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Effects of folic acid and pantothenic acid supplementation to two rations on rumen fermentation, duodenal nutrient flow and serum and milk variables of dairy cows

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Effects of folic acid and pantothenic acid supplementation to two rations on rumen fermentation, duodenal nutrient flow and serum and milk variables of dairy cows

The aim of the present experiments was to investigate the influences of a daily supplementation of 1 g folic acid (**FA**), respectively pantothenic acid (**PA**) on ruminal fermentation, as well as on serum and milk variables. German Holstein cows with one cannula in the dorsal sac of the rumen and another in the proximal duodenum received a ration with a forage to concentrate ratio (**F:C**) of 34:66 (high concentrate, **HC**) in the first part of each experiment and in the second part of 66:34 (high forage, **HF**). Each ration was fed with and without FA or PA supplementation. Thereby, the influences of the ration composition on the synthesis and/or degradation of folates, FA and PA due to ruminal microorganisms were to be investigated. The number of lactating and dry cows was in the first part of the FA experiment four and two, and in the second part four and four. In the first part of the PA experiment it was six and two and in the second part five and four. Regardless of the dietary F:C ratio, both vitamins had rather minor effects on ruminal fermentation as well as on the serum and milk variables. However, irrespective of the ration the FA supplementation increased the serum 5-methyl-tetrahydrofolate concentration. The PA supplementation only increased the duodenal PA flow during the feeding of the HF ration. Irrespective of the ration, the serum and milk PA concentrations as well as the milk PA yields were not influenced by the PA supplementation. However, feeding the HC ration always resulted in higher PA amounts in all considered fluids compared to the HF ration. This was the same for the folate amounts in the serum. Thus, the results of the present experiments show that a 1 g FA supplementation increases the 5-methyl-tetrahyrofolate concentration in the serum, but no positive effects for the dairy cows were detectable, while a 1 g PA supplementation neither increases the PA concentrations in the serum or in the milk. However, the apparent disappearance between mouth and duodenum for PA was between 88 and 97%.

Effekte einer Folsäure- und Pantothensäure-Supplementierung zu zwei Rationen auf die ruminale Fermentation, den duodenalen Nährstofffluss und Serum- und Milchvariablen von Milchkühen

Die durchgeführten Versuche hatten zum Ziel, den Einfluss einer Zulage von 1 g Folsäure (**FA**) bzw. Pantothensäure (**PA**) je Tag auf die ruminale Fermentation sowie auf Serum- und Milchvariablen von Milchkühen zu untersuchen. Deutsche Holstein Kühe mit je einer Fistel im dorsalen Pansensack sowie im proximalen Duodenum erhielten im ersten Abschnitt jedes Versuches eine Ration mit einem Grundfutter-Kraftfutter-Verhältnis (**GF:KF**) von 34:66 (high concentrate, **HC**) und im zweiten Abschnitt von 66:34 (high forage, **HF**) jeweils mit und ohne Vitaminzulage. Damit sollten mögliche Einflüsse der Rationszusammensetzung auf die Synthese und/oder den Abbau von Folaten, FA und PA durch die Pansenmikroorganismen getestet werden. Die Anzahl an laktierenden bzw. trockenstehenden Kühen betrug im ersten Abschnitt des FA-Versuchs vier und zwei, im zweiten Abschnitt vier und vier; im ersten Abschnitt des PA-Versuchs sechs und zwei und im zweiten Abschnitt fünf und vier. Unabhängig vom GF:KF Verhältnis hatten beide Vitamine einen nur geringen Einfluss auf die ruminale Fermentation sowie auf die Serum- und Milchvariablen. Die FA-Zulage steigerte bei beiden Rationen die 5-Methyl-Tetrahydrofolat-Konzentration im Serum. Die PA-Gabe erhöhte den duodenalen PA-Fluss lediglich bei Fütterung der HF-Ration. Ein Einfluss der PA-Gabe auf die PA-Konzentrationen in Serum und Milch, sowie die Milch-PA-Menge wurde bei keiner Ration nachgewiesen. Die Fütterung der HC-Ration resultierte im Vergleich zur HF-Ration allerdings immer in höheren PA-Mengen in den untersuchten Flüssigkeiten. Analog traf dies für die Folatmengen im Serum zu. Zusammenfassend zeigen die Ergebnisse der durchgeführten Versuche, dass die Zulage von 1 g FA die 5-Methyl-Tetrahydrofolat-Konzentration im Serum steigert. Ein positiver Effekt für die Kuh war allerdings nicht nachweisbar. Die Zulage von 1 g PA zeigte weder gesteigerte PA-Konzentrationen im Serum noch in der Milch. Die scheinbaren Verluste an PA zwischen Maul und Duodenum betrugen allerdings 88 bis 97 %.

Contents

Abbreviations

ACP	Acyl-carrier-protein
ADF	Acid detergent fibre
AICAR	Aminoimidazolecarboxamide ribonucleotide
AS	Apparent synthesis
Ash	Crude ash
BW	Body weight
CF	Crude fibre
CoA	Coenzyme A
CP	Crude protein
d	Day
DHF	Dihydrofolate
DIM	Days in milk
DM	Dry matter
DMF	Dry matter flow
DNA	Deoxyribonucleic acid
dTMP	Deoxythymidylate
dUMP	Deoxyuridylate
EE	Ether extract
EP	Endogenous crude protein
FA	Folic acid
FAICAR	Formylaminoimidazolecarboxamide ribonucleotide
F:C	Forage to concentrate ratio
FCM	Fat corrected milk
FGAR	Formylglycinamide ribonucleotide
FOM	Ruminally fermented organic matter
GAR	Glycinamide ribonucleotide
GF:KF	Grundfutter-Kraftfutter-Verhältnis
HC	High concentrate, 34% forage and 66% concentrate on DM basis
HC+FA	High concentrate with folic acid
HC+PA	High concentrate with pantothenic acid
HF	High forage, 66% forage and 34% concentrate on DM basis
HF+FA	High forage with folic acid
HF+PA	High forage with pantothenic acid
i.m.	Intramuscular
LFL	Bayerische Landesanstalt für Landwirtschaft
LSmeans	Least squares means
m	Multiparous
ME	Metabolisable energy
MP	Microbial crude protein
NAD	Nicotinamide adenine dinucleotide
NAN	Non-ammonia-N
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
NEL	Net energy lactation
NFC	Non-fibre carbohydrates

OM	Organic matter
OMI	Organic matter intake
p	Primiparous
PA	Pantothenic acid
RDP	Ruminal degraded protein
SAM	S-adenosylmethionine
SCFA	Short chain fatty acids
SD	Standard deviation
SE	Standard error
SEM	Standard error of means
ß-HBA	*beta*-hydroxybutyrate
THF	Tetrahydrofolate
uCP	Utilisable crude protein
UDP	Ruminally undegraded protein

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1 Introduction

B-vitamins are a heterogeneous group of substances with important coenzyme functions (Schweigert 2000). Thiamin (B_1), riboflavin (B_2), pyridoxine (B_6), cobalamin (B_{12}), niacin, biotin, folic acid (**FA**) and pantothenic acid (**PA**) belong to the group of B-vitamins, whereas this classification is purely historical, as these substances have different chemical structures and metabolic roles (Girard and Matte 2006).

The physiologically active form of FA is tetrahydrofolate (**THF**), which is the central coenzyme for the C_1-metabolism (Michal 1999). Thus, THF participates amongst others in purines and pyrimidines synthesis, as well as in the formation of the primary methylating agent S-adenosylmethionine (Bailey and Gregory 1999).

Pantothenic acid is a component of the important acyl group carrier coenzyme A (**CoA**) and acyl-carrier-protein (**ACP**). Coenzyme A and ACP are available in every cell and are involved in more than 100 reactions e.g. fatty acid synthesis and degradation, pyruvate oxidation and glycerine synthesis (Michal 1999).

It is well established that the microorganisms in the rumen can synthesise B-vitamins (Bechdel et al. 1928). Thus, ruminants have two sources of FA and PA: feed and ruminal synthesis. The Society of Nutrition Physiology could not advise a general supplementation of B-vitamins (Gesellschaft für Ernährungsphysiologie 2001). In the latest edition of Nutrient Requirements of Dairy Cattle the National Research Council (2001) presented first proposals for B-vitamin requirements for tissue and milk. However, it must be considered that the B-vitamin requirements for tissue were extrapolated from requirements of lactating sows. Comparing the proposed requirements (computed for a cow with 650 kg body weight (**BW**) and 35 kg fat-corrected milk) with their ruminal synthesis (calculated from the estimated intake and duodenal flow of each vitamin) it becomes apparent that FA and PA seem to be the sole B-vitamins where the estimated requirements are not met by ruminal synthesis. Therefore supplementations of FA and PA may be beneficial for high-producing dairy cows, whereas, it must be considered that only two studies are available observing the duodenal FA flow in dairy cows (Santschi et al. 2005a; Schwab et al. 2006), and the duodenal flow of PA has only been measured once with growing steers (Zinn et al. 1987). Furthermore,

studies on the impact of FA and PA on ruminal metabolism, blood and milk variables are rare. For dairy cows the influence of an FA supplementation on blood and milk concentrations of FA has been measured in four studies, while PA influence on blood and milk PA concentrations were tested only once. Additionally, the influence of PA on the performance of dairy cows has been tested once and an increase in milk production was established (Bonomi 2000). For FA the influences on performance vary, as studies with and without an effect can be found (Girard and Matte 1998; Girard et al. 2005; Graulet et al. 2007). So far, no studies are available that consider the influences of these vitamins on ruminal metabolism, duodenal nutrient flows, blood and milk variables, as well as the vitamin concentrations in these matrices simultaneously. Results of such a simultaneous survey would be very helpful for a better understanding of the influence of FA and PA on dairy cows.

As B-vitamins are synthesised and degraded by ruminal microorganisms, the composition of the ration may also have an effect on the availability of FA and PA at the duodenum. For FA, the influence of different forage to concentrate (**F:C**) ratios on duodenal FA flow has been measured once, by Schwab et al. (2006). They established that the ration with the highest concentrate and non-fibre-carbohydrate (**NFC**) content resulted in the highest duodenal flows of FA, while the ration with the lowest content of concentrates and NFC led to the lowest flow of FA. However, the effects of varying F:C ratios on supplemented FA have not been proven. So far, an influence of the dietary composition on the duodenal flow of PA has not been observed. Besides FA, the duodenal flows of B_1, B_6, B_{12} and niacin are also influenced by the F:C ratio of the diet (Schwab et al. 2006), thus it is conceivable that the duodenal flow of PA is also influenced.

Therefore, the objective of the present studies was to contribute to a better understanding of FA and PA metabolism in the rumen and the whole animal as will be described in detail in the following sections.

2 SCOPE OF THE THESIS

Deduced from the mentioned gaps of knowledge in the introduction, the scope of this thesis was to establish on the one hand the influence of an FA supplementation and on the other hand the influence of a PA supplementation on selected variables in dairy cows. A further focus was put on the impact of different F:C ratios of the diets on each vitamin. Thus, this thesis is based on the following questions:

1. What influence has an FA or PA supplementation on ruminal metabolism and duodenal nutrient flow with two rations varying in the F:C ratio?

2. What influence has the supplementation of FA respectively PA in the two diets on blood and milk variables?

3. Has the supplementation of FA and PA and the different F:C ratios an influence on the duodenal flow, blood and milk concentration of the respective vitamin?

The influences of an FA supplementation are examined in Chapter 3, while PA is handled in Chapter 4.

Each Chapter starts with a review of current literature, followed by the results of the studies on the impact of an FA respectively a PA supplementation to two diets differing in F:C ratio on ruminal fermentation, nutrient flow at the duodenum, blood and milk variables of dairy cows. Therefore double fistulated dairy cows were either fed with a diet consisting of 34% forage and 66% concentrate or 66% forage and 34% concentrate. The FA and PA experiment were implemented at the same time; thus, each cow either received 1 g FA, or 1 g PA or no supplementation daily.

3   FOLIC ACID

3.1   Folic acid in ruminant nutrition: a review

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Introduction

In general, it is assumed that B-vitamin requirements for ruminants can be met by microbial synthesis in the rumen, even when the animals are fed a diet providing very small amounts of those vitamins. This hypothesis was already established in 1928 by Bechdel et al.. However, since that time average milk and milk component yields have increased drastically (already by about 33% in the last 15 years in the USA), whereas the increase of average dry matter (**DM**) intake was considerably lower (only about 15%; Weiss and Ferreira 2006). Furthermore, feeding strategies changed to support the increase in milk production and milk component yields. Changes in diet composition (from less forage towards more concentrate) influence the microbial population in the rumen, so it is a moot point whether the B-vitamin requirements of dairy cows are still being met.

Folic acid is very important during lactation and for deoxyribonucleic acid (**DNA**) synthesis of fetal and placental tissue during pregnancy (McNulty et al. 1993), therefore a suboptimal supply should be avoided. In agricultural practice in dairy cows, gestation and lactation are concomitant during several months per year, so the avoidance of progressive folate deficiency must be a priority.

Up to now the folate content of feed is rarely analysed and values on microbial folate synthesis are scarce. So it is very difficult to estimate a cow's actual supply with folates (National Research Council 2001). The National Research Council (2001) tried to estimate requirement values of folates for cows, but they had to extrapolate the cows' requirements from data of sows and average vitamin contents found in cows' milk. They estimated a daily folate requirement of 33 mg/d for tissue and 2 mg/d for milk for a dairy cow with a BW of 650 kg and a milk production of 35 kg fat-corrected (4%) milk per day. However, final evidence in the form of studies on cows' requirements is still lacking, as the number of appropriate studies is limited. Therefore this review will present current knowledge of folate metabolism and the influence of FA supplementation on ruminal variables, folate absorption and performance, especially for dairy cows. Areas on which future research should focus will be highlighted.

Chemical structure

The vitamin FA (chemical name pteroylglutamic acid) consists of three parts: a pteri-
dine nucleus, *para*-aminobenzoic acid and glutamic acid (Girard 1998; see Appendix
Figure A1). The name FA is deduced from *folium*, the Latin word for leaf, because
native forms of FA were originally isolated from spinach leaves (Mitchell et al. 1944). In
chemistry the name FA is only used for the synthetic form. It is a stable compound and
the basal structure of a wide family of vitamin coenzymes (Lucock 2000). In nature,
more than 100 compounds, with the basal structure of FA, feature a common vitamin
activity. These pteroylglutamate forms of FA are generally called folates (Bender 1992;
Girard 1998; Finglas et al. 2003). Native folates vary in three chemical characteristics
from FA: first, in the level of reduction of the pteridine nucleus (dihydrofolate or THF);
second, in the character of the onecarbon substituent linked to the N atoms N-5 and
N-10 (for example, formyl, formimino, methyl, methylene or methenyl residues); third,
in the chain length of the glutamyl residues which can be linked to the γ-carboxyl
group of the glutamate via peptide linkages (Wagner 1984; Girard 1998).

Absorption and biochemical functions

There are several excellent reviews on absorption and biochemical functions of folates
(Selhub et al. 1983; Bässler 1997; Scott 1999; Wright et al. 2007). Derived from studies
with non-ruminant animals, two mechanisms of folate absorption from the intestinal
tract seem to exist: an active saturable process and a non-saturable passive process. In
fact, the relative importance of passive absorption changes according to folate supply,
increasing with the amounts of folates available (Selhub et al. 1983; Bässler et al. 2002).
However, folates are perhaps degraded, converted and synthesised in the forestomachs
of ruminants (Zinn et al. 1987), and even absorbed on a small scale (Rérat et al. 1958).
Unfortunately the forms and the availability of the forms present in rumen contents
and duodenal digesta are unknown.

In bovine blood, mainly 5-methyl-THF is found (Anonymous 1992). Cells take up this
compound and demethylate it to THF. To retain THF in cells it must be converted
by folylpolyglutamate synthase to polyglutamate THF, the coenzymic form of folates
(Bässler 1997). Polyglutamate THF is involved in several biochemical pathways in

mammals (Choi and Mason 2000; see Figure A2 in the Appendix). Mainly, folates are donors and acceptors of one-carbon units (Bender 1992; Benevenga 2004). Thus they are involved in the remethylation of homocysteine to methionine, as an essential part of the methylation cycle. This reaction is also vitamin B_{12} dependent, as the catalysing enzyme methionine synthase needs vitamin B_{12} (Scott 1999). Furthermore, the transfer of one carbon units involves folates in the synthesis of purines and pyrimidines and thereby in DNA synthesis and cell proliferation (Bässler 1997). Tetrahydrofolate is regenerated after these catalytic reactions (Bässler 1997). However, folate disappears through urinary excretion and through bile, although a very effective reabsorption by the enterohepatic cycle exists (Bässler et al. 2002). Up to now only one study reported on the urinary excretion of folates in dairy cows (nine animals) after intramuscular (**i.m.**) injection of 0.3 mg FA per kg BW (Girard and Matte 1995). The authors found an excretion of the injected dose of 35.1% after 8 h and 44.2% after 48 h.

A deficit of folates can lead to a decrease in S-adenosylmethionine levels and to an abnormal DNA precursor metabolism resulting in faulty DNA synthesis and a decrease in nicotinamide adenine dinucleotide (**NAD**; James et al. 1994), as a decrease in NAD levels is consistent with an increase in DNA repair activity (James et al. 1989). An indirect lack of folates can be caused by a vitamin B_{12} deficit. This results in an accumulation of 5-methyl-THF called a methyl-trap, as 5-methyl-THF cannot be regenerated to THF (Bässler et al. 2002). If so, cells are unable to conjugate absorbed folate monoglutamates, resulting in a decreased intracellular folate polyglutamate level. Additionally, intracellular folate accumulation declines as only polyglutamates can be retained in cells (Scott and Weir 1976).

As folates influence DNA synthesis and the methionine cycle, they are involved in the metabolic pathways of reproduction and milk protein synthesis; therefore they are very important especially in gestating and lactating cows. An additional special situation for cows is that they have a very high demand for methyl groups in early lactation. Concurrently some precursors for methylated compounds (for example, serine and glycine) are also needed for gluconeogenesis, as the amounts of glucose reaching the small intestine through the digestive system are generally low. So, coincident demand for precursors of methylated compounds leads to competition between different metabolic pathways,

for example, gluconeogenesis, lecithin synthesis, DNA synthesis and remethylation of methionine (Brüsemeister and Südekum 2006; Girard and Matte 2006).

Sources and stability of folates

The following section gives a survey of approximate folate concentrations in some feeds and foods. Different folate contents are given in the literature for the same feedstuffs (Table 1) and for most feeds they are not analysed at all, so only few data are available. It must be considered that as compilations were used in Table 1, the number of samples analysed is not known. Additionally, it must be pointed out that the native folate concentrations in feeds vary due to influences of climate, species, vegetation stage, habitat and fertiliser (Albers et al. 2002). Furthermore, most naturally occurring folates are chemically relatively unstable. Thus folates exhibit a significant loss of activity during harvesting, storage and processing, but measured folate concentrations are also highly influenced by the method used for sample preparation (Gregory 1989). The synthetic form, FA, is more resistant to chemical oxidation (Scott 1999).

The figures generally show very low folate contents in feedstuffs. However, quantities are not the only decisive factor; the folates in the feed must also be available for absorption (Wagner 1984). The so-called bioavailability describes the proportion of an orally administered dose which is available in plasma after absorption (van den Berg 1993). It is difficult to consider the bioavailability of native folates because unknown numbers and amounts of folate metabolites exist in every plant species or feedstuff. For ruminants the assessment of folate bioavailability is more difficult as their microorganisms in the rumen synthesise, but also degrade, ingested folates. The synthesis and degradation of folates in the rumen is crucial for the amount absorbed from the small intestine of ruminants. Up until now the number of studies on rumen folate synthesis and degradation has been very low; therefore for ruminants no values of folate bioavailability from feedstuffs are available. Furthermore the bioavailability of native folates is influenced by different physico-chemical properties and certain feed constituents. For example, polyglutamyl folates have a lower bioavailability than monoglutamyl folates, as polyglutamyl folates must be hydrolysed to monoglutamates before absorption (Seyoum and Selhub 1998). Additionally, the actual amount available for each individual

animal varies depending on differences in intestinal pH or general living conditions (Wagner 1984).

Table 1: Folate content of several feeds given in the literature

Feed	Range of folate content (mg/kg DM)	References
Lucerne meal	1.65 - 5.55	Anonymous (1996), National Research Council (1998)
Lucerne hay	2.60 - 3.40	Anonymous (1996), Schwab et al. (2006)
Barley, whole grain	0.07 - 0.68	Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Maize, whole grain	0.03 - 0.45	Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Maize silage	0.48	Schwab et al. (2006)
Grass hay	0.52	Schwab et al. (2006)
Oats, whole grain	0.04 - 0.58	Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Peas	0.17 - 0.66	Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Rapeseed meal, solvent extracted	0.22 - 2.40	Anonymous (1996), LfL (2004)
Rye, whole grain	0.17 - 0.81	Anonymous (1996), National Research Council (1998), Souci et al. (2000)
Soybean meal, solvent extracted	0.50 - 4.18	Anonymous (1996), National Research Council (1998), LfL (2004), Schwab et al. (2006)
Wheat, whole grain	0.10 - 0.56	Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Whey powder	0.01 - 0.97	Anonymous (1996), National Research Council (1998), Souci et al. (2000)

DM, dry matter; LFL, Bayerische Landesanstalt für Landwirtschaft

Microbial synthesis, degradation and absorption of folates in the gastrointestinal tract of ruminants

It is well known that the microbial activity and the ruminal population are influenced by the level of concentrate in the diet and the type of feed (Hungate 1966). As some bacterial species are able to synthesise folates, and some others need them (Wolin and Miller 1988), different amounts of folates can be synthesised and used in the rumen depending on the feed composition. For steers, Hayes et al. (1966) and Girard et al. (1994) described a relationship between the proportion of concentrates in the diet and the amount of folates in the rumen. High-concentrate diets resulted in an increase of

folates (Table 2). The authors hypothesised that this increase is due to an enhanced microbial activity in the rumen, caused by rapidly degradable carbohydrates. But it must also be considered that concentrations are not necessarily representative of the total amount synthesised in the rumen, as digesta passage and rumen volume could vary between treatments, for example, due to fibre differing greatly in amount and length. Santschi et al. (2005b) could not corroborate this hypothesis for cows, because they found no difference in the amount of folates in the liquid fraction of ruminal content between the high-forage (58% forage) and low-forage (37% forage) diets (Table 2). However, the F:C ratio of the two diets used in the study of Santschi et al. (2005b) was not as extreme as in the studies with the steers (Hayes et al. 1966; Girard et al. 1994) and additionally Santschi et al. (2005b) used diets with more ingredients than Girard et al. (1994) and Hayes et al. (1966; Table 2). Furthermore the steers had a BW of 340 kg (Hayes et al. 1966) and 352 (SE 27) kg (Girard et al. 1994), whereas primiparous and multiparous cows weighed 582 (SE 17) kg and 692 (SE 17) kg (Santschi et al. 2005b), respectively, which resulted in a different DM intake between cows and steers (Table 2). It should be noted that in all three studies folate concentrations in ruminal fluid were very different. One reason for this could possibly be the different diet composition used in the three studies (Table 2). Additionally, Hayes et al. (1966), who found the highest values, used a different sample preparation method from Girard et al. (1994) and Santschi et al. (2005b). The higher values found by Hayes et al. (1966) in the supernatant fraction could result from bacterial content, as the samples were centrifuged after freezing and thawing, which could have destroyed bacterial cells. Contrary to this, Girard et al. (1994) and Santschi et al. (2005b) centrifuged their samples before freezing. Furthermore, Hayes et al. (1966) used a microbiological assay (*Streptococcus faecalis*) to analyse folate concentration in ruminal samples. In contrast, Girard et al. (1994) and Santschi et al. (2005b) analysed their samples by radioassay.

Table 2: Folate content of ruminal material and body weight (BW; at the beginning of the trial) of steers and cows (mean values with their standard errors)

Diet	Animals(n)	BW (kg)		DM intake (kg/d)		Folates in Solid fraction (mg/kg DM)		Liquid fraction (ng/mL)		References
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	
6.3 kg flaked maize[1] + 1.1 kg soybean meal with minerals[1,2]	8	340						186[a]		Hayes et al. (1966)
6.6 kg ground maize[1] + 1.1 kg soybean meal with minerals[1,2]	8	340						180[a]		
7.5 kg ground maize[1] + 1.8 kg long lucerne hay[1] + 0.7 kg soybean meal with minerals[1,2]	8	340						83[b]		
7.5 kg flaked maize[1] + 1.8 kg ground lucerne hay[1] + 0.7 kg soybean meal with minerals[1,2]	8	340						91[b]		
7.4 kg ground maize[1] + 1.7 kg ground lucerne hay[1] + 0.7 kg soybean meal with minerals[1,2]	8	340						144[c]		
70% rolled barley + 30% timothy hay[2]	8	352	27	8.09	0.64	0.81[a]	0.03	53.0[a]	3.8	Girard et al. (1994)
+ 2 mg folic acid per kg BW[2]				7.88	1.35	5.84[c,3]		613.5[c,3]		
30% rolled barley + 70% timothy hay[2]	8	352	27	7.67	1.11	0.66[b]	0.01	40.9[b]	3.0	
+ 2 mg folic acid per kg BW[2]				8.09	0.77	5.84[c,3]		613.5[c,3]		
58% forage (hay silage, maize silage) + 42% concentrate	8[4]	582[4]	17	24.5[a,4]	1.0			4.18[a,4]	0.32	Santschi et al. (2005b)
(cracked maize, soybean meal, protein supplement, minerals)	8[5]	692[5]	17	29.4[b,5]	1.0			3.52[a,5]	0.32	
37% forage (hay silage, maize silage) + 63% concentrate	8[4]	582[4]	17	24.5[a,4]	1.0			4.05[a,4]	0.32	
(cracked maize, soybean meal, protein supplement, minerals)	8[5]	692[5]	17	29.4[b,5]	1.0			3.60[a,5]	0.32	

[a,b,c] Mean values within a trial with unlike superscript letters were significantly different ($P \leq 0.05$).

[1] In these studies no DM values of feed were given; therefore fresh matter values are presented here.

[2] This study was conducted with steers.

[3] In this study values of the solid and liquid fraction of the ruminal content were given as area under the curve; concentrations presented here are calculated on this basis.

[4] Data from primiparous cows.

[5] Data from multiparous cows.

An *in vitro* study by Hall et al. (1955) showed that the degradation of fibrous materials by rumen microorganisms increases (42%) with supplementation of FA (100 mg/20 mL medium). So it seems that cellulolytic microorganisms require folates, which would endorse the findings of Hayes et al. (1966) and Girard et al. (1994), who found decreased folate concentrations with high-forage diets. Studies on folate requirements of microorganisms are rare. Some strains of *Ruminococcus flavefaciens*, a cellulolytic rod, seem to require either FA, THF or *p*-aminobenzoic acid (which is a part of FA; Ayers 1958; Bryant and Robinson 1961; Scott and Dehority 1965; Slyter and Weaver 1977). Also two strains of *Ruminococcus albus* require FA (Bryant and Robinson 1961). Furthermore Hayes et al. (1966) observed that the level of folates in the ruminal fluid correlated negatively with pH. This corroborates the previous findings, because *R. flavefaciens* is sensitive to acid (Miyazaki et al. 1992) and ruminal pH increases with diets rich in fibre. Since these studies were conducted, feeding strategies, genetics and keeping conditions have changed, and it would be interesting to see if similar and if possible even more detailed results relating to species of microorganisms could be obtained today.

Consequent to FA supplementation to steers, Girard et al. (1994) observed an increase in folate concentration in the solid and liquid fractions of the rumen contents compared with a supplement-free diet ($P = 0.0001$ for both fractions; Table 2). However, different from supplement-free diets, the F:C ratio had no influence on the ruminal folate concentration. Furthermore, they observed that neither FA supplementation nor the nature of the diet made a difference to the quantity of protein synthesised per unit of microbial mass (Girard et al. 1994). Chiquette et al. (1993) analysed the effect of FA supplementation on digestibility and ruminal fermentation in growing steers. They noticed a trend ($P = 0.08$) of pH to decline 4 to 8 h after feeding a diet consisting of 70% rolled barley, 30% timothy hay and a supplementation of 2 mg FA per kg BW as compared with the unsupplemented diet. The results showed that the concentration of ruminal acetate and butyrate did not change due to FA supplementation, whereas ruminal propionate concentrations increased ($P \leq 0.05$) after feeding, and the acetate:propionate ratio was numerically higher during the 24 h of observation due to FA supplementation. The apparent digestibility of DM, fibre fractions and crude protein (**CP**) was not influenced by the addition of FA (Chiquette et al. 1993). So it

seems that FA has no major influence on digestibility and ruminal fermentation, but until today these processes have only been tested once (with eight steers), comparing few different diet compositions, so it is difficult to extrapolate these data to other experimental conditions.

Santschi et al. (2005a) and Schwab et al. (2006) determined the daily apparent ruminal folate synthesis for lactating cows without supplementation of FA, whereas Zinn et al. (1987) provided data for growing steers (Table 3). On average, Schwab et al. (2006) calculated 16.2 mg daily apparent folate synthesis and Santschi et al. (2005a) 20.0 mg. Strikingly opposing these results, Zinn et al. (1987) on average calculated a negative daily apparent ruminal folate synthesis of 20.1 mg for growing steers (194 kg BW). These results could be due to the fact that growing male animals which were used in their experiment had a much lower organic matter (**OM**) intake (3.44 kg OM/d) than the adult female animals of Santschi et al. (2005a; 18.4 kg OM/d; calculated from DM intake and ash content of the diet) and Schwab et al. (2006; 18.7 kg OM/d) and due to the differences in the ruminal passage rate. Additionally, the negative values of Zinn et al. (1987) could also result from a dietary effect, as Zinn et al. (1987) fed a diet with a very high concentration of maize grain, in contrast to diets used in the studies of Santschi et al. (2005a) and Schwab et al. (2006; Table 3).

It must be pointed out that disappearance rates, expressed as the amount of folates appearing at the duodenum relative to the quantity given, were very high in all experiments with dietary supplements of FA (about 97%; Zinn et al. 1987; Santschi et al. 2005a). Therefore it is arguable whether unprotected FA can be supplemented effectively. However, one has to keep in mind that disappearance could either be caused by degradation or absorption (Zinn et al. 1987). Indeed, a net flux across the rumen wall was only found if high amounts of FA were present in the rumen. So it seems that the rumen wall is able to absorb FA. However, the efficiency is very low, so net flux across the rumen wall into the blood circulation can be neglected (Girard et al. 2001; Girard and Remond 2003). On the other hand, folates are absorbed at the proximal intestine (Girard and Remond 2003), and therefore they could already be absorbed at the proximal duodenum before the cannula, hence ruminal disappearance of FA could be overestimated (Santschi et al. 2005a).

Table 3 gives a survey of the amount of folates found in the duodenum. The apparent intestinal disappearance (between duodenal and ileal cannula) seems to be very low (Santschi et al. 2005a). Without supplementation of FA, the duodenal flow of folates is lower than the ileal flow, and it rose above the ileal flow only by supplementation of FA (Table 3). So it seems that there is no apparent intestinal disappearance of folates without supplementation of FA and with supplementation the apparent intestinal disappearance approximates 4% (Santschi et al. 2005a). Santschi et al. (2005a) hypothesised that the apparent intestinal absorption of folates is underestimated, as folates are extensively recycled by the enterohepatic cycle and then released between the duodenal and ileal cannula - thus explaining the higher values of folates in the ileal flow.

Generally, Girard et al. (2001) reason that for dairy cows, folate absorption in the gastrointestinal tract is an active saturable process. With dietary supplements of 2.6 g FA/d this process was already saturated, as higher supplementations could not effectively increase the amount of folate reaching the blood circulation. Due to the destruction of folates by microorganisms, and the low importance of passive absorption, they deduced the minor efficiency of folate absorption in ruminants ($< 5\%$; Girard et al. 2001) versus humans (10 to 46%; Zettner et al. 1981). In general it should be considered that at present no studies are available comparing the amount of folates in the ruminal content and the amount in the duodenum, hence no statement can be made on the coherence between the amount of folates in the ruminal content and the amount of folates in the digesta at the duodenum.

Table 3: Folate intake, duodenal flow, ileal flow, apparent synthesis (AS) and body weight (at the beginning of the trial) of steers and cows (mean values with their standard errors)

Diet	Animals (n)	Body weight (kg)		Folate intake (mg/d)		Duodenal flow (mg/d)		Ileal flow (mg/d)		AS[1] (mg/d)		References
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
35% lucerne hay + 10% Sudan grass + 45% maize + 6% molasses + 4% fat												Zinn et al. (1987)
Without folic acid supplementation	3 s.	194		1.2	0.3	1.1[2]	0.3			-0.1[3]		
+ 10 mg of folic acid per d	3 s.	194		11.2	0.3	1.2[2]	0.3			-10[3]		
+ 100 mg folic acid per d	3 s.	194		101.2	0.3	3.8[2]	0.3			-97.4[3]		
44% grass-legume silage + 15% maize silage + 34% high-moisture maize + 5% protein supplement + 2% minerals												Santschi et al. (2005a)
Without folic acid supplementation	4 m.			7.0		27.0	2.0	40.0	3.0	20[3]		
+ 2600 mg folic acid per d	4 m.			2607		106.0	2.0	102.0	16.0	-2501[3]		
35% forage[4] + 65% concentrate with soybean hulls, beet pulp, soybean meal (30% NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	13.7	0.9[5]	28.9[6,7]	2.4[5]			15.2[6,7]	2.0[5]	Schwab et al. (2006)
35% forage[4] + 65% concentrate with maize, barley, soybean hulls, beet pulp, soybean meal (40% NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	12.2	0.9[5]	32.4[6,7]	2.4[5]			20.2[6,7]	2.0[5]	
60% forage[4] + 40% concentrate with soybean hulls, beet pulp, soybean meal (30% NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	12.4	0.9[5]	25.4[6,7]	2.4[5]			13.0[6,7]	2.0[5]	
60% forage[4] + 40% concentrate with maize, barley, soybean hulls, beet pulp, soybean meal (40% NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	12.6	0.9[5]	29.0[6,7]	2.4[5]			16.4[6,7]	2.0[5]	

NFC, non-fibre carbohydrates; s., steers; m., multiparous cows; p., primiparous cows

[1] Apparent synthesis = duodenal flow minus intake.

[2] Values did not differ significantly.

[3] In Zinn et al. (1987) and Santschi et al. (2005a) apparent ruminal synthesis was not calculated by the authors, but daily intake and duodenal flows were given. Furthermore, Santschi et al. (2005a) declare no levels of significance at all, therefore it was not possible to characterise significance in these studies.

[4] Forage = 50% maize silage, 33% lucerne hay, 17% grass hay.

[5] SEM, not SE, was used.

[6] Significant effects of NFC ($P \leq 0.05$).

[7] Significant effects of forage ($P \leq 0.05$).

Folate concentrations in blood, milk and liver

Blood

Table 4 shows serum folate concentrations in different feeding studies with cows. Without supplementation of FA serum folate levels varied between 13.6 and 23.3 ng/mL. In plasma Santschi et al. (2005b) found significantly ($P = 0.005$) lower folate concentration for primiparous cows (12.7 (SE 1.6) ng/mL) compared with multiparous cows (18.4 (SE 1.6) ng/mL). These observations were not affected by the composition of the diet (58 or 37% forage).

Table 4: Average serum folate concentrations (ng/mL) of cows with and without dietary supplementation of folic acid (mean values with their standard errors)

Supplementation	Number of cows	Primiparous		Multiparous		Primiparous + multiparous		References
		Mean	SE	Mean	SE	Mean	SE	
None	14			13.6[1]	1.0			Girard et al.
None	56			23.3[3]	0.6			(1989)[2]
None	8 p. and 12 m.	14.5[1,4]	0.7	17.2[1,4]	0.7			Girard et al.
None	8 p. and 12 m.	14.3[3,4]	0.8	16.8[3,4]	0.8			(1995)[2]
None	10 p. and 11 m.					16.7	1.4	Girard and Matte
2 mg/kg BW	12 p. and 19 m.					25.3[5]	1.4	(1998)
4 mg/kg BW	10 p. and 11 m.					35.5[5]	1.4	
None	18			15.8[6]	1.6			Girard et al.
3 mg/kg BW	16			20.6[5,6]	1.7			(2005)
6 mg/kg BW	16			23.0[5,6]	1.7			

p., Primiparous cows; m., multiparous cows; BW, body weight
[1] Concentration determined in serum from cows before parturition.
[2] Girard et al. (1989) and Girard et al. (1995) declare no levels of significance between cows before parturition and after parturition; therefore it was not possible to characterise significance in these studies.
[3] Concentration determined in serum from cows after parturition.
[4] Significant effect ($P \leq 0.05$) between primiparous and multiparous cows.
[5] Significant differences ($P \leq 0.05$) between control and folate groups.
[6] Concentrations determined in serum from cows 18 weeks after calving.

Without supplementation, serum and plasma folate concentrations increase after parturition, as the maternal-fetal complex no longer requires folates (Figure 1). However, starting on the day of parturition, the cow requires folates for milk production, but it seems that this demand is lower than that for the maternal-fetal complex (Girard and Matte 1999; Graulet et al. 2007). From mating (about two months after previous parturition) to parturition Girard et al. (1989) found a decrease of total serum folates of 40%, indicating that the maternal-fetal complex has an increasing demand for folate. Also, Girard and Matte (1995) discovered a higher demand for folates in the tissues

of lactating and gestating cows than in lactating non-gestating cows. Serum clearance was significantly slower ($P = 0.04$) in non-gestating cows after an intravenous injection of 50 mg FA. However, in another study, Girard and Matte (1999) could not find a difference in serum folate concentrations between gestating and non-gestating cows.

Oral supplementation (Table 4) or i.m. injection of FA significantly increased serum folate concentrations (Girard et al. 1989; Girard et al. 1992; Girard et al. 1994; Girard et al. 1995; Girard and Matte 1998; Girard and Matte 1999; Girard et al. 2001; Girard et al. 2005). Actually, oral doses higher than 0.5 mg FA/kg BW are required to produce a noticeable effect in serum folate concentration, as concluded from a dose-response study with heifers (150 kg BW; Girard et al. 1992). However, the heifers used in the present study were very young and therefore full ruminal function may not have been developed. Comparable investigations have not been carried out again and in all further studies supplementations higher than 0.5 mg FA/kg BW were used.

As shown in Figure 1, FA supplementation increased serum folate concentrations in cows from four weeks before calving until calving. The increase intensified with higher dietary supplementation (Girard and Matte 1999). Unfortunately no further measurements were performed between the initial sampling time four weeks before calving and at the time of calving, so it is not evident if serum folate levels had already increased before calving due to dietary supplementation. After calving, supplemented cows had decreasing serum folate concentrations. The more FA was added to the diet, the sharper the decline, reaching a plateau value sixteen weeks after parturition (Girard and Matte 1999). As well as the influence of FA supplementation, Girard and Matte (1999; $P = 0.0001$) and Girard et al. (2005; $P = 0.02$) showed a significant time effect, as the gain in serum folate concentration due to dietary supplementation was greater in the first eight weeks of lactation than later in lactation (Figure 1). It seems that increasing serum folate concentrations during early lactation could result from a decreased ability of the cells to retain and use folates (Girard et al. 2005). A reason for this may be generally lower serum vitamin B_{12} levels (181 pg/mL) at early lactation compared with 252 pg/mL in the later lactation (Girard et al. 2005); therefore folates can get into the methyl-trap mentioned above.
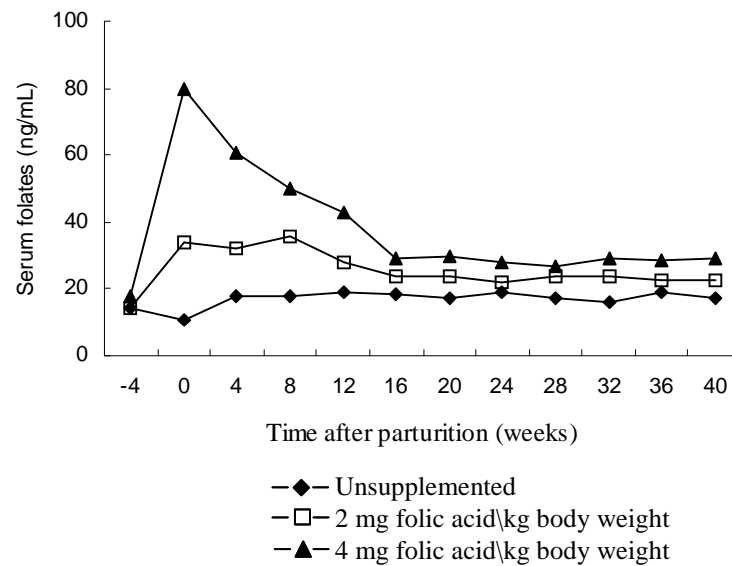
Figure 1: Serum folate concentration of dairy cows fed different daily folic acid supplements (according to Girard and Matte (1999), modified)

Three studies are available dealing with an influence of FA supplementation on packed cell volume and haemoglobin concentrations in blood. In one study with an oral supplementation of 4 mg FA/kg BW an increase ($P \leq 0.05$) in packed cell volume and haemoglobin in primiparous cows was found sixteen weeks after parturition compared with non-supplemented primiparous cows (Girard and Matte 1999). Oral supplementation of FA had no effect on these variables in multiparous cows (Girard and Matte 1999; Girard et al. 2005). These effects could be explained by generally lower vitamin B_{12} levels in primiparous compared with multiparous cows, as folates and vitamin B_{12} are both required for DNA synthesis (as described in the 'Absorption and biochemical functions' section; Girard and Matte 1999). Hence a lack of each individual vitamin or of both vitamins together can delay the maturation of blood cells (Bills et al. 1992). Folic acid supplemented to primiparous cows, which have low vitamin B_{12} levels, may decrease the deficit in DNA synthesis that results in higher packed cell volume and blood haemoglobin values. However, it must be pointed out that changes of packed cell volume and blood haemoglobin due to FA supplementation are smaller than natural changes taking place during lactation (Girard and Matte 1999; Girard et al. 1995). No effects on these parameters were found in either primiparous or multiparous cows with i.m. injections of 160 mg FA once per week (Girard et al. 1995).

Up to now Graulet et al. (2007) are the only authors studying the influence of FA on plasma concentrations of amino acids and glucose. Between week three before calving and week eight after calving, supplementation of FA significantly increased plasma concentrations of alanine, glycine, serine, threonine and total sulfur amino acids. Concurrently, plasma concentrations of glucose and aspartate significantly decreased (Graulet et al. 2007). As aspartate is one of the main N-donors during purine biosynthesis, a decrease in plasma aspartate levels due to FA supplementation could be based on an increased DNA formation. A higher availability of glycine and serine could induce an increase in 1-C-donors for synthesis of methyl-THF (Graulet et al. 2007). So far these are the only explanations for the observed effects. Therefore it would be interesting to conduct further studies on the influence of FA supplementation on plasma concentrations of amino acids and glucose.

As the few available studies show increasing folate concentrations in serum and plasma, it is important to study the influence of FA supplementation on blood variables and thus on whole-animal metabolism.

Milk

Supplementation of FA does not influence feed intake (Graulet et al. 2007; Girard and Matte 1998; Girard et al. 2005). However, the effects of FA supplementation on milk production of cows vary (Table 5). For gestating primiparous and multiparous cows, Girard et al. (1995) found a non-significant increase in milk production of 14% in the last part of lactation due to an i.m. injection of 160 mg FA once per week. However, they could not find an effect on milk production immediately after calving. In contrast, Girard and Matte (1998) found an increased milk production of 6% during the first 100 days of lactation ($P = 0.06$) for multiparous cows receiving 4 mg FA/kg BW and a 10% increase from day 100 to day 200 ($P = 0.05$). For primiparous cows, however, milk production decreased in the first 100 days of lactation ($P \leq 0.08$) with a supplementation of 2 and 4 mg FA/kg BW; in the following lactation no effect could be noticed. Graulet et al. (2007) only studied the first 56 days of lactation; during this time cows fed a supplement of 2.6 g FA/d showed a significant ($P = 0.01$) increase in milk production (Table 5). The effects on multiparous cows could result from folate body

stores depleted by several lactations and gestations. The effects on primiparous cows were explained by their generally lower vitamin B_{12} levels compared with multiparous cows (Girard et al. 1995; Girard and Matte 1998). Graulet et al. (2007) established the hypothesis that higher milk production during FA supplementation results from an improved synthesis of purines and pyrimidines which are necessary for DNA replication. This hypothesis could be supported by the decreased plasma aspartate levels mentioned earlier. These different hypotheses and observations make clear that more studies are necessary to find an explanation for the effects and to discover under which conditions the effects can be reproduced, as Girard et al. (2005) could not find any effect on the milk production of multiparous cows by either supplementing 3 or 6 mg FA/kg BW (Table 5).

Folate occurs in cows' milk mainly as 5-methyl-THF, whereas approximately half of it exists as mono- and the other as polyglutamates (Wigertz and Jägerstad 1995). Almost all folate in cows' milk is bound to specific folate-binding proteins. Generally, the highest milk folate concentrations are found in the colostrum. Starting at parturition, folate concentrations in milk decrease until four weeks after parturition when folate concentrations reach a plateau which is stable until the end of lactation (Girard et al. 1995). All studies with different levels of oral FA supplementations showed significantly increased milk folate concentrations (Girard and Matte 1998; Girard and Matte 1999; Girard et al. 2005; Graulet et al. 2007; Table 5). In humans the transfer of folates into milk is controlled by an active-transport mechanism in the mammary glands. The transfer is linked to the secretion of folate-binding proteins. Once the binding capacity is saturated no further folates can be transferred into the milk (Kirksey 1986). The same seems to be true for cows, as supplementations higher than 3 mg/kg BW could not increase milk folate concentrations while serum folate concentration increased (Girard and Matte 1998; Girard and Matte 1999; Girard et al. 2005; Tables 4 and 5). As observed for serum, the response of milk concentrations of folates to oral supplementation of FA was greater during the first eight weeks after calving then later in lactation (Girard and Matte 1999). However, i.m. injection of FA could not influence milk folate concentrations during the first part of lactation (Girard et al. 1989; Girard et al. 1995). Furthermore, in contrast to the results after an oral supplementation of

FA, i.m. injection of FA tended to increase the folate content only in the colostrum and during progressed lactation (Girard et al. 1995).

At present, only four studies are available dealing with milk components. In these four studies an influence of FA on milk protein and casein was detected for multiparous cows. It seems that i.m. injections and oral supplementations of FA increase milk protein and casein concentrations or yields (Girard et al. 1995; Girard and Matte 1998; Girard et al. 2005; Graulet et al. 2007). The authors explained these effects by depleted folate body stores and generally higher folate requirements because of higher milk production and heavier calves of multiparous cows. Additionally, they hypothesised that the effects on milk protein, similar to the effect on milk production, arise from either an increased synthesis of purines and pyrimidines for DNA synthesis, from an increased secretory capacity of the cells, or from amino acid interconversion which perhaps results in a greater supply of essential amino acids.

It becomes apparent that more studies on supplementation of FA are needed to examine the influence on milk production and milk components and its causes.

Table 5: Influence of oral folic acid supplementation on milk production and composition (mean values and standard errors)

Feed ration	Number of cows	BW (kg) Mean	SE	Milk (kg/d) Mean	SE	Folate content (ng/mL milk) Mean	SE	Protein (g/kg) Mean	SE	Fat (g/kg) Mean	SE	Lactose (g/kg) Mean	SE	References
22% grass legume silage + 15% maize silage + 18% barley + 18% high-moisture maize + 10% soybeans + 15% distillers dried grains + 3% minerals + 1 kg grass legume hay per d														Girard and Matte (1998)
Without folic acid supplementation	11 m.	682[1,2]	126	27.4[2,3]	1.6	37.9[2]	3.6			38.1[2]	0.11	43.0[2]	0.04	
	10 p.	572[1,4]	6	27.1[3,4]	0.7	42.5[4]	3.6			37.5[4]	0.11	43.8[4]	0.04	
+ 2 mg folic acid/kg BW daily	9 m.	682[1,2]	126	28.3[2,3]	1.3	56.1[2,5]	3.6			37.7[2]	0.11	42.4[2]	0.04	
	12 p.	572[1,4]	6	25.3[3,4]	0.8	46.0[4,5]	3.6			38.7[4]	0.11	43.3[4]	0.04	
+ 4 mg folic acid/kg BW daily	11 m.	682[1,2]	126	29.6[2,3]	0.6	48.2[2,5]	3.6			38.0[2]	0.11	43.0[2]	0.04	
	10 p.	572[1,4]	6	25.5[3,4]	0.9	46.6[4,5]	3.6			37.1[4]	0.11	43.3[4]	0.04	
20% grass silage + 20% maize silage + 13% high-moisture maize + 19% barley + 6% wheat + 2% soybean hulls + 4% soybean meal + 4% protected soybean meal + 4% extruded soybeans + 8% minerals														Girard et al. (2005)
Without folic acid supplementation	9	694[1]	11	36.2	1.6	39.3	3.7	31.9	0.8	32.7	1.8	48.3	0.7	
+ 3 mg folic acid/kg BW daily	8	694[1]	11	33.7	1.6	54.6[5]	3.7	33.0	0.8	35.1	1.8	46.5	0.7	
+ 6 mg folic acid/kg BW daily	8	694[1]	11	33.9	1.6	43.1[5]	3.7	32.4	0.8	35.5	1.8	46.5	0.7	
7% grass hay + 27% legume-grass silage + 18% maize silage + 32% cracked maize + 9% soybean meal + 4% maize, wheat, rapeseed products + 3% minerals														Graulet et al. (2007)
Without folic acid supplementation	6	735[6]	25	39.5[7]	1.3	45.6[7]	6.2	30.3[7]	0.6	35.9[7]	1.4	45.1[7]	1.2	
+ 2600 mg folic acid/d	5	755[6]	27	41.8[5,7]	1.6	71.7[5,7]	6.8	29.5[7]	0.7	39.2[7]	1.6	43.5[7]	1.0	

BW, body weight; m., multiparous cows; p., primiparous cows

[1] BW measured at the beginning of the trial, 1 month before calving.

[2] Data from multiparous cows.

[3] This value was calculated and shows the average daily milk production for the whole lactation period.

[4] Data from primiparous cows.

[5] Significant effect between control and folate groups.

[6] BW measured at the beginning of the trial, 3 weeks before calving.

[7] This value is the mean from data determined in the first 8 weeks of lactation.

Liver

After a single supplementation of 2.6 g FA Girard et al. (2001) could not find a sig-
nificant increase in the amount of folates taken up by the liver during a 24 h period
(calculated from folate flow through portal-drained viscera and total splanchnic tis-
sue). Before the supplementation approximately 50% of the portal blood folates were
extracted by the liver; after supplementation only approximately 30% were extracted
(calculated from averaged net flux per h). Graulet et al. (2007) studied the concen-
tration of folates in liver biopsies. Cows receiving a daily supplementation of 2.6 g
FA had significantly ($P = 0.0001$) increased liver folate concentrations of 2.56 mg/g
DNA compared with control cows with 1.50 mg/g DNA during the first eight weeks of
lactation. The results of these two studies led to the assumption that FA supplementa-
tion increases the liver folate concentration but decreases the percentage of extraction
from arterial blood into the liver. Lower percentages of extraction reflect that more
folates are available for post-splanchnic tissues, as for example the mammary glands.
This fact was confirmed by the results of Girard et al. (2001) who found 71% of folates
from arterial blood in post-splanchnic tissues after supplementation of 2.6 g FA and
only 50% without supplementation. Beside the increase in liver folate concentration
Graulet et al. (2007) found higher values of total lipids, triacylglycerol and cholesterol
in the liver during the first two weeks of lactation following a daily supplementation of
2.6 g FA. They explained these higher values with an increased mobilisation of body
reserves during the first weeks of lactation which is necessary to meet the requirements
for the above-mentioned increases in milk production and milk protein yield. Another
explanation for an increase in triacylglycerol could be an inhibition of the ß-oxidation
of fatty acids in the liver, caused by a lack of vitamin B_{12}, as cows receiving FA and
vitamin B_{12} had no increase in triacylglycerol (Graulet et al. 2007). If it can be proven
that FA supplementations increase lipid values in the liver during the first weeks of
lactation, a time when the risk of fatty liver is high, supplementation of FA during this
time would be questionable. Therefore further studies with a higher number of animals
are needed, as up to now only twentyfour multiparous cows have been tested.

Future research directions

For ruminants future research should focus on the determination of the demand for folates. Up until now only requirement values for tissue and milk have been estimated, but they were derived from swine experiments and folate concentrations in cows' milk. Therefore it is necessary to examine the influence of different amounts of FA supplementation under different feeding regimens on rumen variables (for example: pH, volatile fatty acids, microbial population, degradation and synthesis of folates by microorganisms) and available quantity and forms of folates for absorption at the intestinal tract. Additionally, understanding of mechanisms and sites of folate absorption in ruminants is insufficient; some authors mentioned a possible absorption of folates before the duodenal cannula (Zinn et al. 1987; Santschi et al. 2005a), for example, at the beginning of the duodenum or in the abomasum. Furthermore, knowledge on the passage of folates from the intestine to blood and their following distribution to tissues and milk is important. The influences of an oral FA supplementation on amino acids and glucose concentrations in blood were tested only once and significant differences were found, but so far no explanations exist (Graulet et al. 2007). The present review shows that very often controversial results exist, for example, the influence of FA supplementation on milk production or the influence of feed ration on FA availability in the rumen, therefore surveys should be conducted to reassess the variability of previous studies. Also, maximum and minimum daily intake limits are neither available for FA, nor for any other B-vitamin (Anonymous 2005). Indeed, no toxic reactions appeared in any of the experiments mentioned above. As the present review shows, there are many unanswered questions regarding the effects of folates for cows. Therefore the following list points out desirable research areas concerning folates:

1. Studies with different feeding regimens, with and without FA supplementation, should be conducted to assess the influence of the diet on folate degradation, synthesis and absorption in the rumen and the duodenum on the one hand and the influence on digestibility and ruminal fermentation on the other hand.

2. *In vitro* studies with ruminal microorganisms would be helpful to characterise their folate requirements and synthesis.

3. Research on the mechanism and the sites of folate absorption in ruminants is necessary.

4. Experiments on the interactions of physiological stage and folate metabolism in dairy cows are essential.

5. Surveys should be conducted to explain the available effects of FA supplementation on concentrations of amino acids and glucose in blood.

6. Studies to ascertain the whole flow of folates through the body and implications of FA supplementation on the whole organism of dairy cows are crucial.

7. Further studies should focus on the effects of FA supplementation on liver metabolism and milk.

8. Further determinations of folate concentrations in feedstuffs are required to calculate the folate intake.

3.2   Effects of a folic acid supplementation on dairy cows

Introduction

Folic acid belongs to the B-vitamin group and the biologically active forms are folates. Folates are essential for the transfer of one-carbon units from donor molecules into biosynthetic pathways leading to methionine, purine and pyrimidines. Furthermore, they facilitate the interconversion of serine and glycine and play a role in histidine catabolism (Lucock 2000). Rumen microorganisms can synthesise B-vitamins (Bechdel et al. 1928), but it is doubtful if this synthesis always meets the requirements of dairy cows, today (National Research Council 2001). As reviewed by Ragaller et al. (2009; Chapter 3.1) only a few studies have investigated the effects of FA supplementation on rumen metabolism and on blood and milk variables. Most of supplemented FA disappeared between the mouth and the duodenum (Zinn et al. 1987; Santschi et al. 2005a), nevertheless in some studies dietary FA increased milk production (Girard and Matte 1998; Graulet et al. 2007). However, no studies looking simultaneously at the effects of an FA supplementation on ruminal fermentation, duodenal flow of nutrients and blood and milk variables have been conducted. Furthermore, it has been reported (Hayes et al. 1966; Girard et al. 1994; Schwab et al. 2006) that high versus low concentrate diets favour ruminal folate concentrations in ruminal content/fluid and duodenal folate flow. Therefore the present experiment was conducted to investigate the influence of FA supplementations on ruminal fermentation variables and nutrient flow at the duodenum, as well as on blood and milk variables and on blood folate concentrations at dietary F:C rations of (dry matter basis) 34:66 in the first part and 66:34 in the second part.

Experimental methods

Treatments, experimental design and animals

The study was conducted at the experimental station of the Friedrich-Loeffler-Institute in Brunswick with a total of thirteen German Holstein multiparous dairy cows equipped with large rubber cannulas in the dorsal sac of the rumen (inner diameter 10 cm) and simple T-shaped cannulas at the proximal duodenum, close to the pylorus (inner diameter 2 cm). At the beginning of the first part of the experiment the cows had a mean BW of 619 (standard deviation (**SD**) 26) kg and 610 (SD 91) kg at the beginning

of the second part. Due to the limited animal numbers, both dry and lactating cows had to be used. In the first part five cows were in lactation (186 (SD 144) days in milk (**DIM**) at the beginning), while two were dried off. In the second part four cows were in lactation (165 (SD 57) DIM at the beginning) and four were dried off. Lactation numbers ranged from two to five over the whole experiment. Cows were housed in a tethered-stall with neck straps and individual troughs. They had free access to water and a salt block containing sodium chloride. The lactating cows were milked at 05:00 and 16:00 h. In the first part of the experiment the cows received a diet consisting of 34% forage and 66% concentrate (high concentrate, **HC**) on DM basis. In the second part the diet consisted of 66% forage and 34% concentrate (high forage, **HF**). Not ruminally protected FA (a powder with 95% FA, Adisseo, Commentry, France) was supplemented at 1 g/(d x cow) (diets **HC+FA** and **HF+FA**). The experimental design (Table 6) is unbalanced because of different calving dates. Table A1 in the Appendix shows the individual cows used in the different groups.

Table 6: Folic acid experimental design (cows/group)

Group	HC		HF	
	Lactating	Dry	Lactating	Dry
Control	5	2	4	4
FA	5	2	4	4

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; FA, folic acid

The forage consisted of 60% maize silage and 40% grass silage on DM basis. The composition of the concentrate is shown in Table 7. To comply with the envisaged F:C ratio and the maize/grass silage ratio, the DM of maize silage and grass silage was determined twice a week. As maize and grass were ensiled in large bunker silos, all the cows and bulls of the Friedrich-Loeffler-Institute Brunswick experimental station were fed from the same silos. Therefore, the silos were changed several times during the experiment. Forage was offered twice daily in two equal portions at 05:30 and 15:30 h; the pelleted concentrate was evenly distributed over four feeding times per day: at 05:30, 07:30, 15:30 and 17:30 h. The FA supplement was mixed with 100 g of mineral and vitamin mix (Table 7) and two equal portions were manually scattered by hand on

top of the concentrate at 05:30 and 15:30 h. The control animals also received 100 g of mineral and vitamin mix but without FA.

Table 7: Composition of concentrate

Component	Proportion (%)
Wheat grain	25.0
Maize grain	25.0
Soybean meal, solvent-extracted	17.0
Sugar beet pulp, dried	15.0
Pea grain	15.0
Mineral and vitamin mix[1]	2.0
Feed lime	0.7
Urea	0.3

[1] Composition per kg of mix: 175 g Ca, 100 g Na, 50 g P, 30 g Mg, 1 g Fe, 1.3 g Cu, 6 g Zn, 4 g Mn, 0.05 g I, 0.05 g Se, 0.03 g Co, 1,000,000 IU vitamin A, 100,000 IU vitamin D_3 and 4 g vitamin E

To ensure constant ration composition and to avoid refusals the amounts of feed were individually adjusted every day. A standardised digestibility trial with four adult wether sheep (Gesellschaft für Ernährungsphysiologie 1991; 96.6 (SD 8.6) kg BW; n = 4) was conducted to calculate the metabolisable energy (**ME**) concentration of the different types of forage.

Sample collection

Treatments and experiments were conducted according to the European Community regulations concerning the protection of experimental animals and the guidelines of the Regional Council of Brunswick, Lower Saxony, Germany (File Number 33.11.42502-04-057/07). After two weeks of adaptation to the respective diets, two weeks of sample collection followed. During the first sampling week samples of milk, blood and ruminal fluid were taken. Milk yields were recorded daily. Milk samples were collected on the first and the fourth day of consecutive morning and evening milking. From each milking 50 mL were conserved with bronopol and kept at 8°C until analysis of the milk ingredients. Aliquots of additional 800 mL milk were pooled per day and were freeze-dried afterwards (Christ Epsilon 1-15; Christ, Osterode, Germany) and stored at -18°C for fatty acid and folate analyses. Blood samples were taken from a *vena jugularis externa* on the third day of the first sampling week. Due to animal welfare

the frequency of blood samples was kept to a minimum. Folic acid supplementation seems to result in a folate peak 3 to 4 h after feeding (Girard et al. 1992), thus blood samples were taken just before, and three and six hours after, the first morning feed. Until centrifugation blood samples were kept at 15°C. One hour after sampling, the serum was separated by centrifugation at 2123 x g for 30 min at 15°C and kept frozen at -80°C until analysis. Samples of ruminal fluid (approximately 100 mL) were withdrawn from the ventral sac through the rumen cannula using a hand-operated vacuum pump on the second day of the first week of the sampling period. Samples were taken just before first morning feed and 30, 60, 90, 120, 180 and 360 min after the beginning of feeding.

During the second sampling week, duodenal digesta was collected over five consecutive days in two-hourly intervals. At each sampling, four samples of approximately 100 mL were taken through the duodenal cannula from each cow. Immediately afterwards the pH-values were measured with a glass electrode (digital pH measurement device, pH525, WTW, Weilheim, Germany) and the sample with the lowest pH (Rohr et al. 1984) was added to the daily pooled sample from each cow and stored at -18°C. For the calculation of the daily digesta flow, Cr_2O_3 as a flow marker was mixed with wheat flour (ratio 1:4) and 50 g were distributed into the rumen every 12 h, beginning 10 days before the duodenal digesta sampling period, whereas on day 1 before, and then during, the sampling period, 25 g were administered every 6 h. In a comparison of measurements of duodenal flow in dairy cows, the spot-sampling procedure has shown only small differences in flow as compared to the total collection. During the duodenal digesta sampling week, samples of concentrate and forage as well as possibly occurring feed refusals were collected daily and pooled on a weekly basis. Feed samples, refusals and daily duodenal digesta samples were freeze-dried and ground through a 1-mm screen for analysis. Faeces of wethers were dried at 60°C and also ground through a 1-mm screen for analysis.

Analyses

In the feedstuffs and the refusals of the dairy cows, as well as in the faeces of the wethers DM, crude ash (**Ash**), CP, ether extract (**EE**), crude fibre (**CF**) and starch

were analysed according to the methods of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA, 1993) . The method of van Soest et al. (1991) was used to analyse acid (**ADF**) and neutral detergent fibre (**NDF**), which were expressed without residual ash. Folate in the forages, the concentrates and the mineral and vitamin mix was determined using a microbiological assay (*Enterococcus hirae*; Difco 1998a; LUFA Speyer, Germany). After feed, duodenal digesta and milk had already been analysed a closer inspection of the analytical method indicated that *Enterococcus hirae* was not an appropriate species to quantify folates in feed, duodenal digesta and milk. Therefore these variables are not shown and discussed. Immediately after the ruminal fluid collection, the pH of each sample was measured with a glass electrode (pH525, WTW, Weilheim, Germany). Afterwards, the ruminal fluid was centrifuged (30074 x g). In the supernatant $NH_3 - N$ was analysed according to DIN 38406-E5-2 (1998). To measure the short chain fatty acids (**SCFA**) a part of the centrifuged ruminal fluid was acidified with sulphuric acid and centrifuged (18400 x g) again. The supernatant was frozen at -18°C. Before the measurement of SCFA with a gas chromatograph (Hewlett Packard 5580, Avondale, PA, USA) equipped with a flame ionization detector (Geissler et al. 1976) the thawed supernatant was again centrifuged (18400 x g). Nitrogen concentration in freshly thawed duodenal digesta was measured according to the Kjeldahl method; further analyses were carried out with freeze-dried and ground duodenal digesta. The DM and ash contents were analysed in the daily pooled samples by the same methods mentioned above for feedstuffs. The proportion of microbial-N of non-ammonia-N (**NAN**) in the duodenal digesta was estimated by using near infrared spectra according to Lebzien and Paul (1997). Cr_2O_3 was measured by atomic absorption spectrophotometry (Williams et al. 1962) and used to calculate duodenal DM flow. The daily duodenal DM flow was then used as a measure for the preparation of one pooled sample per cow per week. Pooled samples were analysed for NDF and ADF applying the same methods as for the feedstuffs.

Serum samples were analysed for glucose, non-esterified-fatty-acids (**NEFA**), *beta*-hydroxybutyrate (**β-HBA**) and urea by the laboratory of the Cattle Clinic Hanover (University of Veterinary Medicine, Germany). The glucose content was detected using hexokinase (A11A00116, ABX Diagnostics, Montpellier, France), an enzymatic colour

test was applied to analyse the NEFA content (99475409, Wako Chemicals, Neuss, Germany) and an enzymatic UV-test was run to assess β-HBA (RB 1008, Randox Laboratories, Krefeld, Germany) and urea (A11A00075, ABX Diagnostics, Montpellier, France).

Serum samples were analysed for FA, 5-methyl-THF, THF, thiamin, riboflavin, pyri-doxal-5'-phosphate, pyridoxic acid, pyridoxal, pyridoxine, pyridoxamine, PA, nicotin-amide and nicotinic acid by high performance liquid chromatography (W. Bigalke, unpublished method). In the following essential details of the method are given. Sample preparation included protein precipitation with ethanol and fat extraction with n-hexan. After centrifugation (20800 x g) the supernatant was evaporated under a nitrogen stream and the residue was dissolved in the aqueous mobile phase A, fil-trated and injected into a HPLC system (Shimadzu, Kyoto, Japan). B-vitamins were separated using a reversed phase C_{18}-column with mobile phase A containing sodium 1-hexanesulfonate monohydrate and phosphoric acid in ultra-pure water. Mobile phase B consisted of acetonitrile. Quantifications of B-vitamins were performed simultaneously using a multi wavelength detector and a spectrofluorometric detector.

Milk samples were analysed for fat, protein, lactose and urea using an infrared milk analyser (Milkoscan FT 6000 combined with a Fossomatic 5000, Foss Electric, Hillerød, Denmark). The analysis of milk fat composition was carried out in freeze-dried sam-ples after extraction with chloroform/methanol (2:1, v/v) and transesterification with trimethylsulfoniumhydroxide. A gas chromatograph (Hewlett Packard 6890, Palo Alto, CA, USA) equipped with a flame ionization detector and a capillary column (Zebron 7HG-G009-11) was used for the separation of the fatty acids from butyric acid ($C_{4:0}$) to linolenic acid ($C_{18:3}$).

Calculations and statistics

The ME in feedstuffs was calculated according to equation number 1.1.2 of the Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie 2001). Concentrate digestibility was taken from the tabular values for each ingredient (Universität Ho-henheim – Dokumentationsstelle, 1997) whereas the ME concentration of forages was

estimated from the standardised digestibility trial with the adult wether sheep (Gesellschaft für Ernährungsphysiologie 1991).

For the calculation of the daily duodenal DM flow (**DMF**) the following equation was used:

$$DMF\,(kg/d) = \frac{chromium\,application\,(mg/d)}{duodenal\,chromium\,concentration\,(mg/g\,DM)}/1000$$

The daily duodenal flows of OM and OM constituents were estimated by the multiplication of their respective concentrations in duodenal digesta with DMF. The daily duodenal flow of NAN was calculated assuming a mean ammonia-N proportion of total N with N-balanced rations of 4.9% (Riemeier 2004). The utilisable CP (**uCP**) at the duodenum was estimated following Lebzien and Voigt (1999):

$$uCP\,(g/d) = (NAN\,flow\,at\,the\,duodenum\,(g/d)) \cdot 6.25 - endogenous\,CP\,(\mathbf{EP})(g/d)$$

$$\text{where } EP\,(g/d) = (3.6 \cdot kg\,DMF) \cdot 6.25 \text{ (Brandt and Rohr 1981)}$$

Ruminally fermented OM (**FOM**), ruminally degraded CP, undegraded feed CP (**UDP**) and ruminal N balance (**RNB**) were calculated with the following equations:

$$FOM\,(kg/d) = OM\,intake\,(kg/d) - (duodenal\,OM\,flow\,(kg/d) - microbial\,OM\,(kg/d))$$

$$\text{where } microbial\,OM\,(kg/d) = 11.8 \cdot microbial\,N\,(kg/d) \text{ (Schafft 1983)}$$

$$Ruminally\,degraded\,CP\,(g/d) = CP\,intake\,(g/d) - UDP\,(g/d)$$

$$UDP\,(g/d) = 6.25 \cdot (g\,NAN\,at\,the\,duodenum\,(g/d) - g\,microbial\,N\,(g/d)) - EP\,(g/d)$$

$$RNB\,(g/d) = (CP\,(g/d) - uCP\,(g/d))/6.25$$

Fat corrected milk was estimated as follows:

$$Fat\,corrected\,milk\,(kg/d) = ((\%\,milk\,fat \cdot 0.15) + 0.4) \cdot kg\,milk\,yield$$

(Helfferich and Gütte 1972)

The SAS software package (Version 9.1.3., procedure mixed, SAS Institute Inc., Cary, NC, USA) was used to analyse the data. For statistical analysis of rumen and serum variables supplemental FA was considered as a fixed effect. To consider the within-subject factor over all sample times a repeated statement was used. A random statement for the individual cow effect was introduced to consider that a cow had to be used over several periods for different treatments. The OM intake was considered as a fixed regressive component. The restricted maximum likelihood method was used to evaluate

the variances. Degrees of freedom were calculated by the Kenward-Roger-method. To test the differences between the least squares means (**LSmeans**) the "PDIFF" option was applied, using a Tukey-Kramer test for post-hoc analysis. Vitamin concentrations in the serum were analysed using the same model, but without the fixed regressive component OM intake.

For the duodenal and milk variables the model was set up with FA, OM intake and a random statement for the individual cow effect as previously mentioned. Furthermore, for the milk variables the stage of lactation was treated as a fixed effect and additionally implemented in the model. Differences were considered to be significant at ($P \leq 0.05$), and a trend was discussed at ($P = 0.10$). For ruminal, duodenal and milk variables significant levels of the analyses of variances are shown. Significant levels of Tukey-Kramer-Test are only used for blood variables, as, for ruminal variables, no effects were found between controls and their respective treatments at the single measurement times (Tukey-Kramer-test). If not mentioned otherwise, results are reported as LSmeans with standard errors (**SE**).

Results

Because of the scope of this study, the effects of OM intake will not be shown and discussed in the following and the influences of time after feeding and the interaction of FA and time after feeding are only shown and discussed if they were significant.

Intake and chemical composition of feedstuffs

Table 8 shows the arithmetical means of the nutrient composition of the rations. In the Appendix (Table A2) the nutrition composition of the forage is shown. The average nutrient intake per cow (lactating and dry) and day are shown in Table 9. The intended ratio of F:C was achieved over the whole experiment. The two different rations (HC and HF) were neither isonitrogenous nor isocaloric, because the OM intake and the feedstuffs used should have been identical as far as possible.

Table 8: Arithmetic means of nutrient composition (g/kg DM) and metabolisable energy (MJ/kg DM) of the rations (arithmetic means with their standard deviation)

	HC (n = 3)		HF (n = 4)	
	Mean	SD	Mean	SD
OM	936	1.4	931	3.4
CP	185	9.5	142	4.5
EE	26	3.3	32	1.8
CF	115	3.8	166	7.4
ADF	132	3.0	185	2.0
NDF	275	14.8	369	20.6
Starch	372	44.7	292	12.1
ME	12.1	0.0	11.3	0.1

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; SD, standard deviation; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; ADF, acid detergent fibre; NDF, neutral detergent fibre; ME, metabolisable energy

Rumen fermentation variables

As no significant effects or trends at the single measurement times (Tukey-Kramer-test) between the HC and the HC+FA or the HF and HF+FA rations were found for the ruminal variables only the LSmeans over the whole sampling time are presented in Table 10. In general, the influences of FA on the ruminal fermentation variables were negligible for both rations (Table 10). During the HC feeding regime the FA reduced the molar percentage of isovaleric acid by about 0.3 percentage units (a slight but significant change) and tended to lower the molar percentage of isobutyric acid. In contrast, the FA significantly increased the molar percentage of isovaleric acid for the HF ration by about 0.2 percentage units and trends were found for the molar percentages of butyric and valeric acid.

Table 9: Intakes of nutrients (kg/day) by lactating and dry cows fed high concentrate or high forage diets (arithmetic means with their standard deviation)

| | HC | | | | HC+FA | | | | HF | | | | HF+FA | | | |
| | Lactating (n = 5) | | Dry (n = 2) | | Lactating (n = 5) | | Dry (n = 2) | | Lactating (n = 4) | | Dry (n = 4) | | Lactating (n = 4) | | Dry (n = 4) | |
Intake	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	14.17	2.05	8.81	2.63	14.26	2.19	8.51	3.25	13.51	2.60	7.25	1.82	14.48	1.64	7.67	1.20
OM	13.21	1.92	8.18	2.47	13.24	2.05	7.86	3.04	12.49	2.44	6.65	1.67	13.39	1.49	7.07	1.12
N	0.44	0.06	0.27	0.08	0.41	0.07	0.24	0.09	0.31	0.05	0.17	0.04	0.32	0.04	0.17	0.03
NDF	3.79	0.55	2.35	0.70	3.92	0.51	2.47	0.95	4.92	0.87	2.24	0.19	5.17	0.57	2.73	0.47
ADF	1.82	0.27	1.13	0.34	1.95	0.28	1.19	0.46	2.48	0.49	1.33	0.34	2.68	0.31	1.41	0.22
Starch	5.10	0.74	3.16	0.96	5.11	0.65	3.53	1.36	3.94	0.76	2.12	0.49	4.23	0.43	2.25	0.39

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; FA, folic acid; HF, high forage: 66% forage and 34% concentrate on dry matter basis; SD, standard deviation; DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre

Table 10: Influence of folic acid supplementation on ruminal variables averaged over sampling times[1] (LSmeans with their standard error)

	HC					HF				
	Control (n = 7)		FA (n = 7)		P	Control (n = 8)		FA (n = 8)		P
	LSmeans	SE	LSmeans	SE	FA	LSmeans	SE	LSmeans	SE	FA
pH	6.33	0.09	6.33	0.09	0.949	6.37	0.03	6.39	0.03	0.482
NH_3 (mmol/L)	10.20	0.93	9.18	0.93	0.194	9.69	0.32	9.80	0.32	0.738
SCFA (mol %):										
Acetic acid	60.6	0.84	60.3	0.84	0.567	63.3	0.82	62.5	0.82	0.378
Propionic acid	19.3	0.89	19.4	0.89	0.949	18.7	0.30	18.2	0.30	0.153
Isobutyric acid	1.0	0.08	0.9	0.08	0.052	0.9	0.03	0.9	0.03	0.376
Butyric acid	15.3	0.28	14.8	0.28	0.240	14.2	0.66	15.0	0.66	0.097
Isovaleric acid	1.9	0.11	1.6	0.11	0.009	1.7	0.09	1.9	0.09	0.037
Valeric acid	2.4	0.19	2.4	0.19	0.777	1.3	0.09	1.5	0.09	0.058
SCFA (mmol/L)	97.2	4.36	95.9	4.36	0.713	109.0	5.02	104.6	5.02	0.459

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; FA, folic acid; SE, standard error; SCFA, short chain fatty acids
[1] Sampling time: just before and 30, 60, 90, 120, 180, 360 minutes after the first feeding in the morning.

Nutrient flow at the duodenum

Apart from FOM no other variable presented in Table 11 was influenced by FA during the HC feeding regime. The amount of FOM was significantly reduced (-5%) but the proportion of FOM to OM intake only tended to be reduced ($P = 0.060$) in the HC+FA diet compared to the control. With the HF ration only ADF was significantly influenced. Folic acid decreased the ADF flow to the duodenum (-0.08 kg/d) and thus increased the apparent ruminal digestibility of ADF (0.043 kg/kg intake).

As shown in Table 12, with HF feeding the FA had no influence on ruminal CP turnover. However, in the HC ration the FA decreased the flow of microbial CP (**MP**) at the duodenum (-10%) and MP synthesis (g/MJ ME; -11%), whereas for MP synthesis per kg of FOM only a numerical decrease (-7%) could be observed as FOM was also reduced (Table 11). Furthermore, uCP tended to be reduced ($P = 0.087$). As uCP was calculated from MP and UDP, and UDP was not influenced (Table 12), the effect on uCP is totally attributed to the reduced MP synthesis mentioned previously.

Table 11: Influence of folic acid supplementation on nutrient flow at the duodenum, ruminally fermented organic matter (FOM) and apparent ruminal digestibility (LSmeans with their standard error)

	HC					HF				
	Control (n = 7)		FA (n = 7)		P	Control (n = 8)		FA (n = 8)		P
	LSmeans	SE	LSmeans	SE	FA	LSmeans	SE	LSmeans	SE	FA
Duodenal flow (kg/d):										
OM	7.03	0.15	7.13	0.15	0.599	6.44	0.16	6.27	0.16	0.463
FOM	7.14	0.12	6.81	0.12	0.050	5.97	0.13	6.11	0.13	0.377
NDF	1.88	0.12	2.06	0.12	0.168	2.20	0.07	2.10	0.07	0.374
ADF	1.00	0.06	1.08	0.06	0.281	1.12	0.03	1.04	0.03	0.018
FOM of OM intake (%)	61.5	1.27	59.2	1.27	0.060	56.4	1.19	57.9	1.19	0.290
Apparent ruminal digestibility (kg/kg intake):										
OM	0.409	0.0148	0.407	0.0148	0.882	0.398	0.0149	0.419	0.0149	0.342
NDF	0.452	0.0365	0.430	0.0365	0.475	0.483	0.0181	0.504	0.0181	0.423
ADF	0.395	0.0404	0.393	0.0404	0.949	0.476	0.0183	0.519	0.0183	0.030

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; FA, folic acid; SE, standard error; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre

Table 12: Nitrogen flow at the duodenum and efficiency of microbial protein synthesis (LSmeans with their standard error)

	HC					HF				
	Control (n = 7)		FA (n = 7)		P	Control (n = 8)		FA (n = 8)		P
	LSmeans	SE	LSmeans	SE	FA	LSmeans	SE	LSmeans	SE	FA
MP (g/d)	1289	50.8	1166	50.8	0.024	926	42.7	913	42.7	0.833
MP per FOM (g/kg)	180	8.07	168	8.07	0.119	159	7.70	149	7.70	0.370
MP per ME (g/MJ)	8.5	0.33	7.6	0.33	0.003	7.2	0.28	7.0	0.28	0.631
N (g/d)	341	12.6	320	12.6	0.105	256	11.4	254	11.4	0.892
NAN (g/d)	325	12.0	304	12.0	0.105	243	10.8	241	10.8	0.892
uCP (g/d)	1837	73.5	1704	73.5	0.087	1341	65.1	1335	65.1	0.947
MP per RDP (g/g)	0.68	0.03	0.67	0.03	0.884	0.76	0.04	0.77	0.04	0.848
UDP (g/d)	548	34.0	538	34.0	0.698	415	28.2	422	28.2	0.856
UDP per CP intake (%)	21.9	1.33	23.1	1.33	0.197	24.7	1.52	25.5	1.52	0.651

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; FA, folic acid; SE, standard error; MP, microbial crude protein; FOM, fermented organic matter; ME, metabolisable energy; NAN, non-ammonia-nitrogen; uCP, utilisable crude protein; RDP, ruminally degraded protein; UDP, ruminally undegraded feed protein; CP, crude protein

Serum variables

The FA supplementation to the HF ration had no influence on the measured blood variables (Table 13). During the HC feeding regime supplementary FA significantly decreased the serum glucose and urea concentrations (Table 13). The post-hoc analysis for serum glucose concentrations showed a significant difference before ($P = 0.003$) and 360 min ($P = 0.002$) after feeding (Figure 2A), while serum urea concentrations were only significantly lowered at 360 min ($P = 0.014$) after feeding (Figure 2B). But the lower urea concentrations seem to result from the lower N intake of the HC+FA group compared to the HC group (Table 9).

In all serum samples only 5-methyl-THF and no FA or THF could be detected. Figures 3A and B shows the 5-methyl-THF concentrations in serum. For both rations significant influences of FA (for HC: $P = 0.018$; for HF: $P < 0.001$), time after feeding (for HC: $P = 0.009$; for HF: $P = 0.038$) and also an interaction of FA and time after feeding (for HC: $P = 0.004$; for HF: $P = 0.008$) were found. Post-hoc analysis only showed a significant difference 180 min after feeding for both rations (Figures 3A and B). For the HF ration a trend for increased 5-methyl-THF concentrations at 360 min after feeding ($P = 0.078$) was also observed.

Table 13 also shows the effects of FA supplementation on the other measured B-vitamins in serum. With the HF ration the FA had no influence on the other B-vitamins measured, while with the HC ration the FA lowered the serum riboflavin level ($P < 0.001$) from 15 (SE 0.75) ng/mL to 10 (SE 0.75) ng/mL (Table 13). The values of nicotinic acid, pyridoxine, pyridoxamine and thiamin were below the detection limit (W. Bigalke, unpublished data).

Table 13: Effects of dietary supplements of folic acid to dairy cows on serum concentrations of glucose, NEFA, ß-HBS, urea and B-vitamins (ng/mL) averaged over sampling times[1] (LSmeans with their standard error)

	HC					HF				
	Control (n = 7)		FA (n = 7)		P	Control (n = 8)		FA (n = 8)		P
	LSmeans	SE	LSmeans	SE	FA	LSmeans	SE	LSmeans	SE	FA
Glucose (mmol/L)	3.54	0.09	3.09	0.09	0.000	3.26	0.07	3.27	0.07	0.898
NEFA (μmol/L)	100.9	29.6	110.7	29.6	0.472	80.4	7.1	78.8	7.1	0.814
β-HBA (mmol/L)	0.68	0.09	0.73	0.09	0.647	0.78	0.10	0.93	0.10	0.180
Urea (mmol/L)	5.41	0.35	4.47	0.35	0.009	3.53	0.12	3.51	0.12	0.832
Riboflavin	14.84	0.75	10.02	0.75	<0.001	8.00	0.31	7.91	0.31	0.674
Pyridoxal-5'-phosphate	5.62	0.53	4.59	0.54	0.121	4.14	0.50	3.35	0.50	0.154
Pyridoxic acid	5.71	0.81	5.76	0.81	0.836	6.39	0.72	6.33	0.72	0.794
Pyridoxal	3.25	0.36	3.65	0.36	0.068	4.31	0.35	4.17	0.35	0.626
Nicotinamide	318.6	27.3	322.1	27.3	0.941	347.8	26.1	369.4	26.1	0.249
Pantothenic acid	84.1	31.8	120.7	31.8	0.439	82.7	10.2	73.2	10.2	0.367

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; FA, folic acid; SE, standard error; β-HBA, *beta*-hydroxybutyrate
[1] Sampling time: just before and 180 and 360 minutes after the first feeding in the morning.
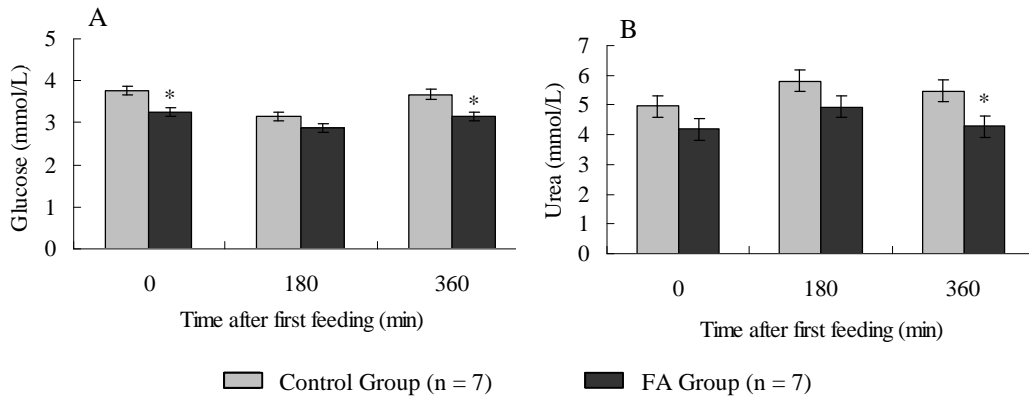
Figure 2: Serum glucose (A) and urea (B) concentrations for the high concentrate (34% forage and 66% concentrate on dry matter basis) feeding groups with and without folic acid supplementation at different time points after feeding *$P \leq 0.05$
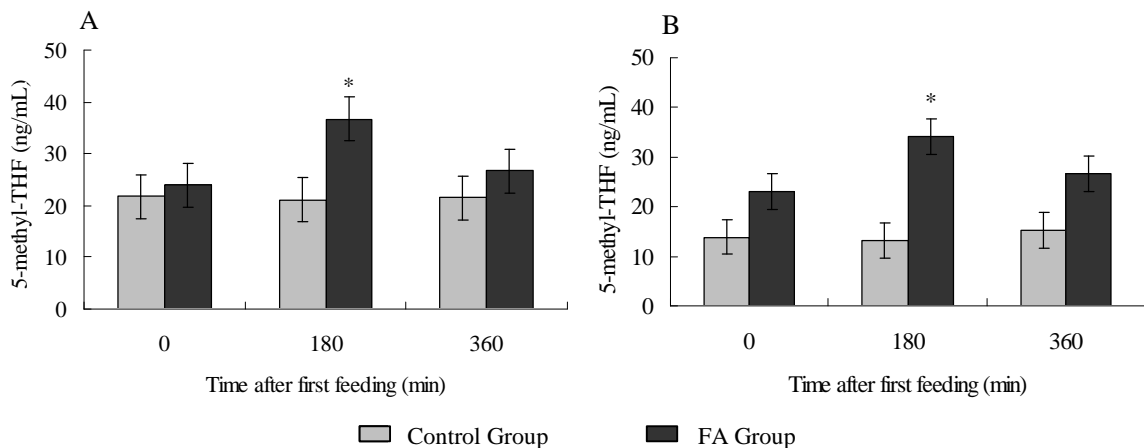
Figure 3: Serum 5-methyl-THF concentrations for the high concentrate (34% forage and 66% concentrate on dry matter basis) feeding groups (n = 7; A) and the high forage (66% forage and 34% concentrate on dry matter basis) feeding groups (n = 8; B) with and without folic acid supplementation at different time points after feeding *$P \leq 0.05$

Milk composition

No significant effects of dietary supplements of FA were observed for milk production, milk content of fat, protein and lactose as well as for yields of milk fat, protein and lactose neither at HC nor at HF ration (Table 14). Only in the HF feeding regime FA supplementation tended to reduce the fat content ($P = 0.097$; Table 14). Urea concentrations tended to be reduced in the HC+FA group in contrast to the HC group ($P = 0.056$; Table 14). In the HC feeding regime, except for $C_{18:3}$, the FA did not

influence the milk fatty acid composition (in the percentage of total fatty acids). $C_{18:3}$ however, was significantly decreased ($P = 0.017$; Table 14).

Table 14: Effects of dietary supplements of folic acid to dairy cows on milk production and composition (LSmeans with their standard error)

	HC					HF				
	Control (n = 5)		FA (n = 5)		P	Control (n = 4)		FA (n = 4)		P
	LSmeans	SE	LSmeans	SE	FA	LSmeans	SE	LSmeans	SE	FA
Milk (kg/d)	25.8	1.15	26.2	1.16	0.722	19.5	0.66	21.2	0.60	0.133
FCM (kg/d)	21.3	1.23	21.6	1.23	0.738	19.7	0.72	21.0	0.66	0.260
Milk composition (%):										
Fat	2.83	0.19	2.95	0.19	0.683	4.06	0.04	3.93	0.04	0.097
Protein	2.97	0.09	3.06	0.09	0.476	3.11	0.06	3.03	0.06	0.181
Lactose	4.56	0.06	4.59	0.06	0.709	4.83	0.05	4.82	0.05	0.885
Yield (g/d):										
Fat	731	57.7	746	57.8	0.834	793	30.5	835	27.9	0.372
Protein	752	45.5	792	45.6	0.549	614	24.4	643	22.4	0.431
Lactose	1177	61.1	1194	61.3	0.678	946	40.9	1021	37.4	0.248
Urea (mmol/L)	4.37	0.32	3.36	0.33	0.056	2.43	0.38	2.00	0.32	0.379
Fatty acids (weight % of total fatty acids):										
$C_{4:0}$	6.7	0.16	6.7	0.16	0.878	7.0	0.23	7.2	0.20	0.531
$C_{6:0}$	4.1	0.29	4.0	0.29	0.668	4.5	0.06	4.5	0.05	0.322
$C_{8:0}$	1.9	0.22	1.9	0.22	0.491	2.0	0.05	2.0	0.04	0.746
$C_{10:0}$	3.9	0.54	3.8	0.54	0.561	3.8	0.10	3.9	0.09	0.627
$C_{12:0}$	6.5	0.75	6.2	0.75	0.435	5.8	0.32	5.2	0.27	0.192
$C_{14:0}$	12.0	0.73	11.9	0.73	0.817	12.2	0.39	12.0	0.33	0.672
$C_{14:1}$	1.7	0.31	2.1	0.31	0.401	1.6	0.18	1.4	0.17	0.326
$C_{16:0}$	29.4	1.26	28.7	1.27	0.531	35.4	0.88	33.8	0.80	0.268
$C_{16:1}$	2.4	0.43	2.4	0.43	0.812	2.1	0.15	2.0	0.13	0.477
$C_{18:0}$	3.9	0.34	3.9	0.34	0.868	4.9	0.47	5.5	0.40	0.374
$C_{18:1}$	22.9	1.39	24.0	1.39	0.385	17.9	0.73	19.5	0.62	0.157
$C_{18:2}$	3.9	0.23	3.9	0.23	0.921	2.3	0.07	2.4	0.07	0.141
$C_{18:3}$	0.8	0.05	0.6	0.05	0.017	0.6	0.04	0.6	0.03	0.882

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; FA, folic acid; SE, standard error; FCM, fat corrected milk

Discussion

Rumen variables

The influence of FA on rumen variables has been reported once using eight steers at two different F:C ratios (70:30 and 30:70; Chiquette et al. 1993). In accordance to the present study Chiquette et al. (1993) found no influences of FA supplementation on pH values in ruminal fluids in both diets. They also found no effects of FA on acetic, propionic and butyric acids concentrations from 1 to 23 h after feeding. Furthermore, the ratio of acetic acid to propionic acid was also not affected. In the present study concentrations of acetic, propionic and butyric acid, as well as the ratio of acetic to propionic acid were also not influenced by FA supplementation (data not shown).

Duodenum

Effects of FA on apparent ruminal digestibility differed at the varying F:C ratios (Table 11). The FA supplementation to the HC diet lowered the amount of FOM and tended to reduce the percentage of FOM to OM intake. To our knowledge FOM has not been considered before, and an explanation for the reduction cannot be given. Contrary to that the FA enhanced the apparent ruminal digestibility of ADF with the HF diet. Feeding a blend of B-vitamins at a F:C ratio of 45:55, Zinn et al. (1987) could not find an influence on the apparent ruminal digestion of OM and ADF. Chiquette et al. (1993) fed F:C ratios of 70:30 and 30:70 and found no effect of FA on the total tract digestibility of DM, NDF and ADF. By contrast, for lambs (BW not given) supplemented with FA at 4.5 mg/(d x animal) Barth et al. (1960) showed a decreased total tract digestibility of DM and OM. In a later work of this research team no influence on total tract OM digestibility could be observed with the same amount of supplemented FA (McLaren et al. 1965). In both studies dietary F:C ratios were not specified. However, some previous *in vitro* studies show either increasing effects of FA on cellulose digestion or a demand for FA or THF by cellulolytic microorganisms (Hall et al. 1955; Ayers 1958; Bryant and Robinson 1961; Slyter and Weaver 1977). Therefore, the supplementation of FA possibly leads to an increased growth of these microorganisms which would lead to an increase in the apparent ruminal digestibility of ADF. The effect on apparent ruminal digestibility of fibre may not exist in the HC

ration, as feeding high amounts of concentrate affect the general conditions for cellulolytic microorganisms and thus, the degradation of cellulose and therefore, not FA could be the first limiting factors for the growth of these microorganisms.

The reason for the reduced efficiency of MP synthesis (g/MJ ME) at HC+FA ration was partly at least, the decreased amount of FOM (Tables 11 and 12) because less energy for MP synthesis was available. The ruminal N balance was always positive (not shown); hence an adequate supply of microorganisms with N was achieved for the control and the treatment group with the result that N deficiency could not be the reason for the decreased MP synthesis in the HC+FA group. The supply with uCP met the requirements of the animals for all rations, apart from the HC+FA group - here the uCP supply was slightly insufficient. Girard et al. (1994) found no influence of 2 mg FA per kg BW on the production of microbial mass and MP, as well as on the quantity of protein per unit of microbial mass at F:C ratios of 70:30 and 30:70 for steers. For a feeding regime with an F:C ratio of 45:55 and a supplementation of a blend of B-vitamins (with FA), Zinn et al. (1987) could also not find an influence on net microbial synthesis. Additionally, *in vitro* no effects of FA on MP synthesis and the efficiency of MP synthesis could be observed at three different F:C ratios of 67:33, 50:50 and 33:67 (Völker 2008).

Serum variables

Up to now, Graulet et al. (2007) are the sole authors studying the influence of dietary FA supplementation on plasma concentrations of glucose and NEFA in cows. According to our results (Table 13) they found no effects on NEFA and decreased plasma glucose concentrations between three weeks before and eight weeks after calving due to dietary FA supplementations of 2.6 g/d. No explanations were given for the lowered glucose levels; but cows fed FA supplementation produced 2.3 kg more milk per day compared to control cows while DM intakes were similar in both groups, which maybe an explanation for decreased glucose concentrations. For humans and rats no (Solini et al. 2006; Mao et al. 2008) or increasing (Prusiewicz-Witaszek and Maciejewska 1994) effects of FA on blood glucose were found. The increase in blood glucose was attributed to a decrease in blood insulin concentration (Prusiewicz-Witaszek and Maciejewska 1994).

Insulin was also decreased in the study of Solini et al. (2006) in overweight humans, but without any effect on glucose. Thus, the results are contradictory. Furthermore, glucose metabolism differs between monogastric organisms and ruminants. Consequently, it would be interesting to consider if FA also has an influence on blood insulin levels in cows and if this provides an explanation for the effect on glucose. The lower urea concentrations of the HC+FOL group seem to result from the lower N intake of this group compared to the HC group (Table 13).

The influence of an FA supplementation on the levels of the other B-vitamins is not well established. Similar to our results, the level of pyridoxal-5'-phosphate in serum of dairy cows (fed with a F:C of 37:63) was not influenced by supplementations of 2 or 4 mg FA per kg BW (Girard and Matte 1999). For riboflavin no further studies are available and at present no explanation can be given.

Milk composition

Generally, the results on milk composition of the present study should not be over-interpreted as this was not the main focus of this study. Three studies are available concerning the influence of oral FA supplementation on milk components. Consistent with our results (Table 14), Girard and Matte (1998) and Girard et al. (2005) could not find an influence of 2 and 4 mg FA per kg BW or of 3 and 6 mg FA per kg BW on milk composition (%) of milk fat, protein and lactose (Table 5). However, Girard and Matte (1998) found that supplementary FA slightly increased the total quantity of milk casein produced by multiparous cows, but supplementation had no effect on the quantity of the other nitrogenous fractions. Girard et al. (2005) could not find any effect on yields of milk components (kg/d). Additionally, Graulet et al. (2007) observed no effect of 2.6 g FA/d on milk composition, whereas yield (g/d) of milk fat showed a trend to greater values and yield of milk protein was significantly greater during the first 56 days of lactation. As milk production was also increased, Graulet et al. (2007) hypothesised that the essential role of FA for the synthesis of purines and pyrimidines for DNA synthesis could be a reason for the increased protein values.

Consistence with the above mentioned results for urea in serum, reduced milk urea concentrations (-33%) seem to result from the lower N intake of the HC+FA group

compared to the control (Tables 9 and 14). Girard et al. (2005) and Graulet et al. (2007) could not find an influence of dietary FA supplementations on milk urea N concentrations. However, for multiparous cows during the first 100 days of lactation Girard and Matte (1998) found decreased milk non-protein-N values due to FA. They hypothesised that this reduction resulted from an improved N utilisation in the rumen and amino acid catabolism in cow tissues. The improved N utilisation in the rumen did not become obvious from the results of the present study (Table 12). The lowered $C_{18:3}$ in milk of the HC+FA group compared to HC group (Table 14) could result from a slightly negative energy balance in the HC group (-0.77 MJ NEL/d) compared to a positive energy balance in the HC+FA group (3.80 MJ NEL/d).

Furthermore, from the results of the present study it is difficult to decide if influences on metabolism result from changes on the ruminal level (Tables 10, 11 and 12) or are a consequence of the increased 5-methyl-THF concentrations in blood (Figures 3A and B), as the influences of FA on ruminal fermentation, as well as on metabolism were rather minor.

Folate content in serum

In all the serum samples only 5-methyl-THF was found, whereas FA and THF were not detectable. Admittedly, in bovine blood mainly 5-methyl-THF is available (Anonymous 1992). In the present study, as well as in literature, FA supplementation higher than 0.5 mg/kg BW always resulted in increased serum folate concentrations (Girard et al. 1992; Girard et al. 1994; Girard and Matte 1998; Girard and Matte 1999; Girard et al. 2001; Girard et al. 2005). Similar to our results, Girard et al. (1992; 1994; 2001) found an influence of the time after FA supplementation on blood folate concentrations as the peak was reached 3 to 6 h afterwards. In the present study preprandial serum concentrations of 5-methyl-THF did not differ significantly in control and FA-supplemented animals, whereas Girard et al. (1994) found higher preprandial serum folate concentrations after supplementations of approximately 0.7 g/d over five weeks in steers fed diets with a F:C ratio of 70:30 or 30:70. In the current study serum 5-methyl-THF concentrations of the HC and HF group varied (Figure 3). In contrast to our results, Santschi et al. (2005b; F:C ratio 37:63 or 58:42) and Girard et al. (1994;

F:C ratio 70:30 or 30:70) found no effect of the F:C ratio on plasma folate concentrations in primiparous (12 or 14 ng/mL) and multiparous (18 to 19 ng/mL) cows, as well as on serum folate concentrations in steers (both 19 ng/mL). Comparing the serum 5-methyl-THF concentrations of the present study with the serum folate concentrations in the previously mentioned literature it is conspicuous that the 5-methyl-THF concentrations in the HC group are on average relatively high (23 ng/mL), while the 5-methyl-THF concentrations of the HF group (13 ng/mL) are rather low. However, it should be noted that in the HC group two cows were nonlactating while in the HF group four cows were nonlactating. This may also be a reason for the huge difference between HC and HF groups, as Girard et al. (1989) observed the highest serum folate concentrations in early lactation and the lowest values at calving.

Conclusion

Results from the present experiment suggest that – regardless of dietary F:C ratio – FA generally had no, or only minor, effects on rumen fermentation and on blood and milk variables. In the serum the supplementation of FA leads to increased 5-methyl-THF concentrations. From the present study the question as to whether or not dairy cows have an advantage from FA supplementation cannot be answered. Therefore studies with a higher number of animals, at different stages of lactation and gestation would be helpful. It would also be interesting to test ruminal protected FA. Furthermore, studies that differentiate between the FA requirements of the ruminal microorganisms and the host are necessary.

4   Pantothenic acid

## 4.1   Pantothenic acid in ruminant nutrition: a review

Introduction

Pantothenic acid was discovered by Williams et al. (1933). Due to its incorporation into CoA and ACP, PA has considerable metabolic importance (Ball 2006). Ruminants have two sources of PA: on the one hand fodder, and on the other hand, ruminal PA synthesis by microorganisms (Bechdel et al. 1928). The National Research Council (2001) made a comparison between ruminal synthesis and daily proposed requirements of a 650 kg cow producing 35 kg of 4% fat-corrected milk. However, tissue requirements were extrapolated from lactating sows as no values were available for ruminants. The calculated ruminal synthesis of 38 mg/d does not cover the proposed daily PA requirements of 304 mg/d for tissue and 121 mg/d for milk production. Because PA is ubiquitous in feedstuffs (Gesellschaft für Ernährungsphysiologie 2001), requirements may be met by PA intake via feed provided that feed-bound PA is not degraded in the rumen. As the small intestine is the major site of PA absorption in ruminants (Rérat et al. 1958), the duodenal flow of PA can be a measure to investigate whether PA intake via feed and ruminal PA synthesis together might meet the proposed requirements. The aim of this review is to give an outline of the current findings on PA synthesis in the rumen, the PA flow to the duodenum, as well as the PA concentrations in the blood and milk of dairy cows. However, due to the scarcity of studies on ruminants, cross-references to studies with non-ruminant animals and humans are, in some cases, unavoidable.

Chemical structure and biochemical functions

The vitamin PA (see Appendix Figure A3) consists of ß-alanin and pantoic acid (Bässler et al. 2002). Already from its name, which is derived from the Greek word *pantos*, meaning "everywhere", it can be deduced that PA is ubiquitous and occurs in nearly every feedstuff (Williams et al. 1933). In nature, the only occurring vitamer of PA is the D-isomer. Except for serum and milk, free PA is rare. Nevertheless, as a part of CoA, PA is available in every living cell (Bender 1992; Pietrzik et al. 2008). As a part of CoA and ACP, the biological activity of PA is closely related to those particular compounds. Bound in CoA, PA is essential for many reactions involved in the release of energy from carbohydrates, fatty acids and amino acids (Figure 4). In carbohydrate

metabolism, acetyl-CoA essentially supplies the tricarboxylic acid cycle with acetyl groups. Furthermore, acetyl-CoA is necessary for the acetylation of amino sugars and choline to form mucopolysaccharides and acetylcholine. Succinyl-CoA is important for the biosynthesis of haemoglobin and cytochromes as a precursor of porphyrin (Ball 2006). Additionally, ACP-bound PA is essentially involved in the biosynthesis of fatty acids (Ball 2006).
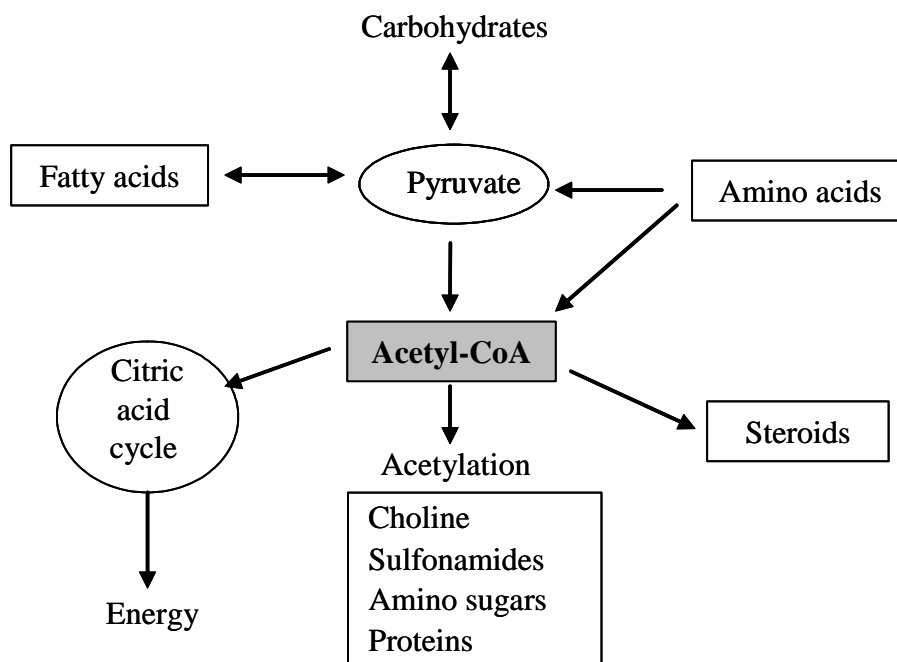
Figure 4: Central position of coenzyme A in intermediary metabolism (Biesalski 1997)

Sources and bioavailability of pantothenic acid

Table 15 shows that PA concentrations vary largely between and within feedstuffs. Values in Table 15 arise from compilations, thus methods of analysis and numbers of samples are unknown. Different analytical methods may result in varying values for PA concentrations, such that a comparison of feedstuffs analysed with different methods is difficult to achieve (Roth-Maier and Kirchgessner 1979). Furthermore, native PA concentrations are influenced by climate, species, vegetation stage, habitat and fertiliser (Albers et al. 2002).

Table 15: Summary of pantothenic acid concentrations of several feeds

Feed	Range of PA (mg/kg DM)	References
Lucerne meal	20.7 - 42.0	Roth-Maier and Kirchgessner (1979), Anonymous (1996), National Research Council (1998)
Lucerne hay	20.1	Anonymous (1996)
Barley, whole grain	3.9 - 11.6	Roth-Maier and Kirchgessner (1979), Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Maize, whole grain	5.7 - 9.1	Roth-Maier and Kirchgessner (1979), Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Oats, whole grain	8.0 - 19.0	Roth-Maier and Kirchgessner (1979), Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Peas	2.2 - 31.1	Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Rapeseed meal, solvent extracted	9.0 - 10.8	Roth-Maier and Kirchgessner (1979), Anonymous (1996), LfL (2004)
Rye, whole grain	1.7 - 17.4	Anonymous (1996), National Research Council (1998), Souci et al. (2000)
Soybean meal, solvent extracted	14.4 - 18.6	Roth-Maier and Kirchgessner (1979), Anonymous (1996), National Research Council (1998), LfL (2004)
Wheat, whole grain	1.4 - 17.5	Roth-Maier and Kirchgessner (1979), Anonymous (1996), National Research Council (1998), Souci et al. (2000), LfL (2004)
Whey powder	5.3 - 123.8	Roth-Maier and Kirchgessner (1979), Anonymous (1996), National Research Council (1998), Souci et al. (2000)

PA, pantothenic acid; DM, dry matter; LFL, Bayerische Landesanstalt für Landwirtschaft

At present, no studies are available simultaneously examining the PA intake, amount of PA at the duodenum and PA content in blood. Thus an estimation of the apparent bioavailability (proportion of PA intake and PA which is available in plasma after absorption) of PA for ruminants is not possible. A determination of the absolute bioavailability (proportion of orally administered PA which is available in plasma after absorption) of PA in ruminants is extremely difficult as PA is both degraded and synthesised in the rumen. Therefore, studies on the distribution of PA with isotop-labeled PA are necessary. So far, no dietary factors are known that affect PA availability (Roth-Maier et al. 2000). For dairy cows it must be considered that, based on a ruminal synthesis of only 38 mg/d, the cow has to ingest nearly 400 mg PA to meet

the proposed PA requirements of 425 mg/d (National Research Council 2001), which is almost impossible in view of the data presented in Table 15. However, the National Research Council (2001) requirements are only first proposals, thus it is questionable whether they are correct.

Microbial synthesis and degradation and absorption of PA in the gastrointestinal tract of ruminants

It is worth noting that most studies on PA in ruminants were published decades ago and very few recent findings are available. The ability of microorganisms in the rumen to synthesise PA has long been known (Bechdel et al. 1928; McElroy and Goss 1941). However, is it difficult to measure the total amount of synthesised PA in the rumen, as the rumen is a dynamic system in which synthesis, degradation, passage along the digestive tract, and possibly absorption happen simultaneously (Kon and Porter 1954). The apparent synthesis of PA (difference between duodenal flow and intake) has only been measured in growing steers and even without supplementation it was negative (-13.5 mg/d; Zinn et al. 1987), indicating that no net synthesis of PA occurred. But growing steers had a low DM intake; hence apparent synthesis in mature animals may be different. Additionally, the major dietary ingredient was maize grain, and as PA synthesis varies with ration composition it would be interesting to evaluate whether other types of diet will lead to positive apparent ruminal synthesis. Table 16 gives a survey of microorganisms (*in vitro*) which synthesise, require or do not need PA. But no studies investigated which microorganisms degrade PA. It should be conducted that the cited studies in Table 16 are older than 48 years, however, more current studies are not available. However, in a field study with ruminally protected and unprotected PA supplementations, ruminal degradation of PA was obvious (Bonomi 2000). Furthermore, Porter (1961) reported that the addition of PA led to a reduced PA *in vitro* synthesis of *Escherichia coli*, whereas the PA synthesis of *Streptococcus bovis* was not influenced. He also found that 100 mL ruminal liquid contained 100 μg PA, which was distributed among the protozoal (10%), bacterial (50%) and supernatant (40%) fractions of ruminal fluid.

Table 16: Survey of microorganisms which synthesise, require or do not need pantothenic acid

Microorganisms	Pantothenic acid		
	Synthesise	Require	No need of
Escherichia coli[1]	X		
Streptococcus bovis[1,2]	X		X
Streptococcus faecalis[2]			X
Streptococcus liquefaciens[2]		X	
8 unclassified strains of *Streptococcus*[2]		X	
Lactobacillus casei[2]		X	
Lactobacillus fermenti[2]		X	
Lactobacillus acidophilus[2]		X	
Lactobacillus plantarum[2]		X	

[1] Porter (1961)
[2] Ford et al. (1958)

The ruminal synthesis of PA is influenced by composition of the diet. With an increasing content of rapidly degradable carbohydrates (starch and sugar), more PA is synthesised, indicating a shift in microbial population from species which consume to species which synthesise PA (Lardinois et al. 1944; Hollis et al. 1954; Hayes et al. 1966; Table 17). It should be considered that the animal numbers (1 to 8) in these studies were rather small and furthermore, studies were older than 40 years. Lardinois et al. (1944) reported a further increase in PA synthesis when urea was added to a diet high in rapidly degradable carbohydrates. Agrawala et al. (1953) found that a purified ration decreased the PA synthesis by about 50% compared to a regular ration (Table 17) and hypothesised that PA synthesis may have been lowered due to a sensitivity to pH changes and/or some lack of essential nutrients and/or rapid utilisation and removal of PA from the rumen. But for this study, calves were used and drastic changes of ration may have a greater impact on this ruminal population in those than in more mature animals. *In vitro*, a correlation ($r = 0.82$) between PA concentration and substrate digestibility was found, which indicates that synthesis of PA was related to total microbial activity (Hayes et al. 1967). Finlayson and Seeley (1983) endorsed this hypothesis for wethers as they established a correlation ($r = 0.94$) between the amount of PA reaching the small intestine and the quantity of microbial DM produced in the rumen, suggesting that most of the PA is associated with the microbial fraction at this

site. The same authors also found that the amount of free PA decreased between the mouth and the proximal duodenum, while the amount of CoA-bound PA increased.

Only Zinn et al. (1987) reported on the influence of PA supplementations on duodenal PA flow. Growing steers (194 kg BW) were fed on a mixed diet (45:55, F:C on DM basis) and PA was supplemented at 0 (PA intake, 24.5 mg/d), 200 and 2000 mg/d, resulting in duodenal PA flows of 11.0, 15.3 and 452.8 mg/d. Compared to the control only 2000 mg PA led to a significant increased duodenal PA flow. Nevertheless, nearly 80% of the supplementation disappeared between the mouth and the duodenum. But it is a debatable point whether disappearance results from degradation or absorption of PA. Previously, ruminal absorption has only been tested in the washed rumen of sheep and it seems that it can be ignored (Rérat et al. 1958), but it has never been tested with PA-supplemented cows. Studies on the site of PA absorption in the small intestine do not exist. Perhaps PA can be absorbed at the proximal duodenum and therefore maybe before the duodenal cannula which would lead to an overestimation of ruminal disappearance. But at least in rats and chickens, the major site for PA absorption seems to be the jejunum (Fenstermacher and Rose 1986). The PA absorption from the intestinal lumen is effected by an active Na^+-dependent carrier mechanism (Fenstermacher and Rose 1986). But the dietary PA concentration showed no influence on the activity of the transporter (Stein and Diamond 1989).

The apparent small intestinal disappearance (between the duodenal and ileal cannulas) of PA has only been measured in wethers. For total PA the apparent small intestinal disappearance was about 50%, however CoA-bound PA disappeared by about 92% while the content of free PA increased fourfold (Finlayson and Seeley 1983). They hypothesised that the disappearance of CoA-bound PA reflects enzymic hydrolysis to yield free PA, most of which is then absorbed by passive diffusion but leaving a residuum of free PA at the terminal ileum larger than that entering the duodenum. Roth-Maier et al. (2000) observed that PA failed to undergo substantial enterohepatic circulation, thus no PA is secreted back into the small intestine.

Table 17: Pantothenic acid concentrations of ruminal material (mean values)

Diet	Number and animal species	BW (kg)	DM intake (kg/d)	PA in Total ruminal content (mg/kg DM)	PA in Liquid fraction (μg/mL)	References
5.45 kg timothy hay[1]	1 cow			3.4		Lardinois et al. (1944)
4.54 kg timothy hay + 1.82 kg maize molasses[1]	1 cow			3.9		
4.54 kg timothy hay + 1.82 kg maize molasses + 0.2 kg urea[1]	1 cow			12.0		
4.54 kg timothy hay[1]	1 cow			5.8		
4.54 kg timothy hay + 0.91 kg maize molasses + 0.91 kg starch[1]	1 cow			5.7		
4.54 kg timothy hay + 0.91 kg maize molasses + 0.91 kg starch + 0.2 kg urea[1]	1 cow			17.9		
4.54 kg timothy hay + 0.91 kg maize molasses + 0.91 kg starch + 0.09 kg casein[1]	1 cow			18.2		
4.54 kg timothy hay + 0.91 kg maize molasses + 0.91 kg starch + 0.09 kg casein + 0.2 kg urea[1]	1 cow			16.3		
42% cornstarch + 25% glucose + 20% cellophane + 4% lard + 5% minerals + 4% urea[2]						Agrawala et al. (1953)
before feeding	6 calves		3.2	10.3		
6 h after feeding	6 calves	181	3.2	16.3		
60% brome-timothy hay + 40% grain mixture[2]		-				
before feeding	1 calve	254	3.2	28.0		
6 h after feeding	1 calve		3.2	27.0		
100% prairie hay[2]	2 lambs		0.6	8.3		Hollis et al. (1954)
53% maize + 45% prairie hay + 2% urea[2]	2 lambs		0.8	14.3		
45% prairie hay + 40% maize + 15% soybean meal[2]	2 lambs		0.8	17.5		
45% sorghum silage + 44% maize + 11% soybean meal[2]	2 lambs	32	0.8	16.4		
53% maize + 45% lucerne hay + 2% soybean meal[2]	2 lambs	-	0.8	22.6		
90% maize + 10% soybean meal[2]	2 lambs	41	0.8	30.1		
44% maize cobs + 25% maize + 20% maize gluten meal + 7% maize syrup + 3% maize oil + 1% CaHPO$_4$[2]	2 lambs		0.7	16.4		
42% maize cobs + 23% maize + 19% maize gluten meal + 6% maize syrup + 6% lucerne ash + 3% maize oil + 1% CaHPO$_4$[2]	2 lambs		0.8	18.7		
6.3 kg flaked maize + 1.1 kg soybean meal with minerals[1]	8 steers	340			2.4[a]	Hayes et al. (1966)
6.6 kg ground maize + 1.1 kg soybean meal with minerals[1]	8 steers	340			3.5[b]	
6.9 kg flaked maize + 1.8 kg long lucerne hay + 0.7 kg soybean meal with minerals[1]	8 steers	340			0.7[c]	
7.5 kg ground maize + 1.8 kg long lucerne hay + 0.7 kg soybean meal with minerals[1]	8 steers	340			0.8[c]	
7.5 kg flaked maize + 1.8 kg ground lucerne hay + 0.7 kg soybean meal with minerals[1]	8 steers	340			1.2[c]	
7.4 kg ground maize + 1.7 kg ground lucerne hay + 0.7 kg soybean meal with minerals[1]	8 steers	340			1.1[c]	

DM, dry matter; PA, pantothenic acid

[a,b,c] Mean values within a trial with unlike superscript letters were significantly different ($P \leq 0.05$).

[1] In these studies no DM values of feed were given; therefore fresh matter values are presented here.

[2] On DM-basis.

Pantothenic acid concentrations in blood, milk and urine

Blood

In serum PA mainly occurs in the free form, whereas in erythrocytes the predominant form of PA is CoA (Pietrzik et al. 2008). Table 18 summarizes published information on blood PA concentrations. For the ease of comparison, also two human studies were listed in Table 18. At present, only two studies are available considering the PA content in the blood of dairy cows. However, differences between the reported values are immense, although cows in both studies (Dubeski and Owens 1993; Bonomi 2000) had a similar milk production and the same analytical method for PA (an indirect enzyme-linked immunosorbent assay) was used. Perhaps a miscalculation in the study of Dubeski and Owens (1993) caused the differences between the PA values. The PA concentrations determined by Roth-Maier and Kirchgessner (1973) and Ray et al. (1947) in the whole blood of calves and sheep correspond to plasma values of Bonomi (2000). As has been shown for humans (Table 18), whole blood normally has a higher PA concentration than plasma, as it also contains the CoA-bound PA of the erythrocytes (Song et al. 1985). Values for calves were lower, possibly because they have no fully developed rumen and therefore less microbial synthesis of PA is possible.

The effects of a PA supplementation on blood PA concentrations vary. For dairy cows, daily supplementations of 100 and 200 mg/cow increased plasma PA concentrations but 50 mg/cow did not. Daily supplementations of 50, 100 and 200 mg rumen-protected PA per cow always resulted in increased plasma PA concentrations compared to the unsupplemented control and in comparison with unprotected supplements (Table 18; Bonomi 2000). Roth-Maier and Kirchgessner (1973) studied the effects of 17, 21, 25 and 45 mg PA per kg milk replacer on the performance of male veal calves, with 17 mg PA/kg milk replacer representing the native PA concentration. Only the highest supplementation level (45 mg/kg milk replacer) increased the whole blood PA content. Roth-Maier and Kirchgessner (1973) further concluded that the PA content of 17 mg per kg milk replacer already met the PA requirements of male veal calves as supplemented amounts did not improve weight gains and feed conversion.

Table 18: Pantothenic acid concentrations in whole blood and plasma of ruminants and women (mean values)

Species (n)	Pantothenic acid			References
	Supplementation	Blood (mg/L)	Blood fraction	
Preruminants:				
Male veal calf (11)				Roth-Maier
80 kg	None[1]	0.267[a] ± 0.029[2]	Whole blood	and
80 kg	4[3]	0.264[a] ± 0.027[2]	Whole blood	Kirchgessner
80 kg	8[3]	0.288[a] ± 0.027[2]	Whole blood	(1973)
80 kg	28[3]	0.355[b] ± 0.029[2]	Whole blood	
110 kg	None[1]	0.265[a] ± 0.019[2]	Whole blood	
110 kg	4[3]	0.260[a] ± 0.016[2]	Whole blood	
110 kg	8[3]	0.290[a] ± 0.024[2]	Whole blood	
110 kg	28[3]	0.304[b] ± 0.019[2]	Whole blood	
140 kg	None[1]	0.273[a] ± 0.026[2]	Whole blood	
140 kg	4[3]	0.281[a] ± 0.026[2]	Whole blood	
140 kg	8[3]	0.260[a] ± 0.024[2]	Whole blood	
140 kg	28[3]	0.346[b] ± 0.023[2]	Whole blood	
Suckling calf (31)	None[4]	0.00003 ± 0.000004[2]	Plasma	Dubeski and
Ruminants:				Owens (1993)
Feedlot steer (16)	None[4]	0.00003 ± 0.000004[2]	Plasma	
Dairy cow (14)	None[4]	0.00002 ± 0.000004[2]	Plasma	
Dairy cow (15)				Bonomi (2000)
1. Farm	None[4]	0.856[a] ± 0.103[5]	Plasma	
2. Farm	None[4]	0.907[a] ± 0.073[5]	Plasma	
3. Farm	None[4]	0.877[a] ± 0.114[5]	Plasma	
1. Farm	50[6]	0.912[a] ± 0.086[5]	Plasma	
2. Farm	100[6]	1.154[b] ± 0.085[5]	Plasma	
3. Farm	200[6]	1.252[b] ± 0.136[5]	Plasma	
1. Farm	50 protected[6]	1.203[b] ± 0.092[5]	Plasma	
2. Farm	100 protected[6]	1.331[c] ± 0.088[5]	Plasma	
3. Farm	200 protected[6]	1.586[c] ± 0.153[5]	Plasma	
Sheep (10)	None	0.418 ± 0.070[5]	Whole blood	Ray et al. (1947)
Women:				
4-5th month of pregnancy (6)	None[4]	0.517 0.432-0.601[7,8]	Whole blood	Ishiguro (1962)
4-5th month of pregnancy (7)	60[6]	0.566 0.454-0.678[7,8]	Whole blood	
7-8th month of pregnancy (6)	None[4]	0.510 0.384-0.636[7,8]	Whole blood	
7-8th month of pregnancy (7)	60[6]	0.497 0.406-0.588[7,8]	Whole blood	
End of pregnancy (6)	None[4]	0.558 0.432-0.684[7,8]	Whole blood	
End of pregnancy (7)	60[6]	0.881 0.629-1.133[7,8]	Whole blood	
nonpregnant + nonlactating (5)	None[4]	0.848 0.562-1.134[7,8]	Whole blood	
4-5th month of pregnancy (6)	None[4]	0.100 0.073-0.127[8,9]	Whole blood	
4-5th month of pregnancy (7)	60[6]	0.097 0.065-0.129[8,9]	Whole blood	
7-8th month of pregnancy (6)	None[4]	0.112 0.066-0.157[8,9]	Whole blood	
7-8th month of pregnancy (7)	60[6]	0.243 0.182-0.303[8,9]	Whole blood	
End (6)	None[4]	0.097 0.062-0.132[8,9]	Whole blood	
End (7)	60[6]	0.254 0.062-0.132[8,9]	Whole blood	
nonpregnant + nonlactating (5)	None[4]	0.118 0.064-0.172[8,9]	Whole blood	
pregnant or	None[10]	0.433[a] ± 0.010[5]	Whole blood	Song et al.
lactating (26)	None[10]	0.108 ± 0.003[5]	Plasma	(1985)
nonpregnant +	None[11]	0.526[b] ± 0.012[5]	Whole blood	
nonlactating (26)	None[11]	0.111 ± 0.004[5]	Plasma	

PA, pantothenic acid

[a,b,c] Mean values within a trial with unlike superscript letters were significantly different ($P \leq 0.05$).

[1] The natural PA content of the milk replacer was 17 mg/kg.

[2] Standard error

[3] mg PA supplementation per kg milk replacer; for the total PA content of the milk replacer the natural PA content has to be added.

[4] PA intake from natural diet was not specified.

[5] Standard deviation

[6] PA supplementation (mg/d)

[7] Bound PA

[8] 95% confidence interval

[9] Free PA

[10] PA intake from natural diet was 5.3 mg/d for pregnant and 5.9 mg/d for lactating women.

[11] PA intake from natural diet was 4.8 mg/d.

For humans, Ishiguro (1962) observed the influence of 60 mg PA per day on the content of bound and free PA in the whole blood of pregnant women. Around the 4th to 5th month of pregnancy PA supplementation did not increase the blood PA content. However, at the 7th to 8th month and at the end of pregnancy the level of free PA increased, while the bound PA only increased at the end of pregnancy due to PA supplementation (Table 18). Compared to non-pregnant women, pregnant women (without PA supplementation) had decreased blood levels of bound PA, but the level of free PA did not differ (Ishiguro 1962; Song et al. 1985).

For ruminants only one field study exists on the influence of PA supplementation on blood variables (Bonomi 2000). It seems that the effects are dose-dependent as supplementations of 50 and 100 mg per cow per day had no influence on either the blood concentrations of glucose, acetic acid, total cholesterol, triglyceride, NEFA, urea N, total protein, albumin, glutamic-oxaloacetic transaminase and γ-glutamyl transferase, or the amino acid profile in plasma (Bonomi 2000). However, supplementations of 200 mg PA per day, as well as supplementations of 50, 100 and 200 mg ruminally protected PA per cow per day increased the glucose content (each by about 5%, 10%, 10% and 12%), decreased the acetic acid concentration (-14%, -15%, -21%, -31%), increased the content of methionine (29%, 50%, 42%, 64%), lysine (12%, 16%, 16%, 22%), isoleucine (25%, 38%, 54%, 57%) and glutatmate (6%, 8%, 8%, 11%) and reduced the content of phenylalanine (-9%, -11%, -15%, -18%), glycine (-15%, -29%, -22%, -33%) and histidine (-14%, -27%, -15%, -31%; Bonomi 2000). From this field study Bonomi (2000) hypothesised that the reduction of acetic acid resulted from a higher utilisation of this acid for lipid synthesis in the udder, as the milk fat content was also increased. Furthermore, PA might have caused an improved energy utilisation that led to the increase of glucogenic amino acids, which, in the unsupplemented diets were needed for protein synthesis (Bonomi 2000). In contrast to cows, rats fed diets with graded levels of PA supplementation (0.16 to 1600 mg/kg diet) had differences in the levels of triglycerides and free fatty acids in serum, and both values decreased with increasing PA levels (Wittwer et al. 1990). However, prior to PA supplementation, these rats had a mild PA deficiency, thus lipid alterations were rather due to the previous deficiency, as a PA deficiency elevates triglyceride and free fatty acid levels. The mechanism for

this elevation is unclear, both CoA or phosphopantetheine may become limiting in an enzyme-circulating pathway, thus elevating circulating lipids (Wittwer et al. 1990). A further study with PA-deficient rats showed that the gluconeogenic capacity is reduced during PA deficiency, as blood glucose and liver glycogen were reduced concurrently (Srinivasan and Belavady 1976). This seems to result from a reduced activity of hexosediphophatase, which controls the rates of glycolysis and gluconeogenesis (Srinivasan and Belavady 1976). This could also be an explanation of the increased glucose concentrations found by Bonomi (2000). If PA is able to enhance gluconeogenesis and thus, blood glucose levels, it would be very interesting for high-yielding dairy cows, because gluconeogenesis is essential for them as nearly all sources of glucose are fermented in the rumen. However, a 700 kg cow with a milk production of 50 L/d requires approximately 3.5 kg glucose daily (Flachowsky et al. 2004). Thus, further studies are warranted.

Milk

Eighty-five to 90% of PA in the human milk occurs in a free form (Song et al. 1984); for cow's milk, no such data is available. Generally, the PA milk concentration is considerably higher than that of the other B-vitamins (Kirchgessner et al. 1965). Literature up to 1965 on PA concentrations in the milk of dairy cows was reviewed and summarized by Kirchgessner et al. (1965). The average PA content in the milk was about 3 mg/L. Since then, only two further studies on milk PA concentrations were published (Kirchgessner et al. 1991a; Kirchgessner et al. 1991b; Table 19). In a compilation by Souci et al. (2000) an average PA content for raw milk of 3.5 mg/L was stated with a range of 2.8 to 4.2 mg/L. At the onset of lactation the lowest milk PA concentrations are found. During the first days of lactation milk PA concentrations increase and peak by about 4 mg/L between the 4th to 14th day. Afterwards concentrations decrease slightly (Lawrence et al. 1946; Pearson and Darnell 1946; Gregory et al. 1958). With supplementations of 0.5, 2 and 16 g PA per cow per day, Marsh et al. (1947) could not increase the PA content in the milk of dairy cows, whereas supplementing 2 g PA to the diet of goats increased the PA concentration of their milk - a supplementation of 0.25 g had no effect. An explanation for this difference between cows and goats was not given.

Thus, up to now, for dairy cows the milk PA content could not be increased due to PA supplementation, however this fact was only tested once, with two cows, more than 60 years ago.

Table 19: Milk pantothenic acid concentrations without pantothenic acid supplementation (means with their standard deviation)

Species (n)	Milk PA (mg/L)		Lactation week	References
	Mean	SE		
Dairy cows (70)	3.66	0.31	n.s.	Holmes et al. (1944)
Dairy cows (32)	2.24	0.87	Colostrum	Pearson and
Dairy cows (32)	3.67	0.57	Milk	Darnell (1946)
Ewes (14)	2.62	0.71	Colostrum	
Ewes (14)	3.66	0.79	Milk	
Dairy cows (8)	3.67	0.65	6 - 10	Kirchgessner
Dairy cows (8)	3.79	0.73	10 - 14	et al. (1991a)
Dairy cows (8)	3.99	1.18	14 - 18	
Dairy cows (8)	3.84	2.12	18 - 22	
Dairy cows (18)	3.49	0.46	10 - 28	
Dairy cows (18)	3.22	0.52	13 - 31	
Dairy cows (18)	3.03	0.73	16 - 34	
Dairy cows (16)	5.01	0.93	~ 7	Kirchgessner
Dairy cows (16)	4.07	1.12	~ 11	et al. (1991b)
Dairy cows (16)	2.98	0.99	~ 15	
Dairy cows (16)	3.23	1.38	~ 19	

PA, pantothenic acid; n.s., not specified

Kirchgessner et al. (1991a; 1991b) reported that neither a prolonged release of bovine growth hormone nor an excess of energy or protein had an influence on PA concentrations in milk. Furthermore, no relationship existed between the PA content in milk and milk fat, total solids, milk-solids-not-fat, caloric value, milk yield and the age of the cow (Lawrence et al. 1946). One field study on the influence of ruminally unprotected and protected supplementations of 50, 100 and 200 mg per cow per day on milk parameters exists (Bonomi 2000). Supplementations of 50 and 100 mg of ruminally unprotected PA showed no influences. The other supplementations showed dose-dependent effects, however the slightest influences were always observed with the ruminally unprotected supplementation: milk production should be increased between 4 to 10%, milk fat and milk protein content both between 4 to 9%, whereas no influence on milk lactose content and fatty acid composition was established. From this field study Bonomi (2000)

assumed that these effects resulted from changes of the haemogram, as PA supplementations increased glucose concentration and some glucogenic amino acids, while acetic acid and some ketogenic amino acids were decreased. The author concluded that the reduced acetic acid concentrations might arise from a better utilisation of this acid for milk lipid synthesis, resulting in an increased lipogenesis. Additionally, the increase in glucogenic amino acids could result from a more efficient energy utilisation, redirecting more amino acids towards protein synthesis. Thus, it seems very interesting to study the influence of PA on milk composition further.

Urine

As for serum and milk, PA in urine occurs mainly in the free form (established for humans; Shigeta and Shichiri 1966). Two previous studies are available considering the excretion of PA via urine in ruminants. Teeri et al. (1951) reported that for heifers fed a 100% roughage ration, PA excretion via urine (between 75 and 83 mg PA per day) always exceeded the intake (by about 17 to 38%). When adding molasses to the ration (amount not specified) the excretion of PA via urine increased significantly (85 to 118 mg PA per day, 134 to 145% of intake). The authors hypothesised that the microorganisms which synthesise PA are favourably affected by the higher amount of rapidly fermentable or degradable carbohydrates in the molasses ration, which was supported by studies of Hayes et al. (1966) and Hollis et al. (1954). For goats, Marsh et al. (1947) found an increased amount of PA in the urine following a PA supplementation of 2 mg/d. Thus, it seems that the PA content in the urine of ruminants is influenced by ration composition and PA intake. Similarly, the urine PA excretions of humans also correlated positively with PA intake (Fox and Linkswiler 1961; Fox et al. 1964; Song et al. 1985). Furthermore, a positive relationship was detected between PA concentrations in the milk and urine of humans (Song et al. 1984). Experiments with humans and rats showed that the urinary excretion of PA is reduced during a PA deficiency (Fox and Linkswiler 1961; Pietrzik et al. 1975). No PA could be detected in the urine of rats fed a diet without any PA for a year (Pietrzik et al. 1975). However, without antagonists, a PA-free situation is not possible in ruminants due to the synthesis of PA by microorganisms.

Conclusion and future research

The importance of PA becomes obvious when considering the variety of metabolic reactions where CoA and ACP are involved. It is therefore surprising that it is still unclear if the PA requirements of dairy cows are being met by intake and/or ruminal synthesis. Furthermore, current studies are hardly available in the literature. Duodenal flow was hitherto only measured in growing steers, thus for dairy cows, which have much higher intakes and duodenal flows of DM and nutrients, the situation may be different. Furthermore, the sole study using dairy cows on the influence of supplementing PA on blood variables, milk production and ingredients, showed vast effects as blood glucose concentrations, milk production and additionally milk fat and protein contents increased (Bonomi 2000). However, this study was implemented under normal working farm conditions and thus the performance was not scientifically-based alone. Due to these facts it is essential to focus future research on the demand for PA. Therefore, it is absolutely necessary to test the influence of different rations on duodenal PA flow, as well as on PA concentrations in blood and milk. Considering these points, the following future research objectives are summarized:

1. Simultaneous determination of duodenal PA flow and PA concentrations in blood and milk at different feeding regimes with and without supplementations of PA.

2. Influence of PA supplementation on ruminal fermentation and ruminal PA synthesis at different rations.

3. Effects of varying ration composition on ruminal PA synthesis. Thus, *in vitro* studies would be helpful to establish which bacteria and possibly protozoa need PA and which degrade it.

4. Influence of PA supplementation on duodenal flows of other nutrients.

5. Mechanisms and sites of PA absorption in ruminants.

6. Course of blood PA concentrations during lactation.

7. Transport mechanisms of PA in mammary glands.

8. Surveys to test whether gestating and lactating, gestating and non-lactating, non-gestating and lactating, and non-gestating and non-lactating cows have different PA concentrations in blood and – if applicable – in milk.

9. Effects of ruminally protected PA.

10. PA analyses of feedstuffs.

4.2 Effects of a pantothenic acid supplementation on dairy cows

Introduction

Pantothenic acid belongs to the group of B-vitamins and due to its incorporation into CoA and ACP it is essential for many metabolic reactions (Pietrzik et al. 2008). In general, it was assumed that for cows no supplementation of PA is necessary as PA is ubiquitous and can be synthesised by microorganisms in the rumen (Bechdel et al. 1928; Gesellschaft für Ernährungsphysiologie 2001; National Research Council 2001). Therefore only few studies considering the influence of PA on ruminants exist. The National Research Council (2001) proposed first PA requirements for tissue (304 mg/d) and milk synthesis (121 mg/d) of a 650 kg cow producing 35 kg of 4% fat-corrected milk per day of 425 mg/d, whereby, tissue values had to be extrapolated from lactating sows. This projection showed that the ruminal synthesis of PA (38 mg/d) is more than 10 times lower then the proposed requirements for tissue and milk. Additionally, about 80% of supplemented dietary PA seem to disappear between mouth and duodenum (Zinn et al. 1987). Nevertheless, Bonomi (2000) observed an increase in milk production due to a daily supplementation of 200 mg PA. Between 1944 and 1966 Lardinois et al. (1944), Hollis et al. (1954) and Hayes et al. (1966) established that, by increasing the concentrate proportion of the diet, PA synthesis in the rumen also increases. More recent investigations on this topic are missing. Therefore, the aim of this study was to determine the influence of a PA supplementation to two diets differing in F:C ratio on ruminal fermentation, nutrient flow at the duodenum as well as on blood and milk variables of dairy cows. Furthermore, the duodenal PA flows and PA concentrations in blood and milk were emphasised in this study.

Experimental methods

Treatments, experimental design and animals

Experiments were conducted in observance to the European Community regulations concerning the protection of experimental animals and the guidelines of the Regional Council of Brunswick, Lower Saxony, Germany (File Number 33.11.42502-04-057/07). A total of fifteen German Holstein dairy cows were equipped with large rubber cannulas in the dorsal sac of the rumen (inner diameter 10 cm) and T-shaped cannulas in the proximal duodenum close to the pylorus (inner diameter 2 cm). The study was divided

into two parts. In the first part eight cows received a HC diet which had a F:C ratio of 34:66 on DM basis. In the second part nine cows were fed a HF diet which had a F:C of 66:34 on DM basis. Each cow received a not ruminally protected PA supplementation (a powder with 98% D-calcium pantothenate, Adisseo, Commentry, France) of 1 g/d (diets **HC+PA** and **HF+PA**). The experimental design is shown in Table 20; it is unbalanced due to different calving dates. Table A3 in the Appendix shows the individual cows used in the different groups.

Table 20: Pantothenic acid experimental design (cows/group)

Group	HC		HF	
	Lactating	Dry	Lactating	Dry
Control	6	2	5	4
PA	6	2	5	4

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; PA, pantothenic acid

The composition of the concentrate was the same as in the FA experiment (Table 7). The forage was composed of 60% maize silage and 40% grass silage (on DM basis). The DM content of the silages was determined twice a week to maintain the appropriate F:C ratio and the maize to grass silage ratio. All the cows and bulls of the experimental station of the Friedrich-Loeffler-Institut Brunswick were fed from the same large bunker silos of grass and maize silages. Thus, the silos were changed several times during the experiment. At 05:30 h and 15:30 h forage was offered in two equal portions, the pelleted concentrate was given at 05:30 h, 07:30 h, 15:30 h and 17:30 h. The PA supplement was mixed into an additional 100 g of mineral and vitamin mix (the same as used for concentrate in Table 7) and one half was top dressed on the concentrate by hand at 05:30 h and the other half at 15:30 h. The control animals received 100 g of mineral and vitamin mix without PA. To ensure constant ration composition the amounts of feed were individually adjusted and avoiding refusals if possible. A standardised digestibility trial with adult wether sheep (96.6 (SD 8.6) kg BW; n = 4; Gesellschaft für Ernährungsphysiologie 1991) was conducted to estimate the ME concentrations of the forages. The cows had free access to water and a salt block containing sodium chloride. They were housed in a tethered-stall with neck straps and individual troughs.

At the beginning of the first and the second part of the experiment the cows had mean BW of 617 (SD 24) kg and 604 (SD 87) kg, respectively. Due to animal numbers, both dry and lactating cows had to be used. In the first part (HC) six cows were in lactation (172 (SD 115) DIM at the beginning), while two were dried off. In the second part (HF) five cows were in lactation (188 (SD 66) DIM at the beginning) and four were dried off. Over the whole experiment lactation numbers ranged from two to five. The lactating cows were milked at 05:00 h and 16:00 h.

Sample collection

Every period consisted of two weeks of adaptation to the respective diets, followed by two weeks of sample collection. Milk, blood and ruminal fluid samples were collected in the first sample week. Milk samples were taken on two days of consecutive morning and evening milking. For the analysis of milk composition 50 mL of each milking sample were conserved with bronopol and stored at 8°C until they were analysed. Aliquots of additional 800 mL were pooled per day according to the milk yield, freeze-dried afterwards (Christ Epsilon 1-15; Christ, Osterode, Germany) and stored at -18°C for fatty acid and PA analyses. Milk yields were recorded daily. The frequency of blood samples was kept to a minimum due to animal welfare. Therefore, on one day blood samples were collected from a *vena jugularis externa* just before, three and six hours after, first morning feed to characterise the influence of feeding on blood PA levels. Until centrifugation blood samples were kept at 15°C. One hour after sampling the samples were centrifuged at 2123 x *g* for 30 min at 15°C and the separated serum was kept at -80°C. On another day just before and 30, 60, 90, 120, 180 and 360 min after the first feeding in the morning approximately 100 mL of ruminal fluid were withdrawn from the ventral sac through the rumen fistula using a hand-operated vacuum pump. For duodenal digesta collection four samples of approximately 100 mL duodenal digesta were taken through the duodenal cannula every two hours for five consecutive days in the second sampling week. Directly after sample collection, the pH values were measured with a glass electrode (digital pH measurement device, pH525, WTW, Weilheim, Germany). For each cow the sample with the lowest pH was added to the daily pooled sample and stored at -18°C as described by Rohr et al. (1984). Cr_2O_3 mixed

with wheat flour was used as a flow marker for the calculation of the daily digesta flow. Every 12 h 50 g of the marker was given into the rumen, beginning ten days before the duodenal sampling period, and 25 g every 6 h one day before, and then during, the sampling period. In a comparison of measurements of duodenal flow in dairy cows (Rohr et al. 1984), the spot-sampling procedure used has shown only small differences in flow as compared to the total collection. Samples of forage, concentrate and feed refusals, if occurring, were collected daily during the duodenal sampling week, pooled on a weekly basis, freeze-dried and ground through a 1-mm screen for analysis. Daily duodenal digesta samples were also freeze-dried and ground (1 mm). Faeces of wethers were dried at 60°C and also ground through a 1-mm screen for analysis.

Analyses

DM, Ash, CP, EE, CF and starch were analysed in the feedstuffs and refusals, as well as in the faeces of the wethers according to the methods of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA, 1993) . Acid and neutral detergent fibre were determined after the method of van Soest et al. (1991) and expressed without residual ash. The LUFA Speyer (Germany) analysed the PA content of forages, concentrate and mineral and vitamin mix using a microbiological assay (*Lactobacillus plantarum*; Difco 1998b). Immediately after withdrawal of the ruminal fluid the pH-value was measured (pH525, WTW, Weilheim, Germany). In the ruminal fluid $NH_3 - N$ was analysed according to DIN 38406-E5-2 (1998). Short chain fatty acids were determined using a gas chromatograph (Hewlett Packard 5580, Avondale, PA, USA) equipped with a flame ionization detector as described by Geissler et al. (1976). Freshly thawed duodenal digesta was used to quantify the nitrogen concentration according to the Kjeldahl method. Freeze-dried and ground duodenal digesta was used for the following analyses. For every day of the sampling week the DM and ash contents were determined by the same methods mentioned above for feedstuffs. According to Lebzien and Paul (1997) near infrared spectra was used to estimate the proportion of microbial-N of NAN in the duodenal digesta. Cr_2O_3 was analysed in the marker and duodenal digesta by atomic absorption spectrophotometry as described by Williams et al. (1962) and was used to calculate the duodenal DM flow. The daily

duodenal DM flow was then used as a measure for the preparation of one pooled sample per cow per week in which concentrations of NDF, ADF and PA were determined by the methods named above for the feedstuffs.

Serum samples were analysed for glucose using Hexokinase (A11A00116, ABX Diagnostics, Montpellier, France), NEFA via enzymatic colour test (99475409, Wako Chemicals, Neuss, Germany), β-HBA by an enzymatic UV-test (RB 1008, Randox Laboratories, Krefeld, Germany) and urea also by an enzymatic UV-test (A11A00075, ABX Diagnostics, Montpellier, France) by the laboratory of the Cattle Clinic Hanover (University of Veterinary Medicine, Germany). In serum PA, FA, 5-methyl-THF, THF, thiamin, riboflavin, pyridoxal-5'-phosphate, pyridoxic acid, pyridoxal, pyridoxine, pyridoxamine, nicotinamide and nicotinic acid concentrations were determined via high performance liquid chromatography (W. Bigalke, unpublished method). Sample preparation included protein precipitation with ethanol and fat extraction with n-hexan. After centrifugation (20800 x g) the supernatant was evaporated under a nitrogen stream and the residue was dissolved in the aqueous mobile phase A, filtrated and injected into a high performance liquid chromatography system (Shimadzu, Kyoto, Japan). B-vitamins were separated using a reversed phase C_{18}-column with mobile phase A containing sodium 1-hexanesulfonate monohydrate and phosphoric acid in ultrapure water. Mobile phase B consisted of acetonitrile. Quantifications of B-vitamins were performed simultaneously using a multi wavelength detector and a spectrofluorometric detector.

Fat, protein, lactose and urea were determined in the milk samples using the infrared milk analyser (Milkoscan FT 6000 combined with a Fossomatic 5000, Foss Electric, Hillerød, Denmark). The analysis of milk fat composition was carried out in freeze-dried samples after extraction with chloroform/methanol (2:1, v/v) and transesterification with trimethylsulfoniumhydroxide. A gas chromatograph (Hewlett Packard 6890, Palo Alto, CA, USA) equipped with a flame ionization detector and a capillary column (model Zebron 7HG-G009-11) was used for the separation of the fatty acids from butyric acid ($C_{4:0}$) to linolenic acid ($C_{18:3}$). The milk PA concentrations were determined by the same methods used for the detection of PA in the feedstuffs and duodenal digesta.

Calculations and statistics

According to the equation number 1.1.2 of the Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie 2001) the ME was calculated. Forage digestibility was obtained from the aforementioned wether balance trial, while the tabular values were used for concentrate (Universität Hohenheim – Dokumentationsstelle, 1997) .

The calculation of the daily duodenal DMF was done as follows:

$$DMF\ (kg/d) = \frac{chromium\ application\ (mg/d)}{duodenal\ chromium\ concentration\ (mg/g\ DM)}/1000$$

The daily duodenal flow of OM and OM constituents was estimated by the multiplication of DMF with their respective concentrations in duodenal digesta. Subtracting the PA intake from the amount arriving at the duodenum, the apparent PA synthesis in the reticulo-rumen was calculated. Assuming a mean ammonia-N proportion of total N with N-balanced rations of 4.9% (Riemeier 2004), the daily duodenal flow of NAN was calculated. According to Lebzien and Voigt (1999) the uCP at the duodenum was estimated as follows:

$$uCP\ (g/d) = (NAN\ flow\ at\ the\ duodenum\ (g/d)) \cdot 6.25 - EP\ (g/d)$$

where $EP\ (g/d) = (3.6 \cdot kg\ DMF) \cdot 6.25$ (Brandt and Rohr 1981)

The following equations were used for the calculation of FOM, ruminally degraded CP and UDP:

$$FOM\ (kg/d) = OM\ intake\ (kg/d) - (duodenal\ OM\ flow\ (kg/d) - microbial\ OM\ (kg/d))$$

where $microbial\ OM\ (kg/d) = 11.8 \cdot microbial\ N\ (kg/d)$ (Schafft 1983)

$$Ruminally\ degraded\ CP\ (g/d) =\ CP\ intake\ (g/d) - UDP\ (g/d)$$

$$UDP\ (g/d) = 6.25 \cdot (NAN\ at\ the\ duodenum\ (g/d) - microbial\ N\ (g/d)) - EP\ (g/d)$$

$$RNB\ (g/d) = (CP\ (g/d) - uCP\ (g/d))/6.25$$

Fat corrected milk was estimated to be:

$$Fat\ corrected\ milk\ (kg/d) = ((\%\ milk\ fat \cdot 0.15) + 0.4) \cdot kg\ milk\ yield$$

(Helfferich and Gütte 1972).

The data was analysed using the SAS software package (Version 9.1.3., procedure mixed, SAS Institute Inc., Cary, NC, USA). For statistical analysis of the rumen and serum variables the following model was used. Pantothenic acid supplement was con-

sidered as a fixed effect and a repeated statement for the within-subject factor over all sampling times was used. The OM intake was treated as a fixed regressive component. Furthermore, the fact that a cow had to be used over several periods for different treatments was taken into account by using the random statement for the individual cow effect. To evaluate the variances the restricted maximum likelihood method was applied and degrees of freedom were calculated according to the Kenward-Roger-method. To test the differences between the LSmeans the "PDIFF" option was used and the Tukey-Kramer test was applied for post-hoc analysis. A modified model was used for the analysis of the duodenal and milk variables as it was set up with PA, OM intake and the random statement for the individual cow effect as previously mentioned. For the variables of milk production and composition, the fixed effect lactation stage was additionally implemented in the model. The duodenal PA flow and milk PA concentrations were analysed with the same model, apart from the fixed regressive component OM intake. Differences were considered to be significant at $P \leq 0.05$, and a trend was discussed at $P < 0.10$. For all variables significant levels of the analyses of variances are shown as post-hoc analyses (Tukey-Kramer-Test) showed no significant effects or trends. If not mentioned otherwise, all values presented are LSmeans with SE.

Results

Due to the scope of this study, the effects of the OM intake will not be shown and discussed and influences of time after feeding and interactions of PA and time after feeding are shown and discussed only when they were significant.

Feeding

Over the whole experiment the proposed ratio of F:C was achieved. The two different rations (HC and HF) were neither isonitrogenous nor isocaloric, because the OM intake and feedstuffs used should have been identical as far as possible. Table 21 shows the mean nutrient composition of the two rations. Native PA content of HC and HF rations differed only slightly (HC = 19.1 mg/kg DM and HF = 17.9 mg/kg DM). The nutrition composition of the forage is shown in the Appendix (Table A2). The mean PA and nutrient intake per cow per day is shown in Table 22. It must be considered that with

the HC ration the PA group had a 10% greater OM intake than the control, as the OM intake of two cows in the control group randomly decreased.

Table 21: Arithmetic means (with standard deviation) of nutrient composition (g/kg DM), metabolisable energy (MJ/kg DM) and pantothenic acid contents (mg/kg DM) of the rations

	HC (n = 3)		HF (n = 4)	
	Mean	SD	Mean	SD
OM	936	1.4	931	3.4
CP	185	9.5	142	4.5
EE	26	3.3	32	1.8
CF	115	3.8	166	7.4
ADF	132	3.0	185	2.0
NDF	275	14.8	369	20.6
Starch	372	44.7	292	12.1
ME	12.1	0.0	11.3	0.1
Pantothenic acid[1]	19.1	1.0	17.9	1.0

DM, dry matter; HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; SD, standard deviation; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; ADF, acid detergent fibre; NDF, neutral detergent fibre; ME, metabolisable energy
[1] without supplementation

Rumen fermentation variables

No effects for the ruminal variables occurred at the single measurement times (Tukey-Kramer-test) between the control groups and their respective treatments. Therefore only the LSmeans over the whole sampling time are presented in Table 23. The ammonia concentrations and the pH values were not influenced by PA in either ration. The PA supplementation to the HC ration significantly increased the molar percentage of acetic acid by about 1.2 percentage units, whereas valeric acid tended to be lowered by about 0.3 percentage untis. Additionally, the total concentration of SCFA significantly decreased due to PA (Table 23). During the feeding of the HF ration the PA supplementation significantly decreased the molar proportion of propionic acid ($P = 0.003$).

Table 22: Intakes of nutrients (kg/day) and pantothenic acid (mg/day) by lactating and dry cows fed high concentrate or high forage diets (arithmetic means with their standard deviation)

	HC				HC+PA				HF				HF+PA			
	Lactating (n = 6)		Dry (n = 2)		Lactating (n = 6)		Dry (n = 2)		Lactating (n = 5)		Dry (n = 4)		Lactating (n = 5)		Dry (n = 4)	
Intake	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	13.84	2.00	8.81	2.63	15.82	1.07	8.48	2.13	13.33	2.37	7.25	1.82	13.83	1.1	7.08	1.32
OM	12.90	1.88	8.18	2.47	14.70	0.99	7.84	2.00	12.32	2.22	6.65	1.67	12.79	1.04	6.49	1.24
N	0.43	0.06	0.27	0.08	0.45	0.03	0.24	0.06	0.30	0.05	0.17	0.04	0.31	0.03	0.16	0.03
NDF	3.70	0.54	2.35	0.70	4.49	0.48	2.24	0.57	4.83	0.81	2.60	0.63	4.95	0.40	2.56	0.37
ADF	1.78	0.26	1.13	0.34	2.19	0.18	1.14	0.29	2.45	0.45	1.33	0.34	2.55	0.21	1.29	0.26
Starch	4.99	0.72	3.16	0.96	6.14	1.05	2.74	0.70	3.89	0.69	2.12	0.49	4.06	0.34	2.02	0.42
PA	264	38.3	168	50.2	1302	20.4	1162	40.7	250	35.8	136	43.8	1251	24.2	1156	62.3

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; PA, pantothenic acid; HF, high forage: 66% forage and 34% concentrate on dry matter basis; SD, standard deviation; DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre

Table 23: Influence of pantothenic acid supplementation on ruminal variables averaged over sampling times[1] (LSmeans with their standard error)

	HC					HF				
	Control (n = 8)		PA (n = 8)		P	Control (n = 9)		PA (n = 9)		P
	LSmeans	SE	LSmeans	SE	PA	LSmeans	SE	LSmeans	SE	PA
pH	6.31	0.08	6.39	0.08	0.226	6.43	0.05	6.44	0.05	0.864
NH_3 (mmol/L)	10.22	0.89	10.03	0.89	0.697	9.70	0.32	10.23	0.32	0.131
SCFA (mol %):										
Acetic acid	60.9	0.84	62.1	0.87	0.042	63.5	0.99	63.9	0.99	0.676
Propionic acid	19.0	0.84	18.4	0.84	0.146	18.3	0.58	17.4	0.58	0.003
Isobutyric acid	1.0	0.08	1.0	0.08	0.053	1.0	0.04	1.0	0.04	0.795
Butyric acid	15.1	0.46	14.7	0.46	0.176	14.1	0.52	14.2	0.52	0.843
Isovaleric acid	1.8	0.20	1.8	0.20	0.744	1.8	0.20	2.1	0.20	0.190
Valeric acid	2.3	0.23	1.9	0.23	0.072	1.4	0.07	1.4	0.07	0.838
SCFA (mmol/L)	95.7	3.71	90.1	3.71	0.031	108.7	3.70	106.9	3.70	0.701

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; PA, pantothenic acid; SE, standard error; SCFA, short chain fatty acids

[1] Sampling time: just before and 30, 60, 90, 120, 180, 360 minutes after the first feeding in the morning.

Nutrient flow at the duodenum

The nutrient flows at the duodenum are shown in Tables 24 and 25. For their interpretation it must be considered that for the HC ration the PA group had a 10% greater OM intake than the control (Table 22) due to a randomly decreased OM intake of two cows in the HC group. As the OM intake was a co-variable in the statistical model, the LSmeans for the amount of OM arriving at the duodenum (Table 24) did not differ between HC and HC+PA as the values were adjusted according to the different OM intake. The apparent ruminal digestibility of OM and amount of FOM show that PA had no influence on the ruminal digestibility of OM. Despite the adjustment according to the OM intake a significantly increased ($P = 0.024$) NDF flow at the duodenum was found in the HC+PA group compared to the control. This effect is attributed to a slightly increased NDF content in the HC+PA ration compared to the HC ration at constant apparent ruminal digestibility of NDF. In contrast to NDF, the apparent ruminal digestibility of ADF tended to be increased ($P = 0.070$) by PA. Therefore, in spite of the fact, that ADF in the HC+PA ration was slightly higher, the amount of ADF at the duodenum was the same as compared to the control (HC). Furthermore, at HC ration PA significantly decreased the efficiency of MP synthesis (Table 25), either expressed per kg FOM (-11%) or per MJ ME (-14%), while the absolute flow (g/d) of MP at the duodenum only tended to be decreased ($P = 0.062$; -12%). The RNB of both groups was positive (not shown); hence in each group an adequate N supply of microorganisms was achieved. Thus, the decreased MP synthesis in the HC+PA group could not be caused by an N deficiency. With the HF diet PA supplementation significantly increased the amount (kg/d) of FOM by 4% and the proportion of FOM of OM intake (5%; Table 24). The PA supplementation had no influence on ruminal CP metabolism when cows consumed the HF diet (Table 25).

Table 24: Influence of pantothenic acid supplementation on nutrient flow at the duodenum, ruminally fermented organic matter (FOM) and apparent ruminal digestibility (LSmeans with their standard error)

	HC					HF				
	Control (n = 8)		PA (n = 8)		P	Control (n = 9)		PA (n = 9)		P
	LSmeans	SE	LSmeans	SE	PA	LSmeans	SE	LSmeans	SE	PA
Duodenal flow (kg/d):										
OM	7.48	0.19	7.42	0.19	0.555	6.43	0.17	6.26	0.17	0.221
FOM	7.40	0.20	7.24	0.20	0.433	5.70	0.13	5.94	0.13	0.001
NDF	1.96	0.10	2.11	0.10	0.024	2.16	0.06	2.15	0.06	0.900
ADF	1.05	0.06	1.06	0.06	0.771	1.09	0.03	1.08	0.03	0.681
FOM of OM intake (%)	60.5	1.70	59.9	1.70	0.707	55.3	1.38	58.2	1.38	0.013
Apparent ruminal digestibility (kg/kg intake):										
OM	0.400	0.0195	0.412	0.0195	0.270	0.386	0.0169	0.410	0.0169	0.181
NDF	0.454	0.0292	0.452	0.0292	0.931	0.475	0.0219	0.485	0.0219	0.606
ADF	0.398	0.0359	0.444	0.0359	0.070	0.479	0.0173	0.489	0.0173	0.511

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; PA, pantothenic acid; SE, standard error; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre

Table 25: Nitrogen and pantothenic acid flow at the duodenum, efficiency of microbial protein synthesis and apparent pantothenic acid synthesis (LSmeans with their standard error)

	HC					HF				
	Control (n = 8)		PA (n = 8)		P	Control (n = 9)		PA (n = 9)		P
	LSmeans	SE	LSmeans	SE	PA	LSmeans	SE	LSmeans	SE	PA
MP (g/d)	1367	68.9	1198	69.9	0.062	915	44.0	949	44.0	0.590
MP per FOM (g/kg)	186	8.04	165	8.04	0.013	165	9.44	158	9.44	0.562
MP per ME (g/MJ)	8.7	0.39	7.5	0.39	0.029	7.3	0.36	7.5	0.36	0.730
N (g/d)	362	15.2	327	15.2	0.131	252	9.8	259	9.8	0.603
NAN (g/d)	344	14.5	311	14.5	0.131	239	9.3	246	9.3	0.603
uCP (g/d)	1946	90.8	1740	90.8	0.137	1316	55.8	1367	55.8	0.526
MP per RDP (g/g)	0.69	0.04	0.66	0.04	0.497	0.77	0.05	0.83	0.05	0.396
UDP (g/d)	578	27.8	543	27.8	0.384	400	22.5	418	22.5	0.487
UDP per CP intake (%)	22.1	1.07	22.8	1.07	0.674	24.4	1.60	26.0	1.60	0.433
PA (mg/d)	141.3	28.58	169.7	28.58	0.207	79.3	26.52	198.5	26.52	0.014
AS of PA (mg/d)	-98.8	24.16	-1097.3	24.16	< 0.001	-120.8	24.58	-1005.3	24.58	< 0.001

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; PA, pantothenic acid; SE, standard error; MP, microbial crude protein; FOM, fermented organic matter; ME, metabolisable energy; NAN, non-ammonia-nitrogen; uCP, utilisable crude protein; RDP, ruminally degraded protein; UDP, ruminally undegraded feed protein; CP, crude protein; AS, apparent synthesis

It was only during the feeding of the HF ration that the PA supplementation could significantly increase the duodenal PA flow (Table 25). In all groups the apparent synthesis of PA was negative, leading to the conclusion that no net PA synthesis took place in the rumen (Table 25). In the HC and HF group 59% and 39% of the ingested PA arrived at the duodenum while with PA supplementation only 13% in HC+PA group and 16% in the HF+PA group could reach the duodenum. Across all groups significant relationships between the duodenal PA flow and MP synthesis (r = 0.48; P = 0.010) or OM intake (r = 0.50; P = 0.003) were found (Figures 5A and B).
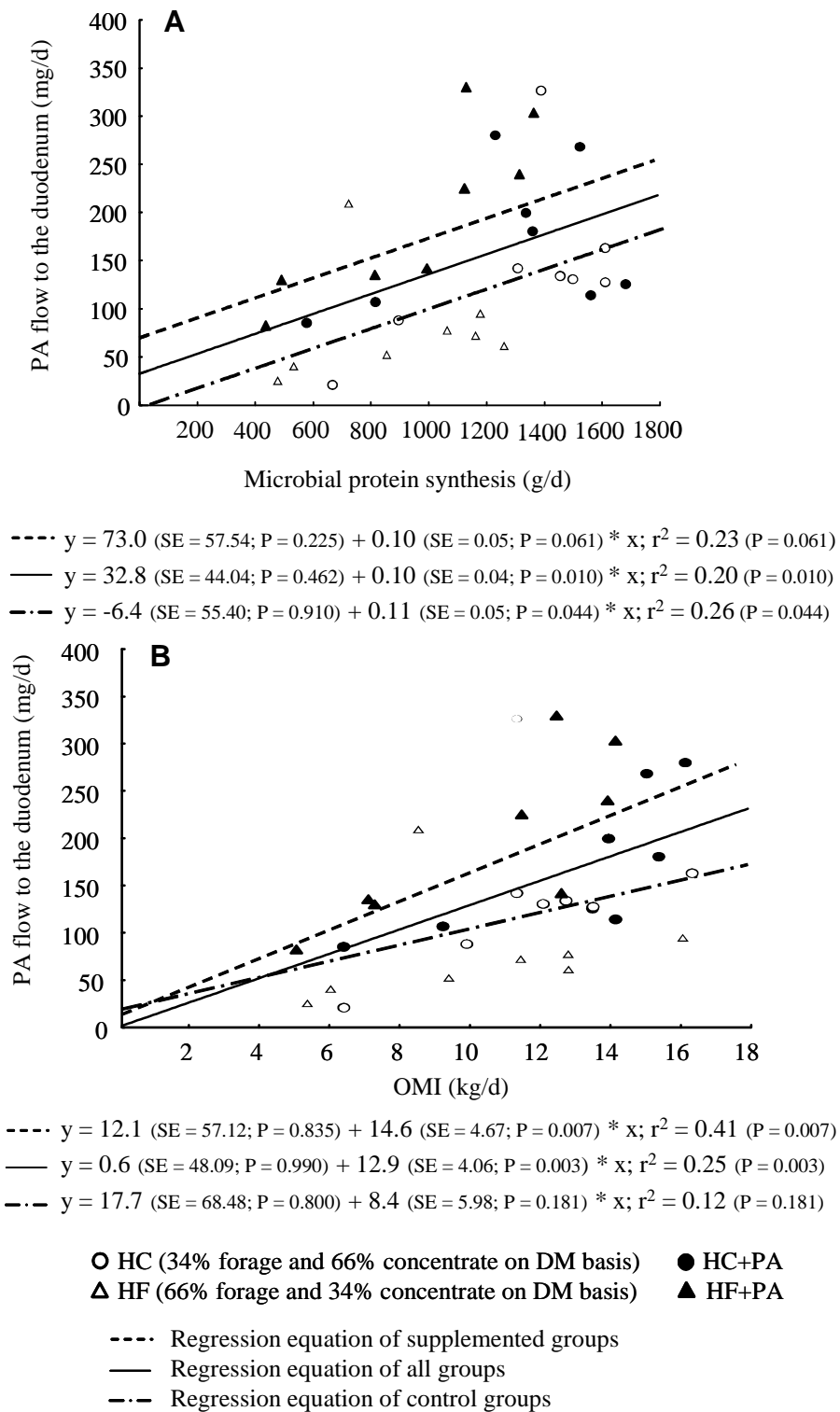
Figure 5: Relation between pantothenic acid (PA) flow to the duodenum and (A) microbial protein synthesis and (B) organic matter intake (OMI)

Serum variables

For the serum variables no significant effects or trends were found at the single measurement times (Tukey-Kramer-Test), thus only the LSmeans over all sampling times are presented in Table 26. Generally, it must be considered that the blood samples were only taken at three time points and thus, are not representative for a whole day. The PA supplementation lowered the serum glucose level when the HC ration was fed, whereas, the PA supplement had no influence on serum variables when cows consumed the HF ration. Furthermore, the serum PA concentrations were not influenced by the PA supplementation for either ration (Table 26) and the serum PA concentrations did not correlate with the duodenal flows of PA. Additionally, no influence of PA on other B-vitamins was observed during the feeding of the HF ration (not shown), whereas supplementing PA to the HC ration resulted in significantly lowered serum riboflavin levels (from 14 to 11 ng/mL). The values of nicotinic acid, pyridoxine, pyridoxamine and thiamin were below the detection limit (W. Bigalke unpublished data).

Milk composition

Apart from few modifications of milk fatty acid composition, i.e. $C_{18:3}$ significantly and $C_{8:0}$ tendentially decreased, whereas $C_{18:0}$ tendentially increased, none of the other milk variables were influenced by supplementing PA to the HC ration (Table 27). During the HF feeding regime the milk protein concentrations were decreased ($P = 0.026$) in cows fed supplementary PA, whereas the milk lactose concentrations were increased ($P = 0.021$; Table 27). Furthermore, $C_{6:0}$ in milk fat significantly increased and $C_{14:0}$ tended to decrease (Table 27). For both rations the supplementation of PA could not increase the milk PA concentrations and yields (Table 27). Due to a higher milk production nearly 78% of PA intake was excreted into the milk with the HC ration, compared to only 44% with the HF ration. With supplementation only 15% of PA with the HC ration and 7% with the HF ration arrived in milk. Furthermore, the milk PA yields did not correlate with the serum PA concentrations and the duodenal PA flows. The ratios between the milk and serum PA concentrations were relatively similar in all groups and ranged from 63.9 in the HF group to 68.8 in the HC+PA group.

Table 26: Effects of dietary supplements of pantothenic acid to dairy cows on serum variables averaged over sampling times[1] (LSmeans with their standard error)

	HC (n = 8)					HF (n = 9)				
	Control		PA		P	Control		PA		P
	LSmeans	SE	LSmeans	SE	PA	LSmeans	SE	LSmeans	SE	PA
Glucose (mmol/L)	3.43	0.08	3.01	0.08	0.003	3.35	0.07	3.41	0.07	0.341
NEFA (μmol/L)	152.0	51.6	116.3	51.6	0.486	87.0	17.0	99.3	17.0	0.153
β-HBA (mmol/L)	0.93	0.24	1.14	0.24	0.510	0.71	0.06	0.69	0.06	0.653
Urea (mmol/L)	5.22	0.30	4.75	0.30	0.117	3.41	0.11	3.53	0.11	0.225
PA (mg/L)	0.096	0.017	0.096	0.017	0.986	0.072	0.008	0.067	0.008	0.627

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; PA, pantothenic acid; SE, standard error; β-HBA, *beta*-hydroxybutyrate

[1] Sampling time: just before and 180 and 360 minutes after the first feeding in the morning.

Table 27: Effects of dietary supplements of pantothenic acid to dairy cows on milk production and composition (LSmeans with their standard error)

	HC (n = 6)					HF (n = 5)				
	Control		PA		P	Control		PA		P
	LSmeans	SE	LSmeans	SE	PA	LSmeans	SE	LSmeans	SE	PA
Milk (kg/d)	26.3	1.44	26.7	1.24	0.842	18.7	0.66	20.3	0.72	0.159
FCM (kg/d)	22.6	1.53	22.9	1.49	0.739	18.6	0.80	20.1	0.87	0.250
Milk composition (%):										
Fat	2.84	0.21	3.09	0.20	0.192	4.92	0.09	3.94	0.10	0.922
Protein	3.02	0.06	2.95	0.06	0.454	3.11	0.04	2.96	0.04	0.026
Lactose	4.56	0.07	4.57	0.06	0.888	4.73	0.11	4.88	0.11	0.021
Yield (g/d):										
Fat	779	75.9	828	74.2	0.302	741	61.3	750	72.3	0.909
Protein	783	21.2	778	20.4	0.782	579	32.3	580	35.3	0.997
Lactose	1201	62.5	1217	55.2	0.839	892	52.8	932	61.5	0.657
Urea (mmol/L)	4.22	0.39	3.80	0.36	0.357	2.41	0.28	2.26	0.31	0.745
Fatty acids (weight % of total fatty acids):										
$C_{4:0}$	6.6	0.25	6.7	0.22	0.777	7.2	0.22	7.4	0.23	0.267
$C_{6:0}$	4.1	0.31	4.0	0.29	0.682	4.4	0.09	4.5	0.09	0.022
$C_{8:0}$	2.0	0.19	1.8	0.19	0.059	1.9	0.04	2.0	0.05	0.681
$C_{10:0}$	4.0	0.48	3.7	0.47	0.341	3.8	0.15	3.9	0.18	0.850
$C_{12:0}$	6.5	0.66	6.2	0.62	0.611	5.5	0.38	5.3	0.41	0.747
$C_{14:0}$	12.0	0.65	12.3	0.62	0.618	12.3	0.17	11.7	0.19	0.055
$C_{14:1}$	1.8	0.29	1.9	0.26	0.816	1.5	0.38	1.6	0.44	0.893
$C_{16:0}$	29.9	1.32	29.9	1.20	0.992	34.8	0.63	33.7	0.75	0.273
$C_{16:1}$	2.5	0.45	2.4	0.43	0.850	2.0	0.22	1.8	0.24	0.464
$C_{18:0}$	3.7	0.41	4.1	0.41	0.056	5.1	0.41	5.4	0.44	0.695
$C_{18:1}$	22.6	1.48	23.1	1.36	0.794	18.4	0.85	20.2	0.95	0.130
$C_{18:2}$	3.9	0.19	3.5	0.18	0.120	2.4	0.07	2.4	0.09	0.956
$C_{18:3}$	0.8	0.04	0.5	0.04	< 0.001	0.6	0.03	0.6	0.03	0.344
PA concentration (mg/L)	6.4	0.68	6.6	0.68	0.784	4.6	0.43	4.5	0.43	0.742
Total PA (mg/d)	186.3	29.96	188.4	29.96	0.939	88.4	8.14	85.2	8.14	0.783

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; PA, pantothenic acid; SE, standard error; FCM, fat corrected milk

Discussion

Studies on the influence of PA on rumen fermentation, duodenal nutrient flow and blood and milk variables are scarce. In many cases, the cited studies are quite old and must therefore be critically considered as analytical methods, feeding regimes and animal performance have continuously developed over the last fifty years (Flachowsky et al. 2002). Furthermore, studies are cited that used blends of B-vitamins. Those blends always included PA, but it must be taken into account that it cannot be ascertained which B-vitamin or B-vitamin interaction caused an effect.

Rumen

Supplementing a blend of B-vitamins (with PA) and vitamin E to male lambs fed on a diet of 61% maizecobs and 39% concentrate and minerals (fibre content, not further specified, of the diet was 28%) had, in accordance with the current study (Table 23), no influence on rumen ammonia or pH, and, moreover no effects on molar percentage and total concentration of SCFA were observed (Perry and Hillier 1969). In an *in vitro* study with a rumen simulation technique and three different rations (F:C ratios: 67:33, 50:50, 33:67) an even lower molar percentage for acetic acid were observed over all rations (Völker 2008). In this study (Völker 2008), the PA supplementation also led to an increase in the number of protozoa. Defaunation results in the decreased molar proportion of acetic acid and an increase of propionic acid (Eugène et al. 2004), thus an increase of protozoa might lead to the opposite effects which would explain the increased molar percentage of acetic acid in the HC+PA group and the decreased molar percentage of propionic acid in HF+PA group (Table 23). Eugène et al. (2004) also showed that defaunation reduces the ruminal concentration of SCFA. However, Santra et al. (2007) found increased concentrations of total SCFA after defaunation. Thus, it would be interesting to consider whether PA may also influence protozoa stains *in vivo*.

Duodenum

Due to the increased FOM (Table 24), more energy for MP synthesis should have been available in the rumen of the HF+PA fed animals compared to the control. But, no effects of PA on the N flow at the duodenum or the efficiency of MP synthesis were observed. However, with the HC ration, where no effect was observed of PA on FOM, PA tended to decrease the flow of MP at the duodenum and decreased the efficiency of MP synthesis (Table 25). Therefore, the higher availability of energy in the rumen with the HF+PA ration may mask a decreasing effect of PA on the MP synthesis. Perhaps this decrease can be explained by an increase of protozoa numbers due to the PA supplementation as in defaunated animals the efficiency of MP synthesis increases (Eugène et al. 2004). No other studies have investigated the influence of a PA supplementation on the nutrient flow at the duodenum, FOM and the efficiency of MP synthesis. Feeding a B-vitamin blend, including PA, Zinn et al. (1987; F:C ratio 45:55) studied the influence on the apparent ruminal digestibility of OM, ADF and N, whereas Perry and Hillier (1969; diet of 61% maize cobs and 39% concentrate and minerals) and Majee et al. (2003; F:C ratio 56:44) considered the influence of PA on the apparent total tract digestion of DM, OM, NDF, CF and CP. These authors could not find any effects.

Serum variables

Studies on the effect of dietary PA supplementation on blood and milk variables of dairy cows are also scarce. To our knowledge only one study by Bonomi (2000) exists; however, this study was conducted on commercial dairy farms and thus, factors potentially affecting response variables were not well controlled. Contrary to our feeding regime, Bonomi (2000) fed no fixed F:C ratio. The cows received 28 kg of forage (fresh matter, consisted of 20 kg maize silage and 7-8 kg lucerne hay; average DM intake 13.8 kg). The amounts of concentrate depended on the milk yield, but were not given. Therefore, this study cannot be reproduced and it is difficult to evaluate the quality of the data. During the first 150 days of lactation Bonomi (2000) found increased plasma glucose concentrations due to PA supplementations of 200 mg/(d x cow), whereas 50 and 100 mg/(d x cow) showed no effect. In this study also ruminally protected

PA in supplementations of 50, 100 and 200 mg/(d x cow) was tested and always resulted in increased plasma glucose concentrations. The results of the current study are different from those of Bonomi (2000), as no influence or decreasing glucose levels were observed; moreover PA supplementation was higher (Table 26). In general it is astounding that such small amounts of PA affected the blood glucose level, as this level is regulated homoeostatically (Stangassinger 2000). However, Wright (1942) reported that PA participates in glucose utilisation, as a supplementation of glucose to fasting rabbits resulted in lower blood PA values. Additionally, Voelkel (1956) noticed an increased glucose utilisation after intramuscular PA injection. This could possibly be an explanation for the decrease of the glucose concentration after supplementing the HC ration with PA. Otherwise, for rats it was established that PA increases the intestinal absorption of glucose by a local action at the brush border of the intestinal cells (Huan and Hung 1972). For ruminants, this fact is questionable, as most of ingested carbohydrates are degraded by ruminal microorganisms and only small amounts of glucose will normally be absorbed from the small intestine. As gluconeogenesis is very important in ruminants (a 700 kg cow with 50 L milk/d requires 3.5 kg glucose daily; Flachowsky et al. 2004), it is interesting that in PA deficient rats the gluconeogenic capacity was reduced (Srinivasan and Belavady 1976). Although, without antagonists a PA deficit will not occur in ruminants, additional PA may increase the gluconeogenic capacity. In agreement to our results (Table 26), a supplementation of a B-vitamin blend with PA (Majee et al. 2003) could not alter the plasma levels of glucose, NEFA and ß-HBA in dairy cows.

In the present study the PA supplementation lowered the blood riboflavin level of the HC+PA group. However, until today, no interactions between riboflavin and PA are known. To explain the observed decrease it has to be established whether PA already affects the duodenal flows of these vitamins and therefore already influences the microorganisms that synthesise or degrade riboflavin or whether the systemic effects of PA are responsible for the effects on this vitamin.

Milk composition

Only Bonomi (2000) studied the influence of 50, 100 and 200 mg unprotected or ruminally protected PA per cow per day on milk composition and found enormous effects with supplementations of 200 mg unprotected and 50, 100 and 200 mg protected PA (milk fat content increased by about 4.4 - 8.5%; milk protein content increased by about 4.2 - 8.5%). These effects could not be confirmed by the results of the present study as no influences on milk fat for both rations and a decrease of milk protein for the HF ration due to PA were observed (Tables 27).

Majee et al. (2003) supplemented two levels of a B-vitamin blend (with PA) to a mixed F:C (56:44) diet and, in accord with the results of the current study with the HF ration, observed an increase in the percentage of lactose and additionally in the yield of lactose when the B-vitamin blend was supplemented at the lower level. However, the higher level of the B-vitamin blend could not change the milk composition or milk component yields. Feeding a ruminally protected blend of B-vitamins at a F:C ratio of 37:63 resulted in increased milk fat yield and greater true protein concentration and yield (Sacadura et al. 2008). For similarly fed mid-lactating cows only the yield of true protein increased and the milk fat percentage decreased. Sacadura et al. (2008) concluded that cows in the early stage of lactation may be more responsive to B-vitamin supplementation due to decreased DM intake relative to milk production and that B-vitamins facilitate an increased efficiency of metabolic functions.

In the current study only a few effects on fatty acid composition in milk were observed (Table 27). May be one explanation for the changes of fatty acids composition during the HC ration could be that PA increased the stepwise saturation of $C_{18:3}$ to $C_{18:0}$, however the processes involved are unknown. Such influences were not found with the HF ration, thus saturation may have occurred in the rumen, as with the HC ration no supplemented PA reached the duodenum. The increase in $C_{6:0}$ with the HF ration due to PA only accounted for 0.1 percentage untis which would have no relevance. Bonomi (2000) could not find any variations in the milk fatty acid composition when PA was supplemented rumen-protected or unprotected.

From the results of the present study it is difficult to decide if influences on metabolism result from changes at the ruminal level, as the influences on both were rather minor,

which may be due to the fact that between 88 to 97% of the supplemented PA disappeared between mouth and duodenum. Therefore, scientifically based studies with ruminally proteceted PA would be interesing. Thus, in future studies it would be helpful to use rumen-unprotected and rumen-protected, as well as a unsupplemented control, to define the site of action.

Pantothenic acid content in duodenal digesta, serum and milk

Until today duodenal PA flows were only measured in growing steers and wethers. When compared with the results of the present study (79 to 141 mg/d; Table 25; 39 to 59% of PA intake) duodenal PA flows of growing steers (11 mg/d; 45% of PA intake; Zinn et al. 1987) and wethers (11 to 40 mg/d; 66 to 424% of PA intake; Finlayson and Seeley 1983) were rather low without PA supplementation, but in relation to PA intake the highest values were found for the wethers. The differences between these studies may result from varying OM and PA intakes and different rations, as well as from general differences between cows, growing steers and wethers. Furthermore, differences possibly arise from varying rates of MP synthesis, because in the present study a correlation between duodenal PA flows and MP synthesis, and in the study of Finlayson and Seeley (1983) between duodenal PA flow and production of microbial DM, was found. Similar to the present study (Table 25), the apparent synthesis (calculated from duodenal folate flows and folate intakes) in the study of Zinn et al. (1987) was always negative, which means that the PA intake was always higher than the duodenal PA flow. For the study of Finlayson and Seeley (1983) positive and negative apparent syntheses were calculated, but positive apparent syntheses were mainly observed with very low PA intakes (0 to 20 mg/d). Zinn et al. (1987) also investigated the effect of 200 and 2000 mg supplemented PA on duodenal PA flows. Supplementations of 2000 mg PA/day increased the duodenal PA flows, as 22% of the supplementation reached the duodenum, while 200 mg did not, as only 3% of the supplementation reached the duodenum. In the present study the influence of PA depended on the rations as, only with the HF ration, was an increase observed (12% of supplementation reached the duodenum), while with the HC ration no increase occurred (only 3% of the PA supplementation arrived at the duodenum; Table 25). However, the control group with

the HF ration had even lower duodenal PA flows than the control group with the HC ration, which probably results from the fact that more PA is synthesised in the rumen with the increasing content of readily available carbohydrates in the diet (Lardinois et al. 1944; Hollis et al. 1954; Hayes et al. 1966; Table 17). Generally, in the study of Zinn et al. (1987) and in the present study substantial amounts of supplemented PA disappear before the duodenal cannula. Disappearance could be caused by degradation or absorption. Until today, ruminal absorption has only been demonstrated for free PA in a washed rumen refilled with a high concentrated B-vitamin solution (105 mg B_1, 85 mg B_2, 983 mg nicotinic acid, 200 mg calcium-pantothenate, 10.4 mg B_{12}; Rérat et al. 1958). Thus, it is not clarified whether absorption is also possible in normal ruminal environments. From studies with rats and rabbits it was established that the main absorption area in the small intestine for PA is the jejunum (Fenstermacher and Rose 1986; Prasad et al. 1999). If this were the same for ruminants an absorption in the proximal duodenum before the duodenal cannula could be ignored, therefore studies with a post ruminal infusion of PA or with ruminal-protected and unprotected PA are needed.

In literature to date, the plasma PA levels for dairy cows differed enormously from 0.00002 mg/L (Dubeski and Owens 1993) to 0.856 to 0.907 mg/L (Bonomi 2000) even though the same analytical method was used (an indirect ELISA assay; Table 18). The serum levels found in the present study differed from these values (0.067 to 0.096 mg/L). It becomes apparent that further studies are required to characterise the blood PA levels of dairy cows. Therefore, analyses of the same blood fraction using the same method would be helpful. From the results of the present study it seems that the PA surplus that arrived at the duodenum due to PA supplementation was not absorbed as serum PA concentrations and milk PA yields were not influenced (Tables 25, 26 and 27). Regardless of PA supplementation, cows fed with the HC ration had considerably higher serum PA levels (about 27%) than cows fed with the HF ration. This may also result from the aforementioned enhanced ruminal PA synthesis during the feeding of high-concentrate diets. In contrast to the present study, after the supplementation of 100 or 200 mg unprotected or 50, 100 or 200 mg rumen-protected PA per cow and day, Bonomi (2000) found an increase in the plasma PA levels (Table 18). In growing

male veal calves 45 mg PA per kg milk replacer resulted in enhanced PA levels of total blood compared to 17 mg PA, which presenting the native PA concentrations of the milk replacer. Whereas no effects were found with concentrations of 21 and 25 mg PA per kg milk replacer (Roth-Maier and Kirchgessner 1973; Table 18).

According to a review by Kirchgessner et al. (1965) and two more recent studies by the same group (Kirchgessner et al. 1991a; 1991b) milk PA concentrations range from 1.7 to 5.5 mg/L, which is similar to the PA values of the present study (4.5 to 6.6 mg PA/L). One previous study (Marsh et al. 1947) tested the influence of a B-vitamin mix, consisted of PA, thiamin, riboflavin and nicotinic acid, on milk PA concentrations in dairy cows and goats. Daily supplementations of each vitamin of 0.5, 2 and even 16 g could not increase milk PA concentrations in dairy cows (Marsh et al. 1947). In goats, daily supplementations of 0.25 g of each vitamin could not increase the milk PA concentrations either; however, 2 g of each vitamin led to an increase. As already reported for duodenal PA flows and serum PA concentrations, milk PA concentrations and yields were higher in both the HC groups than in the HF groups (Table 27) which may also result from the already mentioned increased ruminal PA synthesis with high-concentrate diets.

Conclusion

Regardless of dietary F:C ratio the PA supplementation had only some minor influences on ruminal fermentation. Also the effects on blood and milk variables were only marginal. Apart from the duodenal PA flow with the HF ration, 1 g PA/d could neither increase duodenal PA flow with the HC ration, nor the PA concentrations in blood and milk, as well as milk PA yields with both rations. Thus, from the results of the present experiment a PA supplementation in an unprotected form makes no sense. In the present study, the proposed requirements of the National Research Council (2001) for PA (425 mg/d) could not be achieved at the duodenum, as duodenal PA flows ranged from only 80 to 200 mg/d with the different rations. Thus, it is necessary to study the influence of rumen-protected PA on duodenal PA flows as well as on blood and milk PA concentrations as it seems that dietary PA is extensively degraded in the rumen. However, it is also questionable whether the estimated requirements are correct. For a

better understanding of blood and milk PA concentrations, the absorption of PA from the intestinal tract and the mechanism of PA secretion into the milk must be examined for dairy cows. In this context, it would be interesting to establish whether absorption of PA before the duodenal cannula or in the colon is possible, therefore it would be helpful to use rumen-protected PA.

5   General Discussion

Under consideration of the first proposals for requirements given by National Research Council (2001), it seems that the FA and PA requirements of dairy cows cannot be met by ruminal synthesis (Table 28). However, the proposed requirements for tissue based on lactating sow (175 kg) requirements (National Research Council 1998) adjusted to 650 kg lactating cow weight and the so called milk requirements based to milk folate concentrations adjusted to 35 kg milk production (National Research Council 2001). Studies dealing with the impact of an FA or a PA supplementation on ruminants are scarce and in the available studies only the influences on parts of the metabolism were observed (Chapters 3.1 and 4.1). Additionally, the amount of FA and PA synthesised and degraded in the rumen is influenced by the composition of the ration (Hayes et al. 1966; Schwab et al. 2006). Therefore, the aim of the present study was to investigate the effects of FA or PA supplementation to two different rations on ruminal metabolism, duodenal nutrient flow, as well as on several blood and milk variables of dairy cows. The focus was thereby set on the duodenal flows of FA and PA and their concentrations in blood and milk. Unfortunately after feed, duodenal digesta and milk had already been analysed a closer inspection of the analytical method indicated that *Enterococcus hirae* was not an appropriate species to quantify folates in feed, duodenal digesta and milk. Therefore these variables are not shown and discussed. Accordingly, the cows were fed either with a diet consisting of a 34:66 (high concentrate, HC) or 66:34 (high forage, HF) F:C ratio on DM base. Furthermore, the cows received either 1 g FA or 1 g PA per day or no supplementation.

Regardless of the dietary F:C ratio, the application of each vitamin led to only minor influences on ruminal fermentation, as well as on blood and milk variables (Chapters 3.2 and 4.2). In current literature, the influence of FA and PA on ruminal fermentation and on the metabolism of ruminants has not been studied in detail. The few available studies on FA supplementation accorded with the results of the present study as similarly only minor or no influences were observed (Chiquette et al. 1993; Girard and Matte 1998; Girard et al. 2005; Graulet et al. 2007). The effects of PA on ruminal fermentation have not been studied before and its influences on blood and milk variables only once, in a field study. However, in that study vast effects on metabolism

and performance were observed (Bonomi 2000) which could not be confirmed by other authors and in the present study.

As previously mentioned, the basis for this thesis were the proposed FA and PA requirements of the National Research Council (2001), as it seems from this proposal that these two B-vitamins are the only B-vitamins where the proposed requirements do not seem to be met by ruminal synthesis. Based on the first proposals for requirements given by National Research Council (2001) Table 28 shows the extrapolated requirements for the cows in the current study. In the current study the apparent ruminal synthesis was always negative for PA (Table 28), which illustrates that the apparent ruminal synthesis is not the right variable to assess whether the requirements are met. For this purpose duodenal flows are more suitable.

Table 28: Calculated daily requirements for folic acid and pantothenic acid and duodenal flow and apparent synthesis of pantothenic acid (mg/d)

| | Daily estimated requirement[1] | | | | Duodenal flow[3] | Ruminal synthesis[3] |
	Vitamin	Tissue[2]	Milk	Total		
National Research Council (2001)	FA	33	2	35		7
(650 kg cow, 35 kg FCM)	PA	304	121	425		38
Present experiment:						
HC (619 kg cow, 21 kg FCM)	FA	31	1	32		
HC+FA (619 kg cow, 22 kg FCM)	FA	31	1	32		
HF (610 kg cow, 20 kg FCM)	FA	31	1	32		
HF+FA (610 kg cow, 21 kg FCM)	FA	31	1	32		
HC (617 kg cow, 23 kg FCM)	PA	289	80	369	170	-94
HC+PA (617 kg cow, 23 kg FCM)	PA	289	80	369	194	-1108
HF (604 kg cow, 19 kg FCM)	PA	282	66	348	77	-181
HF+PA (604 kg cow, 20 kg FCM)	PA	282	69	351	275	-977

FCM, 4% fat-corrected milk; FA, folic acid; PA, pantothenic acid; HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis
[1] National Research Council (2001) requirements were based on several sources, while requirements for our study were calculated by dividing the NRC values with the respective weight and milk yield and multiplying this with our cow weights and FCM yields.
[2] Extrapolated from lactating sows.
[3] LSmeans are given; for our experiment, apparent ruminal synthesis were calculated by subtracting intake from duodenal flow.

In accordance with the assumption of the National Research Council (2001), in the current study duodenal flows of PA did not meet the proposed requirements in the control groups (Table 28). On the basis of the measured PA amounts in the milk (Chapter 4.2, Table 27) the so called PA requirements of the National Research Council (2001) seem to be too low, as the PA amounts found in the milk during the present

study (Chapter 4.2, Table 27) were even higher than the proposed requirements (Table 28). Thus the total PA requirements would even increase, which would, on the basis of duodenal PA flows, result in an immense deficiency of PA for dairy cows. Based on the aforementioned facts such a shortage does not seem to be realistic for dairy cows, therefore it is questionable whether duodenal PA flows are the right value to measure the supply of PA on the one hand and on the other hand tissue PA requirements are probably overestimated. Thus, an evaluation of the PA requirements is necessary. The proposed tissue requirements of both vitamins cannot be furhter verified by the results of the present study; however studies to evaluate the tissue requirements of all B-vitamins are imperative as they are based on data from lactating sows. In the present study beneficial effects on the cows due to FA supplementations as found by Girard and Matte (1998) and Girard et al. (2005) were not obvious, but the set-up of this study was not ideal for the investigation of some blood and performance variables, as the focus was put on processes in the rumen and the amount of folates and PA in several bodily fluids. As already established by Santschi et al. (2005a) and Zinn et al. (1987) disappearance between mouth and duodenum might be very high for both vitamins (Chapter 4.2). Therefore, studies with supplementations of ruminally protected FA and PA would be interesting on the one hand, but on the other hand studies on the absorption of both vitamins before the duodenal cannula are also required. However, from the results of the present study it becomes questionable whether supplementations of ruminally protected FA and PA have an impact on dairy cows, as in the current study the increased 5-methyl-THF concentrations in serum had no substantial influence on metabolism and the additional PA which arrived at the duodenum after PA supplementation to the HF diet did not seem to be absorbed. In literature, no studies on the influence of different rations on the PA amount in duodenum, blood and milk are available. From the results of the present study (Chapter 4.2) it can be concluded that more PA arrives in the duodenal digesta, blood and milk with high concentrate diets compared to high forage diets. In the current literature the effects of ration on duodenal folate flows have been proved (Schwab et al. 2006). However, the influences on blood folate concentrations are unconfirmed (Girard et al. 1994; Santschi et al. 2005b).

Summarising the results of the present study and to give answers to the pervious questions (Chapter 2) the following conclusions can be drawn:

- Both vitamins have no or only minor influences on the ruminal metabolism and duodenal nutrient flow, as well as on blood and milk variables.

- It is possible to increase the amounts of folates in the serum by 1 g FA supplementation. However, no beneficial effects on dairy cows are obvious. Therefore, studies with a higher number of non-fistulated cows are recommended.

- The amounts of PA in blood and milk can not be increased by 1 g PA supplementation and the duodenal PA flows only increased during the high forage ration.

- Feeding high concentrate diets results in increased folate concentrations in serum and increased PA amounts in all measured fluids.

- The disappearance of PA between the mouth and duodenum seems to be considerable, thus it must be resolved if an absorption in the rumen is possible, otherwise the application of ruminal protected PA becomes advisable.

6   Summary

Microorganisms in the rumen can synthesise B-vitamins. However, based on the first proposed requirements (National Research Council 2001), folic acid (**FA**) and pantothenic acid (**PA**) seem to be the only B-vitamins for which the ruminal synthesis cannot meet the proposed requirements. Due to the fact that some microorganisms synthesise FA, respectively PA, while others require them it seems that the duodenal flow of each vitamin depends on ration composition.

Therefore, the present experiments investigated the influence of FA and PA on several variables of ruminal fermentation, blood and milk, with a focus on folate and PA amounts reaching the duodenum and milk, as well as the folate and PA concentrations in the blood. These aspects were observed during the feeding of two different rations. To evaluate the influence of FA, respectively PA, German Holstein cows equipped with cannulas in the dorsal sac of the rumen and the proximal duodenum were used. In the first part of the FA-experiment two dry and five lactating cows (186 (standard deviation (**SD**) 144) days in milk (**DIM**) at the beginning) received a diet with 34% forage and 66% concentrate (high concentrate, **HC**), while in the second part a forage to concentrate ratio of 66:34 was fed (high forage, **HF**) to four dry and four lactating cows (165 (SD 57) DIM at the beginning). Furthermore, each cow received 0 or 1 g FA daily. For the examination of the PA influence the same experimental set-up was applied, but the cows received 0 or 1 g PA daily. In the first part of this experiment six cows were in lactation (172 (SD 115) DIM at the beginning) and two were dry, while in the second part five cows lactated (188 (SD 66) DIM at the beginning) and four were dry. Samples of ruminal fluid were withdrawn before and six times after the first morning feeding. Duodenal digesta was colleted over five days in two-hourly intervals. Cr_2O_3 was used as flow marker for duodenal nutrient flow. Blood samples were taken from a *vena jugularis externa* just before as well as three and six hours after the first feeding in the morning. Milk samples were obtained on two days of each period. Milk yields were ascertained daily.

Generally, minor influences of both vitamins on ruminal fermentation and on blood and milk variables were observed. During the feeding of the HC ration FA reduced

the amount of ruminal fermented organic matter. Thus, less energy was available for ruminal microorganisms which resulted in a reduced microbial protein flow at the duodenum. Feeding the HF rations, FA supplementation only increased the apparent ruminal digestibility of acid detergent fibre. With the HF ration FA had no influence on the serum levels of glucose, non-esterified fatty acids, *beta*-hydroxybutyrate, urea, thiamin, riboflavin, pyridoxal-5'-phosphate, pyridoxic acid, pyridoxal, pyridoxine, pyridoxamine, PA, nicotinamide and nicotinic acid. Whereas, supplementing FA to the HC ration lowered the serum glucose and riboflavin levels. Regardless of the dietary forage to concentrate ratio, no effects of FA on milk composition (fat, protein, lactose) were observed. After feed, duodenal digesta and milk had already been analysed a closer inspection of the analytical method indicated that *Enterococcus hirae* was not an appropriate species to quantify folates in feed, duodenal digesta and milk. Therefore these variables are not shown and discussed. The FA supplementation increased the 5-methyl-tetrahyrofolate concentrations in serum.

The PA supplementation slightly increased the molar proportion of acetic acid and lowered the total concentration of short chain fatty acid, as well as the efficiency of the microbial protein synthesis during the feeding of the HC ration. With the HF ration, the PA supplementation reduced the molar proportion of propionic acid and the duodenal starch flow, while the amount of ruminal fermented organic matter increased. Due to the PA supplementation none of the measured blood variables were influenced during the feeding of HF ration, but with the HC ration the glucose and riboflavin levels decreased. Independent of ration, the influences of PA on milk variables were negligible. The PA supplementation only enhanced the duodenal PA flow during the feeding of the HF ration, while with the feeding of the HC ration no increase was observed. Furthermore, the PA supplementation did not influence the serum and milk PA concentrations, or the milk PA yields, regardless of the ration. Apart from the increased duodenal PA flows achieved by supplementing PA to the HF ration, PA amounts or concentrations were always higher in the HC fed animals.

It can be concluded from the results of both experiments that 1 g of each vitamin had only minor influences on ruminal fermentation and metabolism of dairy cows. Furthermore, based on the duondenal flows of PA, the proposed PA requirements were not

met. The PA supplementation in fact only increased duodenal PA flows during the feeding of the HF ration. However, this increase had no effect on the serum or milk PA concentrations. Thus, regarding the results of the present experiment it becomes apparent that the PA supplementations have no influence on the cows. Either PA supplementations were degraded in the rumen or, if PA had been absorbed between mouth and duodenum no influence on the measured variables were observed. By supplementing FA no effects beneficial to the cows in the present experiment became apparent. Therefore, further research is necessary to evaluate the first proposed requirements, as until now they are based on lactating sows and it seems that they are overestimated. Due to the high disappearance rates of PA between mouth and duodenum studies with ruminally protected PA supplementations would be interesting. Furthermore, to prove the influence of each vitamin on performance, studies with a higher number of animals and a more homogeneous stage of lactation are required.

7 ZUSAMMENFASSUNG

Mikroorganismen im Pansen synthetisieren B-Vitamine. Ausgehend von den ersten Bedarfsvorschlägen (National Research Council 2001) scheinen Folsäure (folic acid, **FA**) und Pantothensäure (pantothenic acid, **PA**) dabei die einzigen B-Vitamine zu sein, bei denen die ruminale Synthese den geschätzten Bedarf nicht decken kann. In diesem Zusammenhang muss berücksichtigt werden, dass der Duodenalfluss der betreffenden Vitamine allerdings auch von der Rationszusammensetzung beeinflusst werden könnte. Während bestimmte Mikrooganismen FA bzw. PA synthetisieren, benötigen andere sie für ihren eigenen Stoffwechsel.

Die vorliegenden Untersuchungen hatten zum Ziel, den Einfluss von FA und PA auf verschiedene Fermentations-, Blut- und Milchvariablen zu ermitteln, wobei der Fokus auf die am Duodenum und in der Milch ankommenden Folat- bzw. PA-Mengen, sowie deren Konzentrationen im Blut lag. Getestet wurden die FA-Einflüsse an doppelt fistulierten Kühen der Rasse Deutsche Holstein mit jeweils einer Fistel im dorsalen Pansensack und einer weiteren im proximalen Duodenum. Im ersten Abschnitt der FA-Studie erhielten zwei trockenstehende und fünf laktierende Kühe (186 (Standardabweichung (**SD**) 144) Laktationstage zu Beginn) eine Ration bestehend aus 34 % Grundfutter und 66 % Kraftfutter (high concentrate, **HC**), während im zweiten Abschnitt ein Grundfutter-Kraftfutter Verhältnis von 66:34 (high forage, **HF**) an vier trockenstehende und vier laktierende Kühe (165 (SD 57) Laktationstage zu Beginn) gefüttert wurde. Jede Kuh bekam darüberhinaus entweder 0 oder 1 g FA täglich. Die Untersuchung des Einflusses von PA erfolgte mit demselben Versuchsaufbau, wobei die Tiere entweder 0 oder 1 g PA täglich als Zulage erhielten. Im ersten Abschnitt der PA-Studie waren sechs Kühe (172 (SD 115) Laktationstage zu Beginn) laktierend und zwei trockenstehend, während im zweiten Abschnitt fünf laktierten (188 (SD 66) Laktationstage zu Beginn) und vier trockenstanden. Die Pansensaftentnahme erfolgte direkt vor der ersten Morgenfütterung sowie zu sechs Zeitpunkten danach. Duodenalchymus wurde über fünf Tage in zweistündigen Intervallen gesammelt. Zur Bestimmung des duodenalen Nährstoffflusses wurde als Flussmarker Cr_2O_3 verwendet. Die Blutprobennahme erfolgte aus einer *vena jugularis externa* direkt vor sowie drei und sechs Stunden nach der ersten

Morgenfütterung. An zwei Tagen je Periode wurden Milchproben genommen. Die Bestimmung der Milchmenge erfolgte täglich.
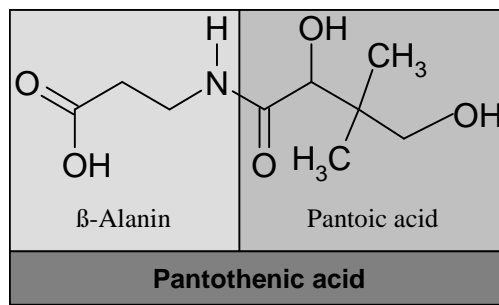
Insgesamt zeigten beide Vitamine einen nur geringen Einfluss auf die ruminale Fermentation sowie auf die Blut- und Milchvariablen. Die FA-Supplementierung zur HC-Ration reduzierte die Menge an ruminal fermentierter organischer Substanz. Dadurch stand den Mikroorganismen im Pansen weniger Energie zur Verfügung, was zu einem verringerten mikrobiellen Proteinfluss am Duodenum führte. Demgegenüber steigerte die FA-Supplementierung zur HF-Ration die scheinbare ruminale Verdaulichkeit der Säure-Detergenzienfaser. Desweiteren hatte bei Einsatz der HF-Ration FA keinen Einfluss auf die Serumkonzentrationen von Glukose, nicht veresterten Fettsäuren, *beta*-Hydroxybutyrat, Harnstoff, Thiamin, Riboflavin, Pyridoxal-5'-Phosphat, Pyridoxinsäure, Pyridoxal, Pyridoxamin, PA, Nicotinamid und Nicotinsäure. Die FA-Supplementierung zur HC-Ration führte hingegen zu einer geringeren Glukose- und Riboflavinkonzentration im Serum. Unabhängig vom Grundfutter-Kraftfutter-Verhältnis konnte kein Einfluss der FA-Zulage auf die Milchzusammensetzung (Fett, Protein, Laktose) nachgewiesen werden. Im Anschluss an die Analyse des Futter, des Duodenalchymus sowie der Milch, zeigte eine genauere Betrachtung das die Spezies *Enterococcus hirae* nicht geeignet ist die Folategehalte im Futter sowie im Duodenalchymus und in der Milch zu bestimmen. Daher werden diese Variablen nicht gezeigt und diskutiert. Die FA-Gabe steigerte die 5-Methyl-Tetrahydrofolat-Konzentration im Serum.

Die PA-Supplementierung steigerte während der Fütterung der HC-Ration geringfügig den molaren Anteil der Essigsäure, wobei die Gesamtkonzentration der kurzkettigen Fettsäuren sowie die Effizienz der mikrobiellen Proteinsynthese reduziert wurden. Bei Einsatz der HF-Ration verringerte die PA-Gabe den molaren Anteil der Propionsäure sowie den duodenalen Stärkefluss, während die Menge an ruminal fermentierter organischer Substanz stieg. Die PA-Supplemetierung zur HF-Ration konnte keine der gemessenen Blutvariablen beeinflussen. Demgegenüber reduzierte die PA-Gabe zur HC-Ration sowohl die Glukose- als auch die Riboflavinkonzentration. Unabhängig von der Rationsgestaltung war der Einfluss von PA auf die Milchvariablen vernachlässigbar. Lediglich beim Einsatz der HF-Ration steigerte die PA-Zulage den duodenalen PA-Fluss, wohingegen bei Fütterung der HC-Ration kein Anstieg festgestellt wurde.

Desweiteren zeigte die PA-Supplementierung bei beiden Rationen keinen Effekt auf die PA-Konzentrationen in Serum und Milch wie auch auf die mit der Milch täglich ausgeschiedenen PA-Mengen. Abgesehen vom gesteigerten Duodenalfluss an PA während der HF-Fütterung zeigte sich, dass die PA-Mengen und Konzentrationen während der Fütterung der HC-Ration vergleichsweise höher waren als während der HF-Fütterung.

Aus den vorliegenden Untersuchungen kann insgesamt geschlossen werden, dass beide Vitamine einen eher geringen Einfluss auf die ruminal Fermentation sowie den Metabolismus der Milchkuh hatten. Basierend auf den duodenalen Flüssen von PA deckte die PA-Supplementierung nicht den geschätzten Bedarf der Milchkuh. Eine Steigerung des duondenalen PA-Flusses lag lediglich bei rauhfutterreicher Fütterung (HF) vor. Diese Steigerung wirkte sich aber nicht auf die Serum- und Milch-PA-Konzentrationen aus. Somit deuten die Ergebnisse dieser Studie darauf hin, dass die PA-Supplementierung keinen Einfluss auf die Kuh hat. Entweder wird die PA-Zulage im Pansen abgebaut oder, sollte die PA-Gabe zwischen Maul und Duodenum absorbiert worden sein, konnten die hier erfassten Variablen keinen Einfluss desselben belegen. Durch die Supplementierung von Folsäure waren anhand der Ergebnisse dieser Studie keine Vorteile für die Kuh ersichtlich. Weitere Studien zur Abschätzung des Bedarfs beider Vitamine sind erforderlich, da die Abschätzungen auf Daten laktierender Sauen basieren und eine Überschätzung vorzuliegen scheint. Aufgrund der hohen Verlustrate von PA zwischen Maul und Duodenum wären somit Studien mit geschützter PA interessant. Zur Überprüfung des Einflusses von FA und PA auf die Leistung von Milchkühen könnten zudem Studien mit einer größeren Tierzahl sowie einem einheitlichem Laktationsstadium hilfreich sein.

8 Appendix

Figure A1: Chemical structur of folic acid

THF = Tetrahydrofolate
DHF = Dihydrofolate
GAR = Glycinamide ribonucleotide
FGAR = Formylglycinamide ribonucleotide
AICAR = Aminoimidazolecarboxamide ribonucleotide
FAICAR = Formylaminoimidazolecaboxamide ribonucleotide

SAM = S-adenosylmethionine
dUMP = Deoxyuridylate
dTMP = Deoxythymidylate

Figure A2: Reactions of the coenzyme tetrahydrofolate (according to Bässler et al. 2002, modified)

Figure A3: Chemical structur of pantothenic acid

Table A1: Individual cows used in the folic acid experiment

Group	HC		HF	
	Lactating	Dry	Lactating	Dry
Control	Elli	Rogate	Inka	Lotte
	Elsa	Fichte	Stella	Wolke
	Emma		Fichte	Elschen
	Urte		Paula	Emma
	Wolke			
FA	Elli	Rogate	Inka	Lotte
	Elsa	Fichte	Stella	Wolke
	Emma		Fichte	Elschen
	Urte		Paula	Emma
	Wolke			

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; FA, folic acid

Table A2: Arithmetic means of nutrient composition (g/kg DM) and metabolisable energy (MJ/kg DM) of the forage (arithmetic means with their ranges)

	Forage[1] (n = 7)	
	Mean	Range
OM	926	921 - 931
CP	130	110 - 175
EE	33	30 - 38
CF	221	209 - 234
ADF	242	232 - 250
NDF	459	435 - 497
Starch	206	121 - 383
ME	10.4	10.3 - 10.6

OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; ADF, acid detergent fibre; NDF, neutral detergent fibre; ME, metabolisable energy
[1] Forage consists of 60% maize silage and 40% grass silage on dry matter basis

Table A3: Individual cows used in the pantothenic acid experiment

Group	HC		HF	
	Lactating	Dry	Lactating	Dry
Control	Elli	Rogate	Inka	Lotte
	Elsa	Fichte	Stella	Wolke
	Emma		Fichte	Elschen
	Urte		Paula	Emma
	Wolke		Reike	
	Ambra			
PA	Elli	Rogate	Inka	Lotte
	Elsa	Fichte	Stella	Wolke
	Emma		Fichte	Elschen
	Urte		Paula	Emma
	Wolke		Reike	
	Ambra			

HC, high concentrate: 34% forage and 66% concentrate on dry matter basis; HF, high forage: 66% forage and 34% concentrate on dry matter basis; PA, pantothenic acid

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