat content ($r = 0.73$, $P < 0.01$). But in cows of the control group, plasma level of β -HBA correlated negatively with that of plasma glucose ($r = -0.63$, $P < 0.05$) and insulin ($r = -0.58$, $P < 0.05$). In cows of the control group, plasma glucose concentration correlated negatively with that of plasma urea ($r = -0.78$, $P < 0.01$). Plasma urea level correlated positively with that of milk urea in cows of the control group ($r = 0.72$, $P < 0.01$) and in those of the fat group ($r = 0.98$, $P < 0.01$).

Table 13. Correlation coefficient between milk and blood parameters of both groups of dairy cows in experiment I¹.

Items	Milk (kg/d)	Milk fat	Milk protein	Milk lactose	Milk urea	Somatic cells	Plasma β-HBA	Plasma NEFA	Plasma glucose	Plasma insulin
Milk fat, %	C ² : -0.04 F ³ : -0.23									
Milk protein, %	C: -0.58* F: 0.01	C: 0.04 F: 0.51*								
Milk lactose, %	C: 0.14 F: -0.02	C: 0.27 F: 0.20	C: 0.16 F: 0.39							
Milk urea, mg/l	C: 0.37 F: -0.08	C: -0.06 F: -0.55*	C: -0.09 F: 0.01	C: -0.34 F: -0.01						
Somatic cells, x 10 ³ /ml	C: -0.70** F: -0.24	C: -0.11 F: 0.02	C: 0.43 F: -0.50*	C: -0.28 F: -0.75**	C: -0.30 F: -0.18					
Plasma β-HBA, mmol/ml	C: 0.38 F: 0.23	C: 0.20 F: -0.29	C: -0.23 F: 0.33	C: 0.06 F: 0.28	C: 0.45 F: 0.48	C: -0.26 F: -0.52*				
Plasma NEFA, μmol/l	C: 0.29 F: -0.21	C: 0.54 F: 0.06	C: -0.38 F: -0.17	C: 0.26 F: -0.07	C: -0.35 F: 0.38	C: -0.25 F: 0.18	C: -0.16 F: 0.32			
Plasma glucose, mmol/l	C: 0.07 F: 0.05	C: 0.18 F: 0.73**	C: -0.09 F: 0.25	C: 0.01 F: 0.25	C: -0.51 F: -0.70**	C: 0.01 F: -0.11	C: -0.63* F: -0.40	C: 0.61 F: -0.09		
Plasma insulin, μU/ml	C: -0.23 F: 0.69**	C: -0.15 F: -0.37	C: 0.22 F: -0.16	C: 0.53* F: 0.22	C: -0.30 F: -0.21	C: 0.11 F: -0.22	C: -0.58* F: 0.05	C: 0.06 F: -0.38	C: 0.22 F: 0.12	
Plasma urea, mmol/l	C: 0.08 F: -0.20	C: -0.13 F: -0.63**	C: -0.21 F: -0.13	C: -0.23 F: -0.04	C: 0.72** F: 0.98**	C: 0.06 F: -0.09	C: 0.59* F: 0.48	C: -0.35 F: 0.35	C: -0.78** F: -0.80**	C: -0.29 F: -0.18

* P < 0.05.

** P < 0.01.

¹ n = 15 (Control group), 16 (Fat group); Results of week 2 to week 15 of lactation.² C: Control group.³ F: Fat group.

4.1.8 ^{13}C content of feeds, milk constituents, and blood CO_2

Due to the substitution of starch with fat, the carbon atoms in the dietary concentrate mixture were depleted in ^{13}C by 0.9 δ -‰ (-20.1 vs. -21.0 δ -‰ for control and fat groups respectively, Table 2) in the fat group compared with the control one. The ^{13}C -content of protected fat was depleted in comparison to tapioca starch by 4.0 δ -‰ (-24.1 vs. -28.1 δ -‰ for added tapioca and protected fat, respectively). The total dietary fat in concentrate mixture was depleted in ^{13}C by 2.6 δ -‰ (-23.8 vs. -26.4 δ -‰ for the control and fat group, respectively).

The mean value of ^{13}C in milk fat, lactose and casein are represented in Table 14. The ^{13}C -enrichment in milk fat of the fat group was significantly decreased in relation to the ^{13}C -content of milk fat in the control group as seen in Fig. 21. This means that fewer carbons of non-fat sources and more carbons of fat source of the diet were incorporated into the milk fat of these cows than in that of the control group. This effect was not seen in case of milk lactose and casein, with an exception at week 14 for casein (Fig. 21).

Table 14. Means of ^{13}C -abundance in milk constituents and blood CO_2 of dairy cows in experiment I (from the 2nd to 15th weeks).

Items	Control n = 15	Fat n = 16	SEM	P
Milk Fat ^{13}C , δ -‰	-20.37	-22.13	0.71	< 0.001
Lactose ^{13}C , δ -‰	-18.12	-18.10	0.65	0.953
Casein ^{13}C , δ -‰	-19.29	-19.51	0.44	0.167
^{13}C in Blood CO_2 ¹ , δ -‰	-6.86	-6.13	0.84	0.078

¹ n = 13 (control group), 12 (fat group).

In contrast to milk fat, the enrichment of ^{13}C in blood CO_2 (which is representative for expired CO_2) increased significantly in the fat group in relation to the control group as shown in Fig. 22. Thus, it can be concluded that the lactating dairy cows of the fat group oxidized more non-fat carbons than those of the control one.

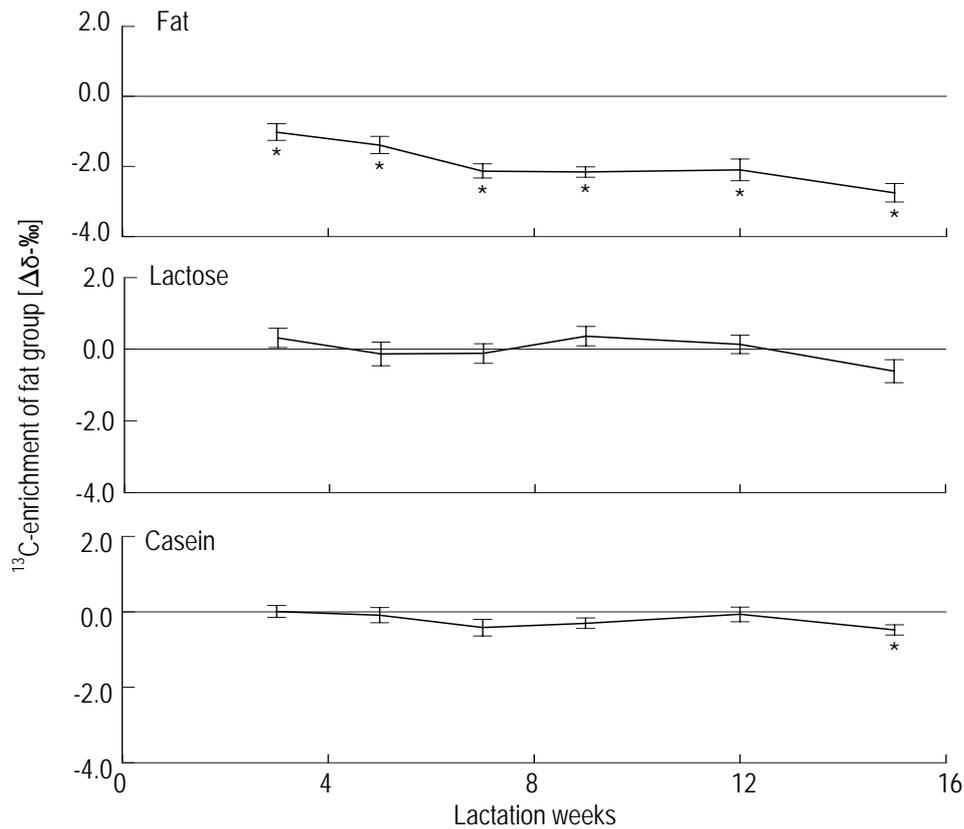


Figure 21. ^{13}C -enrichment of milk fat, lactose, and casein in the fat group in relation to the control one during experiment I. * Difference (fat - control) is significantly different from zero (paired t-test, $P < 0.05$).

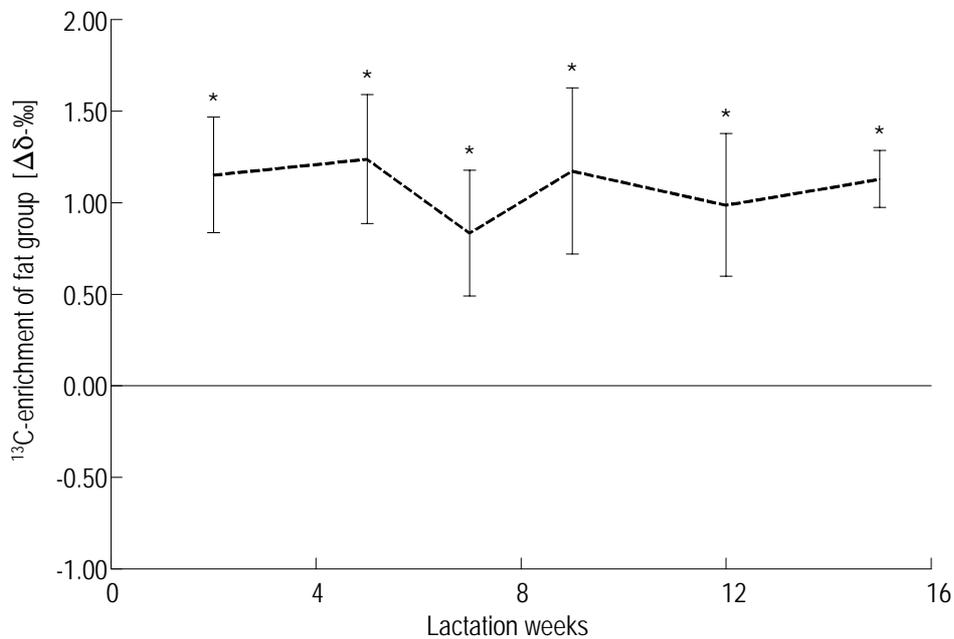


Figure 22. ^{13}C -enrichment of blood CO_2 in cows of fat group in relation to the control one during experiment I. * Difference (fat - control) is significantly different from zero (paired t-test, $P < 0.05$).

4.2 Experiment II

4.2.1 Nutrients intake

Exchange of starch with fat in the diet of dairy cows did not affect the intake of different nutrients significantly except for a decreased intake of starch and increased fat intake (Table 15).

Table 15. Nutrients intake of dairy cows during the two subperiods of experiment II¹.

Items	Control	Fat	SEM	P
DM, kg ·cow ⁻¹ ·d ⁻¹	19.62	18.53	0.80	0.196
Crude fibre, kg ·cow ⁻¹ ·d ⁻¹	2.83	2.72	0.15	0.518
Starch, kg ·cow ⁻¹ ·d ⁻¹	4.32	3.18	0.20	0.001
Fat, kg ·cow ⁻¹ ·d ⁻¹	0.50	1.10	0.05	< 0.001
Crude protein, kg ·cow ⁻¹ ·d ⁻¹	3.28	3.21	0.13	0.609
Utilizable protein, kg ·cow ⁻¹ ·d ⁻¹	3.24	3.12	0.13	0.450
NEL, MJ ·cow ⁻¹ ·d ⁻¹	125.7	125.1	9.02	0.845

¹ n= 8 for both treatments

4.2.2 Milk production

Milk yield and production efficiency

The milk yield, ECM and efficiency of milk production of lactating dairy cows were not significantly affected by diets (Table 16).

Table 16. Milk production, and composition of dairy cows during experiment II¹.

Items	Control	Fat	SEM	P
Milk, kg ·cow ⁻¹ ·d ⁻¹	32.83	31.61	0.75	0.148
ECM, kg ·cow ⁻¹ ·d ⁻¹	34.27	36.23	1.52	0.239
Fat, %	4.26	4.96	0.189	0.008
Protein, %	3.17	2.97	0.11	0.107
Lactose, %	4.98	4.96	0.02	0.203
Urea, mmol ·l ⁻¹	3.28	3.70	0.17	0.044
Fat, kg ·cow ⁻¹ ·d ⁻¹	1.42	1.59	0.09	0.087
Protein, kg ·cow ⁻¹ ·d ⁻¹	1.03	0.93	0.04	0.026
Lactose, kg ·cow ⁻¹ ·d ⁻¹	1.64	1.56	0.04	0.088
Energy, MJ ·cow ⁻¹ ·d ⁻¹	106.7	110.1	4.3	0.443
Acetone, mg ·dl ⁻¹	0.56	0.57	0.19	0.960
Somatic cells x 10 ³ /ml	111.4	109.4	20.43	0.925
Efficiency of milk production ²	0.27	0.29	0.02	0.249

¹ n = 8 for both treatments.

² ECM (kg/d) / NEL intake (MJ/d).

Milk composition

The substitution of starch by protected fat in the diets of lactating dairy cows during the short period of the experiment led to a significant increase in the percentage of milk fat and the fat yield tended to increase (Table 16). The milk protein percentage tended to decrease while its yield reduced significantly. The percentage of milk lactose was not significantly affected, while its yield tended to decrease. Milk urea content increased significantly in comparison to the control group.

4.2.3 Blood parameters

Plasma glucose, insulin and urea

The average of plasma glucose concentrations had a tendency to decrease, while that of insulin was not affected when cows were fed on a high-fat diet. The concentration of plasma urea was significantly higher in the dairy cows fed on a high-fat diet than when the same cows were fed on a starch diet (Table 17).

Table 17. Plasma glucose, insulin, and urea levels of dairy cows during experiment II¹.

Items	Control	Fat	SEM	P
Glucose, mmol/l	3.12	2.93	0.10	0.066
Insulin, μ U/ml	14.29	10.39	3.60	0.315
Plasma urea, mmol/l	4.21	4.90	0.16	< 0.001

¹ n = 8 for both treatments.

Plasma free amino acids

The substitution of starch by protected fat in the diet of dairy cows did not affect the concentration of total free AA. Only a significant decrease in the level of Thr was registered. Also, the concentration of Ile increased non-significantly during the subperiod of fat feeding (Table 18). The NEAA concentrations in the plasma of dairy cows were not affected in the subperiod of high fat intake except for a tendency for the concentrations of Ser and Orn to decrease and the levels of glutamic acid to increase (Table 19).

The ratio of EAA/NEAA was more than 0.6 in both experimental subperiods (control or fat diets) of dairy cows and not affected by the substitution of starch by protected fat in their diet (Table 19).

Table 18. Means of the free-EAA in blood plasma (nmol/ml) of cows during experiment II¹.

AA	Control	Fat	SEM	P
Methionine	27.1	24.9	1.9	0.274
Threonine	98.6	85.9	4.3	0.022
Isoleucine	110.2	129.6	10.7	0.112
Leucine	118.0	118.3	11.0	0.976
Valine	214.9	228.7	13.9	0.353
Lysine	75.0	70.0	5.6	0.399
Phenylalanine	59.2	55.3	3.9	0.345
Histidine	42.4	41.1	0.9	0.192
Arginine	55.3	64.2	3.4	0.754
Total	810.7	818.0	47.8	0.883

¹ n = 8 for both groups.

Table 19. Means of the free-NEAA in blood plasma (nmol/ml) of cows during exp. II¹.

AA	Control	Fat	SEM	P
Serine	79.6	75.3	2.0	0.068
Glycine	262.8	298.0	26.3	0.223
Alanine	193.6	186.7	9.3	0.479
1-Methylhistidine	3.6	3.7	0.6	0.920
3-Methylhistidine	2.2	2.8	0.4	0.175
Glutamic acid	57.1	61.9	2.5	0.088
Glutamine	295.6	303.0	17.5	0.684
Aspartic acid	10.3	10.8	0.9	0.652
Tyrosine	56.6	53.3	4.5	0.495
Ornithine	44.5	37.9	2.3	0.062
Hydroxyproline	15.9	17.5	1.1	0.168
Proline	71.4	77.1	5.5	0.328
Cysteine	ND ²	ND		
Total NEAA	1093	1128	40.6	0.421
Total AA	1904	1946	74.37	0.590
EAA/NEAA	0.74	0.73	0.04	0.784

¹ n = 8 for both treatments.

² Concentrations were not detectable.

4.2.4 Transfer of carbon atoms from glucose into milk constituents and blood CO₂

¹³C content of milk fat, casein, and lactose

As it is shown in Fig. 23, the $\delta^{13}\text{C}$ -excess in C of milk fat, casein and lactose increased after injection of U-¹³C₆-glucose into the jugular vein reaching, their maximal values after 60 h. The highest ¹³C-excess values were observed in milk lactose. The highest recovery rate of infused ¹³C was found in lactose (Table 20). The recovery in lactose was higher (P=0.065) if the cows consumed the fat diet instead of the starch diet.

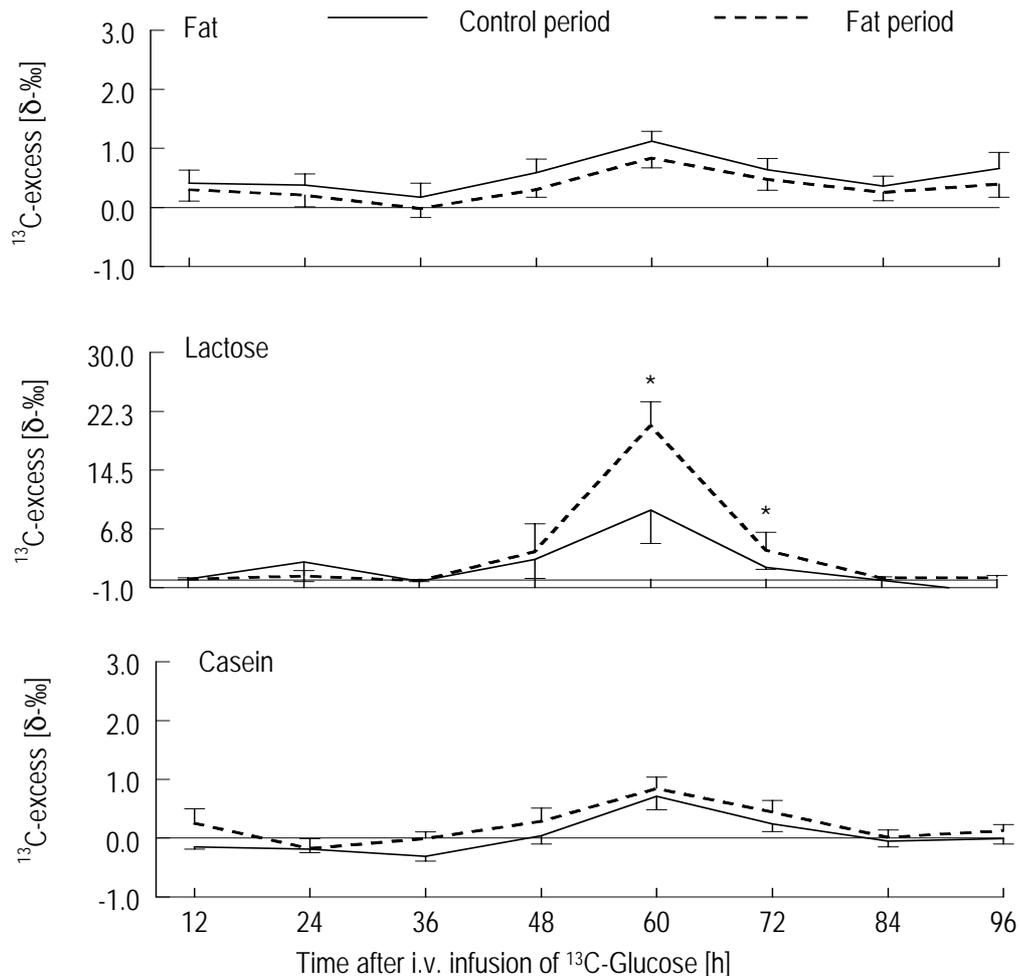


Figure 23. ¹³C-excess in carbons of milk constituents after i.v. infusion of D-[U-¹³C₆]-Glucose in experiment II. Means with SEM (n = 8). * Significant difference (P < 0.05).

Table 20. Recovery rate of ¹³C in milk after i.v. infusion of D-[U-¹³C₆]-Glucose (% of dose).¹

Items	Control	Fat	SEM	P
Lactose	42.7	57.6	5.0	0.065
Casein	0.80	2.90	0.90	0.120
Fat	11.3	9.3	2.7	0.170

¹ (n = 8).

¹³C content of blood CO₂

The ¹³C-content of blood CO₂ increased gradually with time after intravenous infusion of D-[U-¹³C₆]-Glucose and reached its maximal levels after 30 min, decreasing gradually with time till its complete disappearance after about 180 min (Fig. 24). The ¹³C-excess was significantly lower at 10, 15, 20, 30, 45, and 60 min after D-[U-¹³C₆]-Glucose injection ($P < 0.05$) when dairy cows were fed on a high-fat diet than when the same cows were fed on a starch diet. This means that cows with high fat intake oxidised less glucose from the metabolic pool than the cows of the control group.

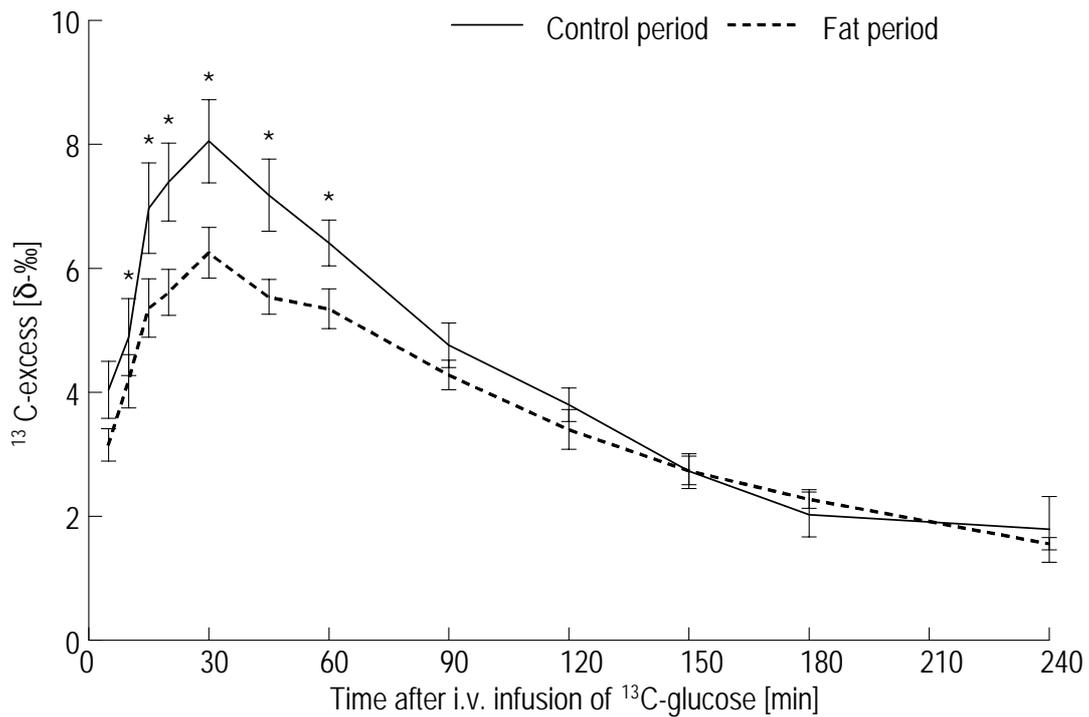


Figure 24. ¹³C-excess in blood CO₂ after i.v. infusion of D-[U-¹³C₆]-Glucose during experiment II. Means (n = 8) with SEM. * Significant difference ($P < 0.05$).

5 Discussion

Regarding the milk production, it is important to know that the milk yield of lactating dairy cows depends on the glucose supply of the mammary gland and the energetic efficiency of milk production depends on the utilization of long chain FA in the mammary gland (KRONFELD 1982). A high yielding dairy cow has to transform large amounts of nutrients to milk as seen in Table 21.

Table 21. Nutrients and energy balance of lactating dairy cow (600 kg BW; 50 kg milk/d)*

Nutrients	Diet	Milk
Dry matter, kg/d	25 to 26	6.0 to 6.5
Carbohydrates, kg/d	15 to 16	2.3 to 2.4
Crude protein, kg/d	4.5 to 5.0	1.6 to 1.8
Crude fat, kg/d	0.6 to 0.8	1.8 to 2.0
Gross energy, MJ/d	400 to 430	155 to 160

* GfE (2001).

Because the glucose requirement of a dairy cow for the daily production of 50 kg milk is more than 3.6 kg (KRONFELD 1982) and absorbed glucose is less than 1.5 kg/d (MATTHE et al. 2001), the cow has to produce more than 2.0 kg glucose from glucogenic precursors like propionate or AA. Furthermore, a net production of about 1 kg fat is necessary.

A many of trials have been carried out to study the effect of dietary fat on milk yield, milk composition and metabolism of dairy cows (as shown in the review). In the most of trials, fat supplement was added to the concentrate mixture to increase the density of energy in the diet. In the present study, dietary starch was substituted by fat on the energy equivalent basis. This means that the ratio of glucogenic to lipogenic energy was reduced without changing the total energy. Thus, the design of this study is not exactly similar to other studies of fat supplementation. Because of the reduced intakes of DM and carbohydrate in the experiments of dietary fat supplementation or postruminally infused FA (e.g. BENSON et al. 2001), as a matter of fact, the dietary fat replaces the starch in those diets as well. Thus, it can be assumed that the supply of propionic acid and other metabolites (microbial AA) for glucose production is affected similarly in the study of fat supplementation and that of starch substitution with fat.

The objective of the present work was to study whether the demands of the mammary gland for glucose and long chain FA can be optimised by the substitution of starch by protected fat in the diets of dairy cows. In other words, does fat influences the homeorhetic regulation of nutrients partitioning for milk production? Regarding this question, it is interesting to know

whether the dietary fat is available in the mammary gland or is predominantly used as an energy source.

For the discussion of the results, the length of the trial must be considered. Experiment I was a long-term trial to study the dietary effect starting immediately p.p. till 100 d of lactation. The periods of experiment II were relatively short and carried out between the 6th and 10th weeks of lactation. For this reason, the responses of cows concerning feed intake, milk and blood parameters in experiment II are not always comparable to experiment I.

5.1 Nutrients intake and energy balance

DM and NEL intakes were not affected significantly in either experiment (Table 6 and 15) by feeding dairy cows on diets containing protected fat instead of starch on energy equivalent basis (Table 1, 4), which is in agreement with the findings of ALLISON and GARNSWORTHY (1998), SMITH et al. (1978), DePETERS et al. (1989), WEST and HILL (1990), and VAZQUEZ-AÑON et al. (1997). CHILLIARD et al. (1993) summarized that the DM intake decreased significantly when cows were fed on diets supplemented with Ca salts of FA. In the present study, there was non-significant decrease in DM intake in both experiments. This may be attributed to an increased secretion of cholecystinin (CHOI and PALMQUIST 1996) that inhibits reticuloruminal motility, which in turn could decrease DM intake (NICHOLSON and OMER 1983). However, many other factors can affect the nutrient intake of a fat-rich diet of dairy cows, such as the influence of fat on ruminal fermentation or the lipostatic control of feed intake (PALMQUIST and JENKINS 1980). The observed non-significant change in the daily intake of NEL by high-fat diet in the present study suggests that DM intake was regulated metabolically in the animal body to achieve an energy homeostasis. A negative feedback signal on the feed intake controlling centres and a reduced palatability were discussed by KOMARAGIRI et al. (1998).

The unaffected milk energy content in the present study is concordant with the findings of MOALLEM et al. (2000) and SCHNEIDER et al. (1990), that reported unaffected milk energy by feeding lactating dairy cows on diets supplemented with Ca salts of FA during the first 150 days of lactation. Consequently, the averages of EB of dairy cows in both groups of experiment I were not significantly affected during the experimental lactation period (Fig. 7, Table 7). These results are in agreement with the finding of BEAM and BUTLER (1998), which showed unaffected EB in lactating dairy cows fed diet supplemented with prilled fat. The relatively non-significant decrease in DM intake with high fat intake in both experiments of the present study suggests that attempts to improve EB by dietary supplementation with protected fat may be ineffective during

the early lactation period. The unchanged EB in cows fed high-fat diets in this study was associated with decreased plasma levels of insulin in experiments I and was also associated with increased plasma levels of NEFA. This is in agreement with the findings of BEAM and BUTLER (1998), RONGE et al. (1988), and SPICER et al. (1990).

The average of subcutaneous fat thickness of lactating cows during experiment I was not affected by high fat intake during the experimental period (Table 7), which is in agreement with the finding of McNAMARA et al. (1995). These authors found no differences in body fat content of cows fed supplemental fat from oil seeds or Ca salts of FA. This effect was also reported in the case of duodenal infusion of vegetable oil for lactating cows (GAGLIOSTRO and CHILLIARD 1991a, b). The dynamics of the curve of subcutaneous fat thickness (Fig. 6) was similar in both groups. But after the 6th week, the drop in fat thickness of the fat group was smaller than in the control one, which may be attributed to inhibition of fat mobilization or stimulation of lipid synthesis. In this period, the milk yield was numerically or significantly higher in this group. Adipose tissue metabolism is subjected to complex homeostatic and homeorhetic controls (VERNON 2003). Pathways of lipid metabolism in ruminant adipocytes are shown in Fig. 25.

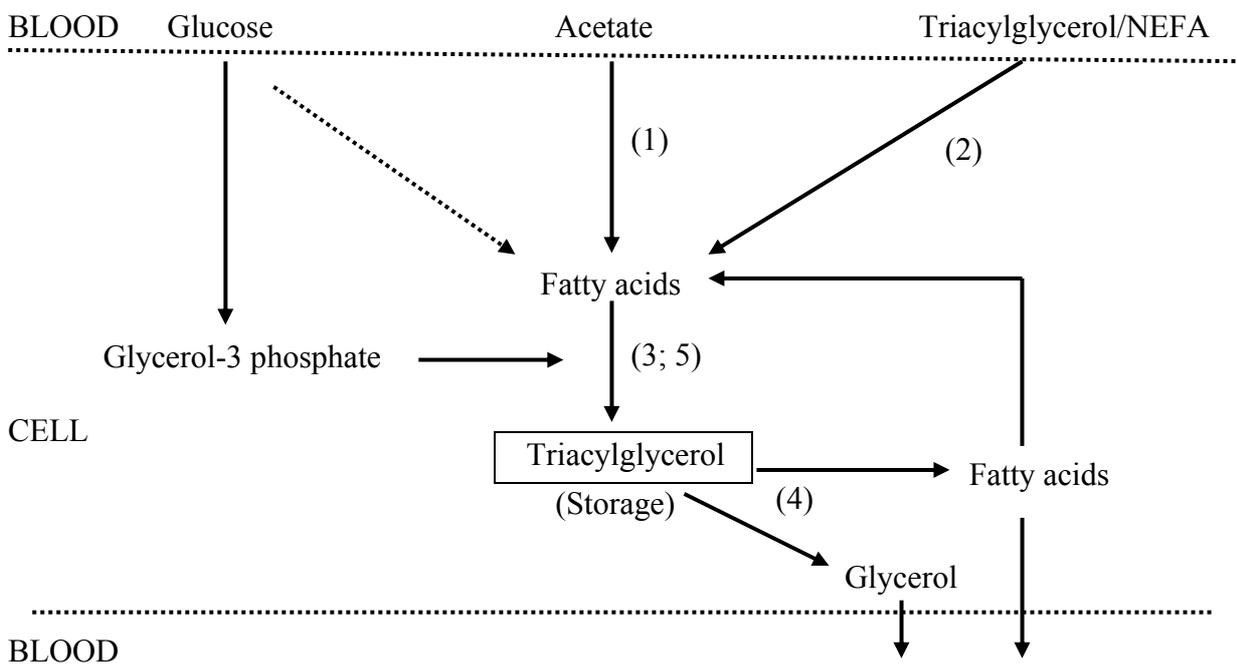


Figure 25. Pathways of lipid metabolism in ruminant adipocytes (VERNON 2003, modified; see text).

As could be seen in Fig. 25, the amount of fat accumulation in adipose tissues results from the equilibrium between de novo synthesis of FA (1), FA uptake (2), FA esterification (3), lipolysis (4), and reesterification of FA produced by lipolysis (5). The rates of FA synthesis are

controlled by the amount of substrate (acetate, glucose) and by concentrations of hormones, such as insulin, glucagon, and noradrenalin (McNAMARA 1997). In cows with positive EB, McNAMARA and BALDWIN (2000) found in-vitro a depressive effect for dietary fat on FA synthesis in adipose tissue. In the current study, the cows were in a negative EB between week 6 and week 10 and the level of the lipogenic hormone insulin in plasma was lower than in control group (Fig. 7, 14). But the possibility of a higher FA synthesis may be considered. Insulin is the most important acute anabolic regulator, however during lactation the ability of insulin to stimulate lipogenesis is diminished (BAUMANN 2000). In this period, FA synthesis is decreased in adipose tissue by lack of nutrients in relation to demand by the mammary gland (BAUMANN 2000). Because the requirement of the mammary gland for acetate and glucose in the cows fed high-fat diet is presumably lower, as a consequence of reduced de novo synthesis of FA (see 5.3.1), a higher synthesis of FA in adipose tissues cannot be excluded. Furthermore, esterification, but not reesterification, is stimulated by dietary fat as discussed by CHILLIARD (1993). Currently, the effect of dietary fat on lipolysis is not fully clear (see 2.3.3). In the present study, the higher level of NEFA in blood (Fig. 16) may be considered as an indicator for a higher absorption of dietary FA and intracellular lipolysis. Likewise, the NEFA concentration in blood can be influenced by the net uptake of NEFA and their secretion as milk fat by the mammary gland.

Overall, it could be hypothesized that the ratio of tissues fat synthesis to fat mobilization was enhanced by fat feeding beyond the 8th week of lactation in the present study. Detailed isotopic studies are required to describe the effect of dietary fat in the different stages of lactation on the total body FA turnover, including lipogenesis and lipolysis in adipose-tissues flux, and interorgan FA recycling.

5.2 Milk production performance and milk composition

Milk yield and efficiency of milk production

An increased milk yield was recorded in most of studies of supplemental fat feeding to high yielding dairy cows during the early lactation period (as shown in 2.3.2.). In experiment I, the average milk yield was increased numerically by partial substitution of starch by protected fat (Table 8). The failure of a high-fat diet to enhance the average NEL intake in this experiment (Table 6) is a plausible explanation for the lack of the significant effect of dietary fat on the mean of milk production. In the first 9 weeks of lactation there existed no significant differences between the treatments (Fig. 8). However, when the cows reached a positive EB (after week 10 of lactation), the milk yield was significantly higher in the high-fat group, suggesting a different

utilization of dietary FA in the different lactation stages. Toward the end of the trial, the dietary FA were secreted in the milk in a higher degree than in the first period of lactation as seen by the lowering of the ^{13}C -enrichment in milk fat (Fig. 21).

In experiment II, the milk yield (Table 16) was slightly lower when the diet of cows was changed from starch to fat, which may be due to non-significant decrease in DM intake and the short duration of the experiment (PALMQUIST and JENKINS 1980). There was a remarkable non-significant increase of FCM in both experiments, which was due to the increase of milk fat content in experiment II and increased milk yield in experiment I (Table 8 and 16). These results are concordant with results of JERRED et al. (1990) and SCHNEIDER et al. (1990) who recorded an increased FCM of cows fed on the high-fat diet. As a conclusion, the partial substitution of starch by fat enhances numerically the FCM yield. The milk production increased significantly beyond the 8th week of lactation if the cows were coming into a state of positive EB.

Milk composition

Somatic cells and acetone

As shown in Table 8 and 16, the somatic cells content of milk in both experiments and the milk acetone level (experiment II) were not significantly affected by the substitution of starch by protected fat, which indicates that the high-fat diet did not affect the udder and animal health (ketosis) respectively. The cows of both experiments were healthy and not suffering from diseases affecting milk quality. The negative correlation between milk yield and cells number in the control group (Table 13) was also summarized by HAMANN and KRÖMKER (1997). This may be as a result of a dilution effect of milk. The same effect was observed as a trend in the fat group (Table 13). In this group, the somatic cells count was negatively correlated with the content of protein and lactose in milk (Table 13). A strong negative correlation between lactose content and somatic cells count was found early as summarized by HAMANN and KRÖMKER (1997). The reason of these negative correlations and also those present between the somatic cell number and the level of β -HBA in plasma needs further examination, especially their interaction with dietary fat. There is a gap in knowledge about the relation between somatic cell count and the metabolism of healthy dairy cows.

Milk fat

The average milk fat percentage and yield of high yielding dairy cows were not significantly affected by the substitution of the starch by protected fat in the diets of experiment I (Table 8), which is in agreement with the review of CHILLIARD et al. (1993) and the studies of GARCIA-BOJALIL et al. (1998), KHORASANY and KENNELLY (1998), and KOWALSKI et al. (1999).

The authors found non-affected milk fat content of dairy cows fed on diets containing Ca salts of palm oil. However, as seen in Fig. 9, the curve of milk fat of lactating cows fed on high fat diet was behind that of cows fed on a low-fat diet, leading to a relatively non-significant decrease of the average milk fat percentage (about 0.27%), which was significant at weeks 11 and 12 of lactation period of the experiment. This decrease in milk fat may be due to the reduced de novo synthesis of FA in the mammary gland. The latter could be attributable to a reduced glucose delivery to the mammary gland. This would reduce NADPH supply for fatty acid synthesis and the availability of glycerol for triacyl esterification as discussed by FAULKNER and CLAPPERTON (1981), FAULKNER and POLLOCK (1989), and GAYNOR et al. (1994). The positive correlation between milk fat content and plasma glucose level (Table 13) supports this assumption. The intra-group variations of plasma glucose level could not linearly be attributed to a variable degree of gluconeogenesis from AA, because plasma glucose level was negatively correlated with milk and plasma urea levels. However, this should not be confused with the fact that plasma and milk urea levels were generally higher in the fat group, indicating an increased gluconeogenesis from AA after fat feeding.

Recent studies have demonstrated that milk fat depression due to fat feeding can also be a consequence of direct inhibition of mammary FA synthesis by products from incomplete or unusual biohydrogenation of polyunsaturated FA in the rumen. PRECHT et al. (2002) found on a material from this study an inverse relation between the content of *cis* Δ 9, *trans* Δ 11 CLA or *cis*-12 isomer in milk fat and the milk fat content ($r = -0.95$ and -0.98). Individual *trans* FA (GRIINARI et al. 1998; PRECHT et al. 2002) and other CLA, like *cis* Δ 12, *trans* Δ 10 CLA (BAUMGARD et al. 2000) have received special attention as the cause of milk fat depression. AHNADI et al. (2002) found that dietary fish oil, as a source of *trans* FA and CLA, reduces the milk fat percentage by inhibiting gene expression of mammary lipogenic enzymes, such as acetyl-CoA carboxylase.

In contrast to experiment I, the milk fat percentage and yield in experiment II were increased significantly when dairy cows were fed during a short period on a high-fat diet (Table 16), which is in agreement with the findings of CHOUINARD et al. (1998), SCHROEDER et al. (2002) and MOALLEM et al. (2000). This increase in milk fat may be due to increased available FA (triacylglycerols and NEFA) for the mammary gland (BINES et al. 1978). The increase in milk fat content by feeding of high-fat diet is a result of greatly increased plasma, very low density lipoprotein and triglycerides concentration, which increases the mammary uptake of long chain FA (YANG et al. 1978). In this case, the uptake of long chain FA exceeds the compensating

reduction of de novo synthesis of FA acids resulting in higher milk fat secretion (PALMQUIST and JENKINS 1980). Obviously, the mammary gland needs time to respond to specific FA (*trans* FA or CLA) to decrease the milk fat content. This may explain to some extent the different effect of high fat intake on the milk fat content in both experiments. Further detailed studies are necessary to give more information about the reasons for these different effects of dietary fat on milk fat content in both experiments of this study.

Milk protein

In experiment I, the average milk protein percentage was significantly decreased by 0.21% in the lactating dairy cows fed on a high-fat diet compared with those fed on a high-starch diet, which was also significant from the 7th week till the end of lactation period (Table 8, Fig. 10). But milk protein yield was not affected by the diets. In the control group of this experiment, milk yield was negatively correlated to its protein content, showing a dilution effect (Table 13). This effect was not seen in the fat group. This can be explained by the positive correlation between plasma insulin level and milk yield (Fig. 26). The uptake of AA by the mammary gland is insulin dependent as will be discussed below. Likewise, during experiment II, milk protein percentage had a tendency to decrease and its yield also decreased significantly when cows were fed on a high-fat diet (Table 16). This depression in milk protein percentage is well documented in most of the previous studies as previously discussed under 2.3.2. In contrast, BAUMGARD et al. (2000), ENJALBERT et al. (1997), and MOALLEM et al. (2000) found unaffected milk protein content when feeding supplemental fat to lactating dairy cows but RODRIGUEZ et al. (1997a) and WU et al. (1993, 1994) found a decreased milk protein content with unaffected milk protein yield with high fat intake of lactating dairy cows.

The depression in milk protein content may be due to many factors (WU and HUBER 1994). The substitution of starch by protected fat may leads to decreasing of the rumen microbial protein synthesis (Table 12 and Fig. 19), which provides the basis for about two third of the milk protein (CLARENBURG 1992). However, the diets were formulated so that the amount of utilizable protein at duodenum was nearly identical and the presumably two first limited AA, methionine and lysine, were supplemented in protected form. Thus, the duodenal passage of AA was presumably not significantly different. On the other hand, AA could be exhausted by gluconeogenesis to meet requirements for glucose. The increased urea flux (Table 12) and reduced levels of some AA (Table 10, 11 and Fig. 18) support this assumption.

In comparison to the starch diet, the insulin level in plasma was reduced when cows were fed on a high-fat diet (Table 9, 17). The uptake of AA by the mammary gland is a function of both

insulin and somatotropin levels. These hormones act synergistically (CASPER and SCHINGOETHE 1989) in the process of nutrient partitioning. The decreased levels of plasma insulin may negatively affect the AA uptake by the mammary gland (MACKLE et al. 2000). The effect of fat on endocrine regulation of AA uptake by the mammary gland seems to be the main reason for the reduced protein content of milk. However, because the total production of milk protein was not different between the cow groups, it could be concluded that the amount of substrates used for protein synthesis was not a limiting factor in this study.

Milk urea

The average concentration of urea in the milk of dairy cows fed on a fat diet in both experiments increased significantly (11% and 13% in experiments I and II, respectively) in comparison to those fed on the control diet (Table 8, 16). CANT et al. (1993), CHOW et al. (1990) and DePETERS and CANT (1992) reported an increased milk NPN of lactating cows fed on diets supplemented with fat. Also, it increased quadratically as increased amounts of FA infused in the abomasums of dairy cows in the study of LaCOUNT et al. (1994). On the other hand, our finding is not in agreement with the findings of DRACKLEY et al. (1998) and RODRIGUEZ et al. (1997b) that reported no effect on milk urea N by feeding supplemental fat, or by infusing of long chain FA in the abomasum of dairy cows in the findings of CHRISTENSEN et al. (1994). As it was expected, the milk urea level was positively correlated with the urea level in blood plasma (Table 13). The milk urea content in experiment I was synchronized with plasma urea content, which was clear when the milk urea levels curve was compared with that of plasma urea (Fig. 12, 17). This is in conformity with the finding of GUSTAFSSON and PALMQUIST (1993). The increase in milk urea level may be due to the transfer of increased plasma urea by passive diffusion to milk (Table 9, 17). The latter results most likely from enhanced gluconeogenesis from AA. This assumption is confirmed by the increased urea flux (Table 12) and the changed level of some free AA in blood plasma (Fig. 18)

Milk lactose

The average of milk lactose percentage was not affected by the substitution of starch by protected fat in either experiment (Table 8, 16), which agrees with other studies (as shown in 2.3.2). In experiment I, the milk lactose content increased at the beginning of lactation for cows fed on a high-fat diet in comparison to the other cow group till the 6th week of lactation. Then this increase disappeared, which was associated with a higher milk production of fat-fed cows (Fig. 8, 11). In comparison to the control group, the average yield of milk lactose of lactating dairy cows fed on a high-fat diet had a tendency to increase during experiment I ($P = 0.072$). The results are

in agreement with the report of FAHEY et al. (2002) and RODRIGUEZ et al. (1997a) that showed an increased milk lactose production during the lactation period of cows fed on diets supplemented with Ca salts of FA. The reason of this increased milk lactose in fat group of experiment I will be discussed in 5.3.1. In contrast, when cows were fed on high fat diet during experiment II, the yield of milk lactose had a tendency to decrease to about 95% of the value of cows fed on a starch diet. This decrease in milk lactose yield in the second trial coincided with the slightly decreased milk yield of cows fed on the fat diet. An explanation for these differing results in both trials is not easily to give. This result demonstrates that the metabolic adaptation must be considered in the interpretation of the effects of dietary fat intake on milk lactose. Overall, however, the effect of high-fat diets on milk lactose content is negligible, feeding of dairy cows on high fat diet may sometimes increase the milk lactose yield.

5.3 Metabolism

5.3.1 Intermediary metabolic parameters

Glucose

Plasma glucose concentration of lactating dairy cows was numerically ($P < 0.08$) but not significantly reduced by the substitution of starch by protected fat during the experimental lactation period of experiment I (Table 9). This effect was significant at the 5th and 9th weeks of lactation (Fig. 13). In experiment II, plasma glucose concentration had a tendency to decrease with high fat intake. The results in both experiments are in contrast with the findings of DRACKLEY et al. (1998) and BETMAN et al. (1996) that reported a tendency to increase in plasma glucose concentrations of lactating cows with high fat intake. The authors attributed this increase to the appearance of insulin resistance. SIMAS et al. (1995) recorded a reduced liver glucose release by feeding of dairy cows on diets contained 2.5% of DM as Ca salts of FA. This is in agreement with the study of BUCKLEY et al. (2000), FAHEY et al. (2002) and KOWALSKI et al. (1999).

The milk production of dairy cows depends on the amount of available glucose in the mammary gland, which is used primarily for lactose synthesis. Because the glucose transporters in the mammary gland, but not in adipose tissues, are independent on insulin (BALDWIN and SMITH 1983), it can be expected that the significantly lower concentrations of plasma insulin (Table 9 and Fig. 14) in cows fed on a high-fat diet enhanced the direct use of the glucose for milk synthesis, while glucose utilization in extra mammary tissue is reduced. The added fat may reduce the glucose oxidation in the pentose phosphate pathway, because the incorporation of dietary FA into milk fat reduces the de novo synthesis of FA and consequently the requirement of

NADPH. This effect increases the availability of glucose for lactose synthesis (Fig 1), and consequently for milk production.

Model calculation of glucose-sparing effect of protected fat

This sparing effect of protected fat on glucose requirement was assumed in experiment I, which led to a certain increase in milk lactose output (Table 8). The following model calculation demonstrates the glucose-sparing effect of protected fat in the diets of lactating dairy cows. Assuming that:

- The generation of 12 mol of NADPH needs one mol glucose (180 g) (NELSON and COX 2001).
- The C₂ elongation (28 g) in the process of FA synthesis requires 2 mol of NADPH (NELSON and COX 2001).
- About 20% of tapioca starch is absorbed as glucose in the small intestine and about 75 % is fermented in the rumen resulting in 0.379 mol propionate per mol glucose (VOIGT 1975).
- Microbial protein yield in the rumen is about 220 g (about 2 mol AA) per kg of fermentable starch (HAGEMEISTER et al. 1981).
- Gluconeogenesis of glucose from propionate or AA (alanine) requires 4 mol ATP, and the oxidation of glucose supplies 38 mol ATP per mol of glucose (BERGNER 1996).

Consequently,

- The synthesis of 168 g fat (6 x 28) requires 180 g of glucose, or the inclusion of additional 730 g of fat in the diet of fat group of experiment I (Table 1) can save 780 g glucose (180/168 x 730).
- The inclusion of 1770 g of starch in the diet of control group supplies $1770 \times 0.2 \times 1.1 = 389$ g (2.16 mol) of glucose plus $1770 \times 0.8 \times 1.1 \times 0.379/2 = 295$ g (1.64 mol) of glucose, totally 684 g glucose (3.8 mol). In addition, 0.36 mol = 64 g glucose ($1770 \times 0.2 \times 180/1000$) can be produced from microbial AA. The synthesis of total 2 mol of glucose from propionate or AA (1.64 + 0.36) needs an ATP equivalent of 0.21 mol of glucose ($2 \times 4/38$). From this, a total net production of 3.95 mol ($3.8 + 0.36 - 0.21$) of glucose (711g) can be produced.

For comparison, about 780 g of glucose were saved by the inclusion of fat in the diet of the fat group and about 711 g of glucose are available in case of inclusion of about 1770 g starch in the diet of the control group. Theoretically, the net glucose supply was higher by about 69 g in the fat group than in the control one. However, the plasma glucose levels were slightly decreased

after fat feeding. Therefore, it can be assumed that fat can only partially substitute glucose in the mammary gland and not in other tissues. Additionally, the suboptimal conversion of dietary fat to milk fat counteracts the glucose-sparing effect. This may explain that the glucose-sparing effect of fat may not be at all evident in some experiments, e.g. experiment II of the present study.

In conclusion, added fat can spare glucose by lowering the glucose requirement for FA synthesis in lactating dairy cows, subsequently increasing the available glucose in the mammary tissues for synthesis of lactose, and though increasing milk production.

Fate of ^{13}C - glucose infused intravenously

In experiment II, the concentrations of ^{13}C in blood CO_2 after i.v. infusion of ^{13}C -glucose in cows fed on the control diet were significantly higher than when cows were fed on a high-fat diet (Fig. 24). This result suggest that the dairy cows fed on a high-fat diet oxidized less glucose than when cows were fed on a high starch diet. This is confirmed by the opinion of YANG et al. (1978), showing in vitro a decreased glucose oxidation in adipose tissue of lambs and sheep fed on a high-fat diet. This decreased glucose oxidation when dairy cows were fed on a high-fat diet confirms the theory of glucose sparing in the metabolism of cows fed fat-enriched diets. Also, in the same experiment, as seen in Fig. 23 and Table 20, the average of ^{13}C -excess in milk fat of lactating dairy cows was significantly lower ($P = 0.001$) after i.v. infusion of ^{13}C -glucose during the period of high fat intake than that of low-fat intake. Thus, the proportion of glucose transferred into milk fat was also lower in the fat group than in the control group. On the other hand, the average of ^{13}C -excess in milk casein was significantly higher ($P = 0.013$) and tended to be higher in milk lactose ($P = 0.066$) in dairy cows with high fat intake than with low fat intake. The increased ^{13}C -excess in milk lactose and casein and lower ^{13}C -excess in the expired CO_2 and milk fat of cows fed high fat diet means that the glucose of the metabolic pool was used in a higher proportion for synthesis of lactose and casein than for oxidation processes or synthesis of milk fat.

Plasma insulin

In both experiments, the mean of plasma insulin concentrations decreased for the lactating dairy cows with high fat intake. The difference was significant in experiment I (Table 9 and Fig. 14) and not significant in experiment II (Table 17). The reduction in insulin release may be due to a lower supply of glucogenic precursors like propionate, or an excessive clearance of insulin from the body tissues (STAPLES et al. 1998). Our finding is in conformity with the finding of STAPLES et al. (1998), reporting a 25% reduction in plasma insulin concentrations when protected fat is fed to lactating dairy cows, and is not in agreement with the judgements of BLUM

et al. (1985) and also KHORASANI and KENNELLY (1998), who reported increased plasma insulin concentrations in dairy cows with high fat intake. However, SCHNEIDER et al. (1990) showed unaffected plasma insulin concentration in lactating dairy cows fed on high-fat diets. Plasma insulin concentrations of dairy cows with high fat intake are presumably positively related to feed intake and the tissues EB. These concentrations depend on the glucogenic supply, net portal-drained viscera release of insulin and liver removal as recorded in the study of BENSON and REYNOLDS (2001).

The milk yield was positively correlated in the fat group with the plasma insulin concentration (Fig. 26, Table 13). This could be the result of higher glucose production or lower sensitivity of insulin receptors in cows with the highest milk yield. The results would also support the finding of MACKLE et al. (2000) on the role of insulin in extraction efficiency of AA in the mammary gland. The milk protein content is not reduced with increased milk yield in this group as seen in the control group (Table 13). These findings demonstrate the anabolic effect of insulin in lactating dairy cows.

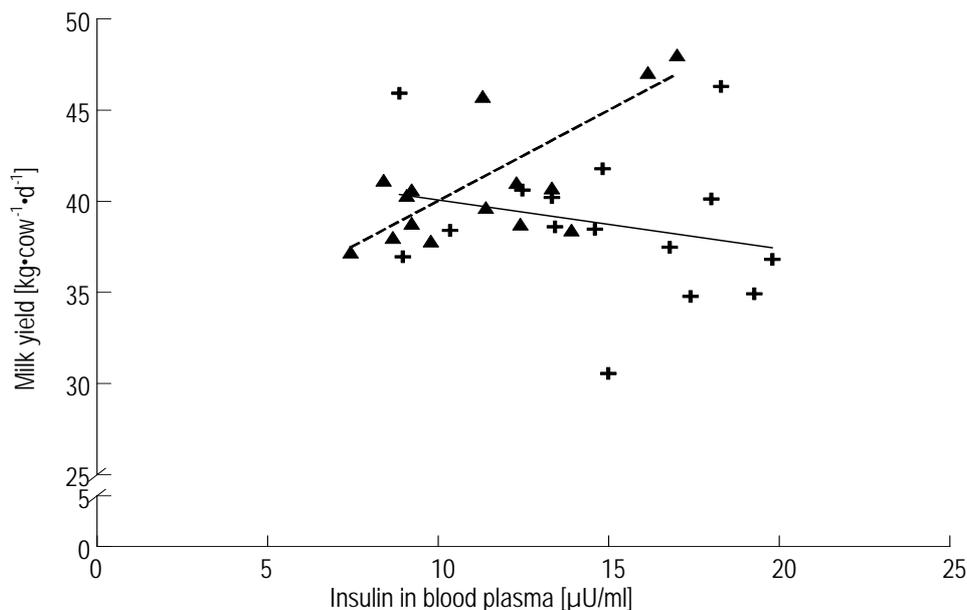


Figure 26. Relation between milk yield and insulin levels in blood plasma of cows in experiment I. Means of 15 and 16 cows sampled weekly in lactation week 2 to 15 for control and fat group. + — Control group [Milk yield = $42.7 - 0.27 \times \text{insulin}$; $r = -0.233$; $P = 0.403$]. ▲ - - - Fat group [Milk yield = $30.1 + 0.995 \times \text{insulin}$; $r = 0.689$; $P = 0.003$].

Plasma β -HBA and NEFA

The plasma concentrations of β -HBA were increased significantly by the substitution of starch by protected fat in experiment I (Table 9). This increase was observable during most of the experimental lactation weeks (Fig. 15). The increased β -HBA in plasma of cows fed on high-fat

low starch diet may be attributed to many factors, such as a lower supply of glucogenic nutrients (FERGUSON and SNIFFEN 1991) as a result of starch substitution, increased plasma NEFA concentration (CANT et al. 1993) and enhanced hepatic urea synthesis (GRUMMER and CARROLL 1991). However, the expected positive correlation between β -HBA and NEFA that was recorded in the study of CANT et al. (1993) was not clear in the present study (Table 13). That means that the NEFA were not the main source of β -HBA as in fasting animals. The higher level of β -HBA might be as a result of its synthesis in the liver from the accumulated acetyl-CoA, because the utilization of acetyl-CoA for FA synthesis in the mammary gland is reduced and the entry of acetyl CoA into TCA-cycle is decelerated because the intermediary products of this cycle are used for gluconeogenesis. In the control but not in the fat group of dairy cows, β -HBA concentrations were negatively correlated with those of plasma glucose and insulin (Table 13). This indicates the difference in glucose utilization between the cows of the two groups. As demonstrated in experiment II, glucose was not preferentially directed into TCA cycle in the dairy cows with high fat intake (Fig. 24). Apparently, the degradation of β -HBA in extra hepatic tissue may take place independent of glucose. β -HBA is transformed in three steps into Acetyl-CoA, which can be used for FA synthesis.

Plasma concentrations of NEFA were increased significantly by the replacement of starch by protected fat in the diet of lactating dairy cows in experiment I. This increase was significant during most of the experimental lactation weeks (Table 9 and Fig. 16). In general (see Fig. 25), increased concentration of NEFA in plasma may be attributed to either hydrolysis of increased blood triglycerides (DRACKLEY 1999) or inefficient uptake of FA by tissues because of FA spilling into blood during the hydrolysis of lipoproteins, where it is catalysed by the insulin dependent lipoprotein lipase (MARTIN et al. 1983; McNAMARA 1997). It can be expected that dietary fat increases the entry of lipoprotein into the lymphatic pathway and finally into the venous blood and decreases the antilipolytic action of insulin in comparison to starch.

5.3.2 Transfer of carbon atoms from fatty acids into milk constituents and blood CO₂

The ¹³C-abundance was lower in the diet of the fat group than in that of the control group due to its lower abundance in fat than in starch (3.1.6) as seen in Table 2. The enrichment of ¹³C in milk fat decreased significantly in the lactating dairy cows fed on a high-fat diet in comparison to cows fed on high-starch diet (Table 14, Fig. 21). However, this was not seen in milk lactose and except at the 15th week of lactation in milk casein (Fig. 21). This means that the dietary added fat was incorporated into milk fat and little carbon atoms were transferred into milk lactose or casein. Only at the end of the experiment, carbon atoms were transformed from fat into lactose

and casein. Theoretically, a channelling of carbon atoms from FA after their oxidation via TCA cycle to oxalacetate, and to glucose via gluconeogenesis is possible (see 2.4) and was demonstrated by SCHULZE et al. (1992) for endogenous fat. The results of the present study show that the including of fats in diets for high-yielding dairy cows did not enhance the transfer of carbon atoms from FA into lactose during the period of negative EB. However, a net transfer of carbon atoms into milk lactose and casein take places probably in the period of positive EB.

The enrichment of ^{13}C in blood CO_2 was higher for the lactating cows fed a high-fat diet than for those fed on a starch diet, which is seen in Fig. 22. From this, it was confirmed that the dairy cows fed on a high-fat diet obtained most of their energy requirements from other sources (e.g. acetate) than long chain FA, either dietary or mobilized FA. This result is in agreement with the finding of CUMMINS and RUSSELL (1985) *in vitro*, who showed a decreased oxidation of lipids in bovine adipose tissue when cows were fed on a high-fat diet.

In conclusion, the lactating mammary gland has an enormous capacity to use the preformed FA. Oxidation of FA is negligible and most of the dietary FA appear in milk fat.

5.3.3 Protein metabolism

Microbial protein in the rumen

As shown in Table 12, the milk allantoin content and consequently the calculated ruminal microbial N had a tendency to decrease in lactating dairy cows in experiment I that were fed on a diet containing protected fat instead of starch. The microbial N decreased significantly in the 10th week and tended to decrease at the 6th week of the experimental lactation period (Fig. 19). The substitution of starch by protected fat in the diets of lactating dairy cows supplies less fermentable matter and, consequently, little additional energy for rumen fermentation and thus may reduce microbial protein synthesis. On the other hand the efficiency of microbial protein synthesis (g microbial-N/kg fermentable organic matter) may increase by inhibition of the protozoan population in the condition of fat supplementation. The defaunating effect of lin seed oil was recently described by using of a concentrates-rich diet in the study of UEDA et al. (2003). We have used protected fat and the protozoan number was not estimated in the present study. However, KLUSMEYER et al. (1991) demonstrated earlier the positive effect of protected dietary fat on the efficiency of microbial protein synthesis. Hence this compensatory effect on the yield of microbial-N can explain the absence of significant difference in microbial-N yield at the 14th week of lactation between the animals of the two groups (Fig. 19).

Plasma free amino acids

The concentrations of free essential AA in plasma of lactating dairy cows were influenced by diets in experiment I and II as shown in Tables 10 and 18 as well as Fig. 18. Particularly, it was noticed that the levels of BCAA rose significantly with dietary fat supplementation, as found earlier by OLTJEN et al. (1971). In contrast, MOHAMED et al. (1988) found a decrease in the level of BCAA in plasma. The BCAA have been shown to be important in the regulation of AA and protein metabolism (LOBLEY 1992), and BCAA or their keto acids may even be involved in inter-organ (liver and mammary gland) regulation of nutrients partitioning (LAL and CHOUGH 1995). They provide nearly 50% of the EAA that are normally present in the milk protein and Ile could be a limiting AA in milk production (ROBINSON 1999). In contrast to the other AA, the BCAA are not predominantly oxidised in the liver but in a variety of extra-hepatic tissues including the mammary gland (BLOUIN et al. 2002). Insulin directs the AA toward the muscular tissues. Therefore, the response of plasma BCAA concentrations to the dietary fat supplementation may be due to decreased plasma insulin level. Furthermore, BCAA are taken up by the mammary gland in excess of their direct output in milk protein (MEPHAM 1982). Thus, BCAA provide an intermediate for glycolytic and Krebs cycle pathways and contribute to the pool of NEAA required for protein synthesis in the mammary gland. The low insulin level in plasma of fat-fed dairy cows could decrease the intramammary use of the BCAA. Because the milk protein output was not enhanced in the fat group (Table 8), it can be suggested, that the BCAA were not limiting AA and the effect of BCAA on milk protein yield were limited by the lack of other AA under the dietary conditions.

The decreased Met concentration in experiment I was significant during both the 6th and 14th weeks of lactation (Fig. 18). Met is very important for the synthesis of carnitine, apo-protein B, and phosphatidylcholine, which play important roles in the transport of FA to hepatic mitochondria and in the formation of very low density lipoproteins (OVERTON and PIEPENBRINK 2000). These syntheses could be enhanced by the high fat intake. The tendency to decreased levels of His in plasma of cows fed a fat diet is in agreement with the finding of CHRISTENSEN et al. (1994). His was identified as the first limiting AA in milk production of dairy cows fed on grass silage-cereal based diet (KORHONEN et al 2000). The decreased His level may be due to increased efficiency of its utilization as limiting AA. In both experiments of this study, the plasma concentration of Lys was not affected significantly. The significant increase in the total EAA in plasma of lactating cows fed a high-fat diet in experiment I may be attributed to the significant increase in the concentrations of BCAA in blood, but this total EAA was not affected in experiment II.

The increased concentration of 1-methylhistidine with high fat intake of dairy cows may be due to decreased activity of digestive peptidase in the small intestine (GSDL 2001) or due to increased protein catabolism because 1-methylhistidine is a component of anserine, which is a normal constituent of the muscle of many vertebrates (ZAPP and WILSON 1938). The concentration of 3-methylhistidine in plasma increased non-significantly in both experiments in lactating dairy cows fed on a high-fat diet, which may indicate that protein catabolism might be enhanced somewhat in these cows. The plasma concentrations of Ser were decreased significantly in experiment I, which was significant during both week 6 and 14 of lactation (Fig. 18) and had a tendency to decrease in experiment II. This decrease may be as a consequence to decreased Met, where Met acted as methyl-donor, which gave the methyl group to phosphatidylethanolamine to produce phosphatidylcholine and homocysteine. Then, in the presence of Ser, homocysteine can be converted to cysteine (EMMANUEL and KENNELLY 1984).

The ratio of EAA to NEAA in blood plasma was more than 0.60 for lactating dairy cows of both experiments, indicating an efficient protein supply (PALMQUIST and CONRAD 1978) for cows in both experiments for both diets (Tables 11 and 19). The average ratio of the total EAA to NEAA in experiment I was increased significantly for the lactating dairy cows fed on the high-fat diet in comparison to those fed on the control diet. The significant increase in the EAA/NEAA ratio of cows fed on a fat diet in the presence of reduced AA such as Met, His, and Ser indicates an enhanced hepatic gluconeogenesis (CANT et al. 1993; CASPER et al. 1988).

Plasma urea and urea kinetics

In experiment II, the plasma concentrations of urea were increased significantly by about 0.7 mmol/l when dairy cows were fed the fat diet instead of the starch diet (Table 17). This increase was also found as a tendency ($P = 0.094$) in experiment I (Table 9). Fig. 17 shows that there were significant differences between groups during the 7th and 10th weeks of lactation of dairy cows in experiment I. This confirms the findings of RODRIGUEZ et al. (1997b), CANT et al. (1993), PALMQUIST and CONRAD (1978) and CHRISTENSEN et al. (1994), and does not agree with the findings of AVILA et al. (2000) and BUCKLEY et al. (2000) which showed unaffected plasma urea concentrations when feeding lactating cows on diets supplemented with Ca salts of FA. ROMO et al. (2000) recorded lower blood urea concentrations for cows infused abomasally with long chain FA.

The isotope studies with $^{15}\text{N}_2$ -urea have shown that the substitution of starch by protected fat in the diet increased significantly the rate of urea flux by $0.39 \text{ g N/kg BW}^{0.75}$ (Table 12). It has been shown previously that abomasal infusion of dairy cows with vegetable oil significantly increased the net portal drained viscera release and liver uptake of NH_3 and numerically the net release of urea by the liver (BENSON et al. 2002). High fat intake of lactating dairy cows led to elevated hepatic utilization of AA for gluconeogenesis in the study of CANT et al. (1993). From these previously mentioned facts, two factors could be responsible for the increased rate of urea flux and urea concentration in plasma and milk: 1. The decreasing of starch as an energy supply in the diet containing protected fat that led to decreased ammonia fixation by microbes in the rumen (Table 12). 2. The increased catabolism of some AA in the liver led to an increase in the rate of urea synthesis (OLTNER and WIKTORSSON 1993). Because the diets were formulated to have the same N-balance in the rumen, the second reason should be of more importance. However, the higher intake of crude protein in the fat group (29 g N/cow or $0.22 \text{ g N/kg BW}^{0.75}$, Table 6) and the problems to calculate the rumen N balance (GfE 2001) should also be taken into account for discussion. Regarding these considerations and the relatively small increase in plasma urea level and urea-flux, it can be concluded that the oxidation of AA is not excessively stimulated by the substitution of starch by fat.

5.4 Conclusion

Lactating dairy cows utilize fat by 1) direct incorporation into milk fat, 2) deposition in adipose tissues and 3) oxidation (PALMQUIST 1994). The current study was designed to examine if protected fat instead of starch in the diets of dairy cows in the early lactation period is used predominantly as energy fuel or is transferred directly to milk fat. Further, the effect of dietary fat in comparison to starch on the glucose and protein metabolism of dairy cows was to be studied.

The ratio of glucogenic to lipogenic nutrients decreases by the addition of fat, because the dietary fat supplies no glucose or glucogenic precursors. This can enhance the development of metabolic disorders like ketosis. On the other hand, the mammary gland seems to show more preference for long chain FA than for acetate for milk fat synthesis. Thus, fat can save glucose because the requirement of glucose for fat synthesis (NADPH production) is reduced, as was confirmed in the present study. A higher portion of available glucose in the metabolic pool is used for lactose synthesis and a lesser amount is oxidized and incorporated into milk fat in cows fed on a high-fat diet in comparison to those fed on a control diet.

This means that the availability of glucose for milk synthesis is higher in cows with a high fat intake, which could also be the result of a lower insulin level in the blood plasma. Insulin is involved in the mechanisms of glucose partitioning, which is directed toward body tissues and not to the milk synthesis. Thus, the inclusion of fat in diets causes, in comparison to a starch diet, an enhancement in lactogenesis, which is reflected in higher milk production. This effect exists at times in which the daily energy deficit is lower than 20 MJ NEL and not in the period of highly negative EB. On the other hand, the cows are not able to produce more milk protein. The increase in milk production even diluted the milk protein level. However, the negative correlation between milk protein content and milk yield is absent, presumably as a result of increased plasma insulin level in cows with higher milk yield. The increased urea synthesis and altered level of free AA in blood plasma may be attributed to enhanced hepatic gluconeogenesis from AA. The increase of BCAA in blood indicates that the dietary fat influences the extra-hepatic metabolism of AA, presumably including the intramammary use of BCAA.

The increased level of NEFA in blood of lactating dairy cows fed on diets contained protected fat may be as a result of higher FA intake and changing of FA turnover in the adipose tissues. In addition, the uptake of FA and/or synthesis of FA by the mammary gland may be influenced by specific FA, which is manifested as a reduced milk fat content.

Even today, only a limited understanding of fatty acid oxidation exists for dairy cattle fed supplemented fat. As seen from the isotope studies, the dietary fat was predominantly incorporated into milk fat and was oxidized to a lesser extent than other energy sources. Net transfers of carbon atoms from dietary fat into lactose or casein do not occur in the period of negative EB, but is possible in the period of positive EB.

Overall, it can be concluded that the oxidation of dietary fat is negligible and most of dietary FA are incorporated into milk fat. The inclusion of protected fat in the diets of dairy cows in early lactation supports the homeorhetic control of nutrients partitioning in the direction of milk synthesis. This effect is augmented when the cows reach the state of positive EB. Further studies are necessary to examine the specific effects of dietary fat on the hormonal control of nutrients utilization and the AA metabolism to prevent the depression of milk protein content.

6 Summary

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Effect of fat in comparison to starch in an isoenergetic diet on the metabolism of high yielding dairy cows

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The aim of these experiments was to study the effect of dietary protected fat instead of starch on the metabolism of dairy cows during the early lactation period, especially whether the ratio of glucogenic to lipogenic nutrients could affect the supply of glucose in the metabolism of dairy cows. Therefore, the study was conducted in two experiments. Experiment I was carried out on two groups of high-yielding dairy cows ($n = 16$ in each group) during the first 15 weeks of the lactation period. The dairy cows were fed semi-ad libitum on isoenergetic and isoproteineous rations based on corn silage. The proportion of fat energy to the total energy of the ration was 6.7% for the control diet and 15.8% for the fat diet. About 2.9 kg of tapioca (about 1.8 kg starch, $-24.1 \delta\text{-}\text{‰}^{13}\text{C}$) in the diet of the control group were substituted by about 0.7 kg of fat (as Ca salts of palm, soybeans and sunflower oil FA and protected soybeans, $-28.1 \delta\text{-}\text{‰}^{13}\text{C}$) in the diet of the fat group of cows. In both groups, 5 cows were fitted with permanent catheters in the coccygeal vein (median caudal vein). The milk yield of the dairy cows was measured three times daily, in addition to the daily nutrients intakes. Milk, feed, and blood samples were taken weekly to estimate the milk and feeds composition, different metabolic blood parameters, ruminal microbial protein synthesis, urea-flux, and the ratio of ^{13}C to ^{12}C in milk components and blood CO_2 . Experiment II was carried out on 8 dairy cows during the 6th to 10th week of the lactation period. The dairy cows were subjected to two consecutive subperiods (cross-over) and fed on two consecutive rations (protected fat or starch). The dairy cows were injected intravenously with a single dose of D-[U- $^{13}\text{C}_6$]-Glucose in each subperiod.

In experiment I, the substitution of starch by protected fat tended to increase the milk production (38.8 vs. $41.5 \text{ kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$, $P = 0.075$) and milk lactose output (1.82 vs. $1.96 \text{ kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$, $P = 0.072$). Milk urea content increased (3.57 vs. 3.96 mmol/l , $P = 0.040$) but that of milk protein decreased (3.12 vs. 2.91% , $P = 0.001$). The energy balance and the efficiency of milk production (ECM/NEL intake) of cows were not significantly affected by the starch substitution. The drop in the thickness of subcutaneous fat was lower ($P < 0.05$) between the 6th and 10th week

of lactation. The substitution of starch by protected fat led to a tendency to decrease of the microbial protein synthesis in the rumen (235 vs. 209 g N·cow⁻¹ · d⁻¹, P = 0.086) and plasma glucose concentrations (3.20 vs. 2.83 mmol/l, P = 0.080) but decreased the plasma insulin concentrations (14.8 vs. 11.6 μU/ml, P = 0.010), increased the plasma levels of β-hydroxybutyrate (1.16 vs. 1.91 mmol/l, P < 0.001) and NEFA (211 vs. 307 μmol/l, P = 0.006). The total urea-flux in the animal body, measured by isotope dilution (¹⁵N), increased (1.69 vs. 2.08 g N · kg LM^{-0.75} · d⁻¹, P = 0.031) and the urea levels in blood were non-significantly increased (5.12 vs. 5.70 mmol/l, P = 0.094). The level of the free essential AA Met (23.3 vs. 19.6 nmol/ml, P = 0.040), Ser (87.2 vs. 73.4 nmol/ml, P = 0.016), and His (44.1 vs. 39.4 nmol/ml, P = 0.06) decreased. The levels of branched-chain AA in blood plasma increased (Ile, P < 0.001; Leu, P < 0.001; Val, P = 0.005). In comparison to the control group, the enrichment of milk fat with ¹³C was lower (P < 0.001), while that of blood CO₂ was higher in the fat group (P < 0.05). In experiment II, the ¹³C-excess in blood CO₂ was lower till one hour after i.v. infusion of ¹³C-glucose when cows were fed on the high-fat diet than when fed on the starch diet (P < 0.05). In comparison to the starch diet, the ¹³C-recovery increased in lactose (P = 0.065) in case of fat diet..

The results indicate that the substitution of starch by protected fat can save glucose in the intermediary metabolism for lactose synthesis in the mammary gland. The dairy cows used FA predominantly for milk fat synthesis and not for oxidation. From the increased urea-flux and changing of the plasma concentrations of free AA, it can conclude that the amino acid catabolism was enhanced. Therefore, the decreased levels of limiting amino acid methionine and histidine in plasma must be taken into account during ration formulation of lactating dairy cows. As a conclusion, the protected fat in the diets of lactating dairy cows supports the homeostatic control of nutrient partitioning toward the milk synthesis especially in case of low energy deficit and in the periods of balanced and positive energy balance.

7 Zusammenfassung

Die Wirkung von Fett im Vergleich zu Stärke in einer isoenergetischen Ration auf den Stoffwechsel der Hochleistungskuh

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Schlüsselworte: Milchkuh, geschütztes Fett, Stärke, stabile Isotope, Stoffwechsel.

Das Ziel der Untersuchungen bestand darin, die Wirkung von Fett als Energiequelle mit der von Stärke in der Futtermischung auf den Stoffwechsel der Milchkuh in der Früh-Laktation zu vergleichen. Insbesondere sollte die Frage beantwortet werden, ob durch die Verringerung des Verhältnisses von glucogenen zu lipogenen Nährstoffen die Glucoseversorgung der Kuh beeinflusst wird. Dazu wurden zwei Versuche durchgeführt. Im Experiment I erhielten in zwei Gruppen eingeteilte Milchkühe in den ersten 15 Laktationswochen in Einzelfütterung semi-ad libitum isoenergetische und isoproteinogene Rationen auf der Basis von Maissilage. In der Tagesration der Fettgruppe (n = 16) waren 2,9 kg Tapioka (1,8 kg Stärke, $-24,1 \delta\text{-}\text{‰}^{13}\text{C}$) der Ration der Kontrollgruppe (n = 16) durch 0,7 kg Fett (Ca-Salze langkettiger Fettsäuren aus Palmfett und Soja-/Sonnenblumenöl, und geschütztes Sojabohnenschrot, $-28,1 \delta\text{-}\text{‰}^{13}\text{C}$) substituiert. Der Anteil der Fettenergie an der Gesamtenergie der Rationen betrug 6,7 % (Kontrollgruppe) und 15,8 % (Fettgruppe). In jeder Gruppe waren 5 Kühe mit einem permanenten Katheter in der *V. coudalis mediana* ausgestattet. Die Milchleistung der 3-mal täglich gemolkenen Kühe wurde täglich registriert, die Rückenfettdicke wöchentlich. In wöchentlichen Abständen wurden Futter-, Milch-, und Blutproben entnommen. Gemessen wurden: Nährstoffaufnahme, Milchinhaltstoffe, Blutparameter, Allantoin Ausscheidung in der Milch, Harnstoff-Flux sowie das $^{13}\text{C}/^{12}\text{C}$ -Verhältnis in Milchinhaltstoffen und Blut- CO_2 . Im Experiment II wurde an 8 Milchkühen in der 6. bis 10. Laktationswoche in einer Überkreuz-Versuchsanlage mit obigen Behandlungen der Verbleib von Glucose-C nach einmalig i.v. infundierter D-[U- $^{13}\text{C}_6$]-Glucose verfolgt.

Im Experiment I bewirkte der Austausch von Stärke- durch Fettenergie eine nicht signifikante Erhöhung der Milchleistung ($38,8$ vs. $41,5 \text{ kg} \cdot \text{Kuh}^{-1} \cdot \text{d}^{-1}$, $P = 0,075$) und des Laktoseaustrags ($1,82$ vs. $1,96 \text{ kg} \cdot \text{Kuh}^{-1} \cdot \text{d}^{-1}$, $P = 0,072$). Der Harnstoffgehalt in der Milch stieg an ($3,57$ vs. $3,96 \text{ mmol/l}$, $P = 0,040$), während der Milchproteingehalt abfiel ($3,12$ vs. $2,91 \%$, $P = 0,001$). Die Energiebilanz und Effizienz der Milchbildung (ECM/NEL- Aufnahme) waren unverändert. Der

Mikrobenproteintrag im Pansen war tendenziell verringert (235 vs. 209 g N · Kuh⁻¹ · d⁻¹, P = 0,086). Der Abfall der Rückenfettdicke war in der 6. bis 10. Laktationswoche in der Fettgruppe geringer (P < 0,05). Im Blutplasma waren der Gehalt an Glucose nicht signifikant (3,20 vs. 2,83 mmol/l, P = 0,080) und der Insulinspiegel signifikant vermindert (14,8 vs. 11,6 µU/ml, P = 0,010). Die Spiegel von β-Hydroxybutyrat (1,16 vs. 1,91 mmol/l, P < 0,001) und NEFA (210 vs. 310 µmol/l, P = 0,006) waren erhöht. Der durch Isotopenverdünnung gemessene Harnstoff-Flux im Gesamtkörper war größer (1,69 vs. 2,08 · g N · kg LM^{-0,75} · d⁻¹, P = 0,031) und der Spiegel von Harnstoff im Blutplasma tendenziell erhöht (5,12 vs. 5,70 mmol/l, P=0,094). Im Blutplasma waren die Spiegel an freiem Methionin (23,3 vs. 19,6 nmol/ml, P=0,040) und Serin (87,2 vs. 73,4 nmol/ml, P = 0,016) reduziert. Der Gehalt an Histidin sank tendenziell (44,1 vs. 39,4 nmol/ml, P = 0,06). Die Gehalte der verzweigkettigen Aminosäuren waren erhöht (Ile, P < 0,001; Leu, P < 0,001; Val, P = 0,005). Der Kohlenstoff im Milchfett war geringer mit ¹³C angereichert, der im Blut-CO₂ demgegenüber stärker angereichert (P < 0,05). Im Experiment II war bis 1 h nach der i.v. Verabfolgung von ¹³C-Glucose der ¹³C-Excess im Blut-CO₂ bei der Fettration geringer als bei der Stärkeration (P < 0,05). Im Vergleich zur Stärkekütterung war die Wiederfindungsrate des Glucose-¹³C in der Lactose bei der Fettfütterung erhöht (P = 0,065).

Die Ergebnisse zeigen, dass durch den Austausch von Kohlenhydratenergie durch Fettenergie Glucose im Intermediärstoffwechsel eingespart wird, die für die Synthese von Lactose in der Milchdrüse zur Verfügung steht. Die Kühe nutzen die langkettigen Fettsäuren vorwiegend für die Milchfettsynthese, nicht für die oxidative Energiegewinnung. Aus dem erhöhten Harnstoff-Flux und dem veränderten Spiegel freier Aminosäuren im Blutplasma kann auf einen gesteigerten Aminosäurenkatabolismus und eine Beeinflussung des Aminosäurestoffwechsels geschlossen werden. Der Zufuhr der für die Milchproduktion erst- oder co-limitierenden Aminosäuren, wie Methionin und Histidin, ist bei der Rationsbilanzierung unter Nutzung von Futterfetten besondere Beachtung zu schenken. Es ergibt sich die Schlussfolgerung, dass mit der Diät zugeführtes pansenstabiles Fett die homeorhetische Regulation der Nährstoffverteilung bei der laktierenden Kuh günstig beeinflusst, was insbesondere in der Periode einer geringen negativen bzw. ausgeglichenen und positiven Energiebilanz zutreffend ist.

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