Introduction

Niacin is of great importance in the metabolism due to its incorporation into the coenzymes NAD and NADP[1]. Both forms of niacin, nicotinic acid (NA) and nicotinamide (NAM) can be converted into the coenzymes, although they contain only NAM as a reactive component.

Apart from feed as a source of niacin, nearly all species are able to synthesize the vitamin[1-3] from tryptophan[3] and quinolinate[4]. Since micro-organisms are able to produce niacin as well, ruminants have an additional supply due to their rumen microbes[5]. Ruminal synthesis of niacin was estimated to be 1804 mg/d for a 650 kg cow producing 35 kg of 4% fat-corrected milk/d[6]. This seems to cover the requirement definitely, which was assumed to be 256 mg/d for tissues and 33 mg/d for milk production, thus 289 mg/d in total[6]. Therefore, it was concluded that a general supplementation could not be advised[6,7]. But tissue requirements are estimated based on data from lactating sows and have not been experimentally determined[6]. Furthermore, synthesis might vary, for example, when different feeding regimens are applied[8]. Indeed, numerous studies showed positive responses to a niacin supplementation. On the other hand, a lot of research has been done where administration of niacin did not have any effect. Therefore current literature is reviewed here to distinguish the vitamin's impact on cow performance and metabolism. The aim of this review is to present the state of knowledge on niacin synthesis in the rumen and the amount of niacin arriving at the duodenum, niacin's mode of action on ruminal and several blood parameters as well as its influence on milk production and composition. Where possible, conclusions are drawn from experiments and gaps of knowledge are identified. Cognition of these processes would facilitate a decision on necessity and time of a niacin supplementation.

To our knowledge, the last detailed review available on niacin (NA and NAM) in dairy cow nutrition was done in 1993[9]. Therefore in this review studies newer than 1990 are used to show developments. But in some cases (rumen, duodenum), older literature was included as a comparison with few new results available. Only significant effects ($p < 0{\cdot}05$) and tendencies ($p < 0{\cdot}10$) are mentioned, unless otherwise noted. In all studies, supplemental niacin was not rumen-protected.

Rumen

Niacin in the rumen

In Table 1, niacin concentrations in ruminal contents from several studies are summarised. In interpretation of the results, it has to be kept in mind that different analytical methods for niacin determination exist (for example, colorimetric, microbiological and HPLC methods[10]). This could lead to different results as was proven for cereal-based foods analysed by microbiological and HPLC method[10].

Niacin concentration in the rumen was enhanced if pure NA or NAM were supplemented[11,12], while the highest intake via feed components did not necessarily force the highest concentration in the rumen[8,13]. Santschi *et al.*[8] found no difference in total niacin content in the rumen when comparing rations with a forage-to-concentrate ratio (F:C ratio) of 60:40 or 40:60. However, they noticed an effect on the concentrations of each vitamer. Although no NAM was present in the feed, it was found in the rumen. Furthermore, NAM was significantly increased with the low-forage ration. NA decreased numerically and hence total niacin content was not affected. Earlier work showed an effect of the F:C ratio on ruminal niacin concentrations, which was highest in the all-concentrate ration[14] (data not shown). Thus, there is evidence that ruminal niacin concentrations and/or the concentrations of each vitamer are influenced by niacin supplementation and the F:C ratio.

Some studies have been conducted to measure ruminal synthesis of niacin. Microorganisms use aspartate and dihydroxyacetone phosphate for niacin production[4]. It is extremely difficult to measure real synthesis; therefore apparent synthesis is calculated by subtracting the intake from the amount reaching the duodenum. Some data are given in Table 2. It can be assumed that there is an influence of type of feed. Zinn *et al.*[15] mentioned a stimulating effect of starch on the ruminal synthesis of all B-vitamins. Schwab *et al.*[16] found a significant effect of the non-fibre carbohydrate (NFC) content of feed on niacin synthesis, while the F:C ratio had no effect. But the effect of NFC might also reflect large differences in niacin intake (Table 2). In the above-mentioned studies where an effect of the F:C ratio on ruminal niacin concentrations was found[8,14], duodenal niacin flow was not measured, therefore it was not possible to calculate apparent synthesis to compare these values.

In all studies listed in Table 2, the ration with the highest niacin content within a study resulted in the lowest apparent niacin synthesis. It was stated that there seems to be an optimal concentration. Synthesis will occur below this level and above it, excess niacin

Table 1: Niacin concentrations in the rumen of cattle

Reference	Feeding ration	Niacin content of feed (mg/kg DM)	Niacin intake (mg/d)	Niacin concentration in the rumen	Vitamer	Studied fraction
Riddel *et al.*(1985)[11]	55% wheat straw, 45% concentrate (corn starch, dextrose, soyabean meal)				NA and NAM †	Whole rumen content
	without niacin	6	50	102 – 114 mg/kg DM [a*]		
	with 6 g NA	697	6060	119 – 155 mg/kg DM [b*]		
Abdouli & Schaefer (1986)[13]	27% lucerne hay; 73% barley	64	868	0·48 mg/l fluid + 2·32 mg NAD/l [a]	NA and NAM †	Rumen fluid
	29% lucerne hay 69% oats	19	166	0·32 mg/l fluid + 1·51 mg NAD/l [b]		
Campbell *et al.* (1994)[12]	60% forage (lucerne haylage, corn silage) 40% concentrate (corn, soyabean hulls and meal)					
				0 mg/l fluid [a]	NA	
	without niacin	n. d.	-	0 mg/l fluid	NAM	
	with 12 g NA	n. d.	+ 12000 NA	14 mg/l fluid [b]	NA	Rumen fluid
				0 mg/l fluid	NAM	
	with 12 g NAM	n. d.	+ 12000 NAM	14 mg/l fluid [b]	NA	
				0 mg/l fluid	NAM	
	with 6 g NA and 6 g NAM	n. d.	+ 6000 NA + 6000 NAM	12 mg/l fluid [b]	NA	
				0 mg/l fluid	NAM	
Santschi *et al.* (2005)[8]	60% forage (mixed silage, corn silage), 40% concentrate (corn, soyabean meal)	26	520	143 mg/kg DM	NA	Solid associated bacteria
				77 mg/kg DM [a]	NAM	
	40% forage (mixed silage, corn silage), 60% concentrate (corn, soyabean meal)	23	453	137 mg/kg DM	NA	
				94 mg/kg DM [b]	NAM	
	60% forage (mixed silage, corn silage), 40% concentrate (corn, soyabean meal)	26	520	173 mg/kg DM	NA	Liquid associated bacteria
				86 mg/kg DM [a]	NAM	
	40% forage (mixed silage, corn silage), 60% concentrate (corn, soyabean meal)	23	453	161 mg/kg DM	NA	
				123 mg/kg DM [b]	NAM	
	60% forage (mixed silage, corn silage), 40% concentrate (corn, soyabean meal)	26	520	0·08 mg/l fluid	NA	Particle free fluid
				0·53 mg/l fluid	NAM	
	40% forage (mixed silage, corn silage), 60% concentrate (corn, soyabean meal)	23	453	0·09 mg/l fluid	NA	
				0·62 mg/l fluid	NAM	

NA, nicotinic acid; NAM, nicotinamide; n.d., not determined

[a,b] Values with unequal superscripts within a study differ significantly ($p \leq 0{\cdot}05$)

[*] Depending on different sampling times after feeding (0 to 8 hours), means differed significant at 4 and 6 h after feeding

[†] The vitamin content was determined via microbiological assay, where it is not possible to distinguish between the vitamers

is degraded by the bacteria[17]. This might be the reason why in two studies with cows and feedlot calves where 6 or 2 g NA/d were supplemented[11,15] only 2% and 20%, respectively, of the amount added reached the duodenum. Santschi *et al.*[18] reported a ruminal disappearance rate for niacin of 98·5% as well. The fate of niacin that disappeared from the rumen is not clear. Zinn *et al.*[15] suggested either degradation or absorption. It is not completely clarified if absorption of vitamins could take place in the rumen. Erickson *et al.*[19] found free NAM to be absorbed at 0·98 g/h from a dilution in a washed rumen of cows. NA was not absorbed, because it is ionised under a physiological pH. But usually, most of the niacin is bound in the bacterial fraction[8,19,20]. Therefore, under normal circumstances, no absorption should take place from the rumen[18]. Yet it has to be kept in mind that with niacin supplementation, a high amount of usually free niacin reaches the rumen. Thus, some absorption might occur. However, in the work of Campbell *et al.*[12], supplementation of NAM gave significantly higher duodenal values of niacin than NA. If only NAM is absorbed from the rumen at normal ruminal pH values[19], the opposite would be expected. Consequently, ruminal degradation might be the reason for the high disappearance rate of supplemented niacin from the rumen. Another possible explanation could be that niacin is absorbed in the proximal duodenum, before the duodenal cannula. In man, niacin is absorbable from the stomach as well[21]. To our knowledge, no studies concerning absorption from the abomasum are available.

In summary, niacin concentrations and apparent synthesis in the rumen are affected by niacin supplementation and the ration fed. But it is not known which feed component most influences niacin in the rumen. If niacin is supplemented, only a small part reaches the duodenum. Ruminal absorption might occur, but does not seem to make a large contribution. Ruminal degradation or absorption in the abomasum or before the duodenal cannula seems more likely.

Effect of niacin on rumen metabolism

In contrast to ruminal bacteria it is assumed that protozoa are not able to synthesize niacin and need to cover their requirements from feed or bacterial synthesis[22]. Doreau and Ottou[22] observed no effect of 6 g NA on bacteria, but an increase of protozoa[22]. This especially concerned *Ophryoscolecidae*, but *Isotrichidae* were not affected. Increasing protozoal numbers, especially *Entodinia* (family *Ophryoscolecidae*), may increase

Table 2 : Apparent synthesis of niacin in the rumen of cattle and flow at the duodenum

Reference	Feeding ration	Niacin suppl. (g/d)	DM intake (kg/d)	Niacin intake with feed (mg/d)	Duodenal niacin flow (mg/d)	Apparent synthesis (mg/d)[†]
Riddel *et al.* (1985)[(11)‡§]	55% forage (wheat straw) 45% concentrate (corn starch, dextrose, soyabean meal)	0	8·7	50	85	35
		6 NA	8·7	6060	138*	-5922
Miller *et al.* (1986)[(32)‡§]	12% lucerne meal, 88% corn grain, urea	0	6·7	204[‖]	589	386
	13% lucerne meal, 87% wheat grain	0	7·0	357 [‖]	785	428
	13% lucerne meal, 87% oat grain, urea	0	7·4	163 [‖]	750	586
	13% lucerne meal, 87% barley grain, urea	0	6·5	485 [‖]	664	179
	13% lucerne meal, 87% sorghum grain, urea	0	7·3	295 [‖]	813	518
	11% lucerne meal, 89% corn grain	0	6·2	93 [‖]	557	485
	70% lucerne meal, 30% corn grain	0	6·3	314 [‖]	753	439
Zinn *et al.* (1986)[(15)‡§¶]	45% forage (lucerne hay, Sudan grass) 55% concentrates (corn, molasses, fat)	0	3·4	67	277	210
		0·2	3·4	267	207	-60
		2	3·4	2067	401	-1666
Campbell *et al.* (1994)[(12)††]	60% forage (lucerne haylage, corn silage) 40% concentrate (corn, soyabean hulls and meal)	0	19·9	n. d.	1716 NA 0 NAM	
		12 NA	19·9	> 12000 NA	3187 NA[‡‡] 0 NAM	
		12 NAM	19·9	> 12000 NAM	4902 NA[‡‡] 0 NAM	
		6 NA + 6 NAM	19·9	> 6000 NA + 6000 NAM	3922 NA[‡‡] 0 NAM	
Santschi *et al.* (2005)[(18)]	58% forage (grass-legume silage, corn silage) 42% concentrate (corn, soyabean meal, protein supplement)	0	19·8	465 NA 0 NAM	1334 NA 1242 NAM	912 NA 1259 NAM
	B-vitamin blend infused post-ruminally	1·17 NAM	19·8	465 NA 1173 NAM	1815 NA 1140 NAM	

Table 2 continued

Reference	Feeding ration	Niacin suppl. (g/d)	DM intake (kg/d)	Niacin intake with feed (mg/d)	Duodenal niacin flow (mg/d)	Apparent synthesis (mg/d)[†]
Schwab *et al.* (2006)[16]	35% forage (corn silage, lucerne and grass hay) 65% concentrate (soyabean hulls and meal, beet pulp) in total 30% NFC	0	21·3	620 NA [§§‖‖] 1399 NAM [§§‖‖]	1209 NA 1256 NAM[§§]	589 NA -143 NAM[‖‖]
	35% forage (corn silage, lucerne and grass hay) 65% concentrate (corn, barley, soyabean hulls and meal, beet pulp) total 40% NFC	0	22·2	489 NA [§§‖‖] 838 NAM [§§‖‖]	1504 NA 1370 NAM[§§]	1015 NA 532 NAM[‖‖]
	60% forage (corn silage lucerne and grass hay) 40% concentrate (soyabean hulls and meal, beet pulp, blood meal, fat) total 30% NFC	0	18·1	462 NA [§§‖‖] 727 NAM [§§‖‖]	1016 NA 892 NAM[§§]	555 NA 165 NAM[‖‖]
	60% forage (corn silage lucerne and grass hay) 40% concentrate (corn, barley, soyabean hulls and meal, beet pulp, blood meal, fat) total 40% NFC	0	19·8	363 NA [§§‖‖] 221 NAM [§§‖‖]	1134 NA 837 NAM[§§]	771 NA 615 NAM[‖‖]

DMI, dry matter intake; NA, nicotinic acid; NAM, nicotinamide; n.d., not determined; NFC, non fibre carbohydrates

* Significant differences ($p \leq 0{\cdot}05$) between control and niacin groups. In the paper of Santschi *et al.*[18], level of significance was not declared, furthermore Zinn *et al* [15] and Miller *et al.*[32] did not calculate the apparent synthesis, therefore it was not possible to characterise significances in these studies.

† Apparent synthesis = Duodenal flow minus intake

‡ In these studies apparent ruminal synthesis was not calculated by the authors, but daily intake and duodenal flows were given, therefore apparent synthesis was calculated by us.

§ The vitamin content was determined via microbiological assay, where it is not possible to distinguish between the vitamers.

‖ Level of niacin intake differed significantly ($p \leq 0{\cdot}05$).

¶ In this study, the vitamer applied was not named. It was just stated that niacin was supplemented. But since the term niacin is occasionally also used as synonym for NA[45], it is assumed that NA was fed in this survey.

†† In this study, concentrations per litre duodenal digesta were given, but the authors stated that on average duodenal content had a DM content of 6·65% and a daily DM flow of 16·3 kg. Based on this, values presented here are calculated.

‡‡ Significant differences between control versus niacin and NA versus NAM ($p \leq 0{\cdot}05$).

§§ Significant effects of forage ($p \leq 0{\cdot}05$).

‖‖ Significant effects of NFC ($p \leq 0{\cdot}05$).

bacterial numbers as well, because *Entodinia* are able to regulate the ruminal environment by consuming starch[19]. Others also found a significant increase in total protozoa in the rumen fluid due to niacin feeding[23-25], which was once primarily attributable to increases in numbers of *Entodinia*[25]. Therefore, an effect of niacin on the microbial population is likely, but might be mainly on protozoa.

As a result of this probable effect of niacin on microbial population, ruminal N-metabolism could also be affected. A stimulating effect of niacin on microbial protein synthesis has been observed *in vitro*[26] and *in vivo*[23,24]. In contrast, in some *in vivo* studies no influence was seen on microbial protein production, either on the total amount or on the efficiency[12,15].

Whereas some *in vivo* trials[22,27,28] showed no niacin effect on ammonia concentration in the rumen, other *in vitro*[26] and *in vivo*[23,24] experiments showed a decreasing effect of niacin on rumen NH_3 – N. An interaction of fat and niacin towards increasing ammonia concentrations in the high fat, and decreasing values in the low fat, diet after niacin feeding was also found *in vivo*[27]. It is known that ammonia fixation of the rumen bacteria and fungi occurs largely via NADP- or NAD-linked glutamic dehydrogenase, and possible assimilation of ammonia via NAD^+-dependent glutamic dehydrogenase was also shown for protozoa[29]. This might be favoured by a niacin supplementation.

The fermentation pattern of carbohydrates might also be altered due to a possible niacin effect on microbial population, resulting in a change in volatile fatty acid (VFA) production in the rumen. Results for *in vivo* experiments are presented in Table 3. Butyrate was the VFA which was mostly but inconsistently affected, but there were also influences on acetic and propionic acid; in some surveys, no effect was seen at all. The effect of niacin on butyrate might be induced by the effect on rumen protozoa, since the presence of some protozoa species led to more butyrate produced[30]. This would match with the work of Doreau and Ottou[22], who observed higher protozoal counts and an increase in molar proportion of butyrate. But it is contrary to Samanta *et al.*[24], who observed higher protozoal counts and a decrease in molar proportion of butyrate. Thus, the effect of niacin on protozoa might not be the main reason for its effect on VFA.

In total, the responses of ruminal parameters to niacin feeding vary greatly. Ottou and Doreau[31] concluded that response differences could be due to the level of niacin supplementation, but this was not obvious here, since niacin concentrations varied in an equal range in all studies. Furthermore, Ottou and Doreau[31] listed dietary conditions, diurnal variations in the concentration of rumen protozoa, micronutrients and other

growth factors as an explanation. It must also be kept in mind that measuring ruminal concentrations is dependent on time after feeding, which was not equal for all studies cited. This might explain some of the differences obtained and it cannot be excluded that some of the observed niacin effects are rather due to high diurnal variations in the rumen than a response to niacin.

Table 3: Effect of niacin on ruminal total VFA concentrations and molar proportions of individual VFA in cattle

Reference	Control ration	Niacin / day	Niacin effect
Campbell *et al.* (1994)[12]	60% forage (lucerne haylage, corn silage) 40% concentrate (soyabean hulls and meal, corn)	12 g NA	No effect
	60% forage (lucerne haylage, corn silage) 40% concentrate (soyabean hulls and meal, corn)	12 g NAM	No effect
	60% forage (lucerne haylage, corn silage) 40% concentrate (soyabean hulls and meal, corn)	6 g NA 6 g NAM	No effect
Christen-sen *et al.* (1996)[27]	40% forage (lucerne haylage, corn silage) 60% concentrate (corn, soyabean hulls and meal) total 2·8% fatty acids	12 g NA	C_2 (↓) C_4 ↑ Inter-action with fat
	40% forage (lucerne haylage, corn silage) 60% concentrate (corn, soyabean meal, whole raw soyabeans, tallow) total 5·9 % fatty acids	12 g NA	C_2 (↓)
Doreau & Ottou (1996)[22]	60% forage (corn silage, grass hay) 40% concentrate (soyabean meal, rapeseed meal, urea)	6 g NA	C_4 ↑
Madison-Anderson *et al.* (1997)[28]	50% forage (lucerne hay, corn silage), 50% concentrate (corn, barley, soyabean meal)	12 g NA	No effect
	50% forage (lucerne hay, corn silage), 50% concentrate (corn, barley, extruded soyabeans) 3% of DM as unsaturated fat	12 g NA	No effect
Samanta *et al.* (2000)[24]	Corn, ground nut-cake, wheat bran and straw as forage, amounts were not specified	400 mg NA/kg concentrate	total VFA ↑ C_3 ↑ C_4 ↓
Kumar & Dass (2005)[23]	50% forage (wheat straw) 50% concentrate (soyabean cake, wheat bran, corn)	100 mg NA/kg feed	total VFA ↑
	50% forage (wheat straw) 50% concentrate (soyabean cake, wheat bran, corn)	200 mg NA/kg feed	total VFA ↑

VFA, volatile fatty acids; BW, body weight; NA, nicotinic acid; NAM, nicotinamide; C_2 , acetic acid; C_3 , propionic acid; C_4 , butyric acid; C_5 , valeric acid; iso-C_5 , iso-valeric acid
(↓) tendency,

Duodenum

The amount of niacin reaching the duodenum varies less than does the concentration in the rumen. Duodenal flow values for niacin are given in Table 2. From these data it can be concluded that a niacin supplementation led to higher niacin values reaching the duodenum[11,12,15,18]. But the extent to which this occurs varies and is low. A loss of nia-

cin occurs even when the vitamin is infused into the abomasum[18] but to a lower extent. This indicates abomasal or duodenal absorption before the duodenal cannula. Niacin flow at the duodenum was higher than daily niacin intake after postruminal niacin supplementation, even if the total amount given did not reach the duodenum[18]. This was not the case when niacin was added to the ration[11,12,15]. Therefore, it is likely that an oral niacin supplementation is highly degraded in the rumen and might also suppress niacin synthesis. A higher amount seems to reach the duodenum when it is infused post-ruminally.

The type of feed might modify the amount of niacin reaching the duodenum. Schwab *et al.*[16] found an effect of the F:C ratio. The high-forage ration decreased NAM content in duodenal fluid significantly, and tended to decrease NA content. The NFC content had no effect. Apparent synthesis of niacin in the rumen was affected by NFC, but not by the F:C ratio. This further indicates that the NFC effect on apparent synthesis might be due to different niacin intake, and that the F:C ratio could be important. But more information is lacking.

Even if given post-ruminally, NAM seems to convert to NA. After NAM supplementation only the amount of NA was enhanced at the duodenum, while NAM was even lower than in the control group[18]. The authors concluded that this was due to the acidic environment in the abomasum which may transform NAM to NA. Additionally, supplementation of NAM in feed enhanced the amount of niacin arriving at the duodenum to a higher extent than did NA[12].

Apparent absorption of niacin in the duodenum was not influenced by the type of feed[32] and accounted for 67%[32], 79%[15] and 84% (73% of the NA and 94% of the NAM)[18] of the amount reaching the duodenum. When supplemental niacin was fed, Riddell *et al.*[11] observed a higher amount of niacin reaching the duodenum, but excretion with faeces was equal. Therefore, the authors concluded that absorption in the duodenum must have been higher in the supplemented group. But no measurements were taken in the large intestine, thus results could also be due to a higher degradation or absorption in the large intestine. In other studies, a B-vitamin blend was supplemented, either in the feed or post-ruminally, but did not influence absorption in the duodenum[18].

Little knowledge is available concerning the mechanism of absorption. New research in human subjects suggests that the mechanism for NA absorptions in physiological amounts is dependent on an acidic pH and a specialized Na^+-independent carrier-

mediated system[33]. In higher concentrations, diffusion was observed to be the main mechanism in rats[34]. For NAM, absorption was suggested to occur via diffusion at twice the rate of NA[35], but new research on NAM absorption is not available. Furthermore, it is not known if the same mechanisms take place in ruminants.

Briefly, niacin feeding enhances the amount reaching the duodenum. But not the whole quantity supplemented reaches the duodenum, even after post-ruminal infusion. This provides evidence for abomasal or duodenal absorption before the duodenal cannula. Furthermore, there might be influences of the type of feed and vitamer given. Apparent absorption in the duodenum seems to be high, but the mechanism of absorption has not yet been studied in ruminants.

Blood

Niacin in blood

Data concerning blood niacin concentrations are given in Table 4. Obviously, concentrations vary in a wide range. A reason for this might lie in difficulties of vitamin analysis and / or in different blood fractions examined.

There is disagreement about the existence of NA in blood. Whereas Campbell *et al.*[12] found both vitamers, Kollenkirchen *et al.*[36] stated that only NAM was present in blood of sheep. In two studies, only values for NAM were named[37,38]. It was not stated whether only NAM was found, or if only NAM was analysed. The metabolism of niacin in the body might provide an explanation for this discrepancy. There appears to be no direct conversion of NA to NAM. NA is first converted to NAD, and NAM is then produced from hydrolysis of excess NAD[39]. Part of the NAM formed is reutilised to NAD, but NAM is produced in excess to supply extra-hepatic organs with niacin[40]. Therefore, NAM seems to be the main transport form of niacin in blood[4], although the NA that escaped liver metabolism is also transported to various cell types in the body[41].

The difference in niacin content of the analysed blood fractions between control and niacin-supplemented groups was significant in three studies[37,38,42], but not in the others[12,43,44]. Campbell *et al.*[12] found a significant difference between the vitamers. Addition of NA enhanced both NA and NAM, while feeding NAM had a decreasing impact on blood NA and NAM concentrations. This was not expected, since the NAM-supplemented group had the highest duodenal values of niacin; at this point it is not explainable, why this should result in the lowest niacin content of plasma. For rats, it

Table 4: Niacin concentrations in blood of cattle

Reference	Feeding ration	Niacin supplement (g/d)	DMI (kg / d)	Niacin intake (g/d)	Niacin concentration of blood (µg/ml)	Blood fraction
Driver *et al.* (1990)[38]†	45% forage (lucerne hay and silage), 55% concentrate (ground corn and oats, heat-treated soyabean meal)	0	21·4		0·7 NAM	Plasma
		6	20·1		1·0 NAM*	
	45% forage (lucerne hay and silage), 55% concentrate (ground corn and oats, heat-treated whole soyabeans)	0	19·3		0·6 NAM	
		6	20·4		1·0 NAM*	
Martinez *et al.* (1991)[43]†‡	40% chopped lucerne hay, 60% concentrate (beet pulp, whole cottonseed and –meal, corn, wheat, molasses) total 2% fat	0	23·8		14·3	Whole blood
		12	23·3		17·3	
	40% chopped lucerne hay, 60% concentrate (beet pulp, whole cottonseed and –meal, corn, wheat, molasses, fat) total 4% fat	0	23·6		8·1	
		12	23·2		9·7	
Lanham *et al.* (1992)[44]†‡	40% forage (corn silage, Bermuda grass hay), 60% concentrate (corn, soyabean meal)	0	19·8	0·69	1·1	Plasma
		approx. 6 ‖	16·7	5·14	1·3	
	40% forage (corn silage, Bermuda grass hay), 60% concentrate (corn, soyabean meal, whole cottonseed)	0	17·4	0·56	1·3	
		approx. 6 ‖	17·2	5·23	1·3	
Campbell *et al.* (1994)[12]	60% forage (lucerne haylage, corn silage) 40% concentrate (corn, soyabean hulls and meal)	0	19·9		0·9 NA + 1·2 NAM	Plasma
		12 NA	19·9		1·3 NA¶ + 1·3 NAM	
		12 NAM	19·9		0·6 NA ¶ + 0·9 NAM	
		6 NA + 6 NAM	19·9		1·0 NA + 1·0 NAM	
Ottou *et al.* (1995)[42]‡	79% forage (corn silage, hay) 21% concentrate (beet pulp, wheat, barley, rapeseed meal, soyabean meal, molasses), with niacin infused into the proximal duodenum	0	18·4		0·6	Plasma
		6 NA	19·3		2·5 *	
	77% forage (corn silage, hay) 19% concentrate (rapeseed meal, soyabean meal), 3·5% rapeseed oil infused into the proximal duodenum	0	17·9		0·4	
	3·5% rapeseed oil and niacin infused into the proximal duodenum	6 NA	17·7		2·4 *	