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Post-ruminal or intravenous infusions of carbohydrates or amino acids to dairy cows 1. Early lactation

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The objectives of this study were to compare the effects of post-ruminal and intravenous infusions of wheat starch or glucose (CHO) or a mixture of amino acids (AA) on milk protein yield, nitrogen utilisation, plasma metabolites and mammary extraction rate of dairy cows in early lactation. Eight cow, ruminally fistulated, was assigned to two 4 × 4 Latin squares during 14-day periods, where the last 7 days were for infusions. Infusions were: (1) starch in the abomasum (SP), (2) glucose in the blood (GB), (3) AA in the abomasum (AP), and (4) AA in the blood (AB). The experiment started 54 ± 4 days (mean ± S.E.) post partum (milk yield 33.4 ± 1.7 kg). Daily amounts of nutrients infused were 378, 365, 341, and 333 g for SP, GB, AP and AB, respectively. The cows were fed a basal diet consisting of a concentrate mixture and grass silage (55:45 on dry-matter (DM) basis), and DM intake was 17.2 kg/day. Milk production was affected by site of infusion within substrate, whereas infusion substrates within infusion site (CHO or AA) were of minor importance. Compared with SP infusion, GB infusion increased ($P < 0.05$) milk protein yield and concentration by 55 g and 1 g/kg. The AB infusion tended to ($P < 0.10$) increase milk yield and ECM and increased ($P < 0.05$) protein yield and concentration by 1.8 and 2.2 kg, 83 g and 1.1 g/kg compared with AP infusion, respectively. Nitrogen balance data indicated higher losses of metabolic faecal nitrogen (MFN) by abomasal than by intravenous infusions, and an increased ($P < 0.05$) catabolism for AP and AB infusions compared with SP and GB infusions. GB infusion did not increase ($P > 0.10$) plasma glucose or insulin concentrations above that of SP infusion. Compared with the SP infusion, the GB infusion had minor effect on plasma AA. AP infusion increased ($P < 0.05$) plasma non-essential AA (NEAA) concentration compared with AB infusion, whereas infusion site of AA had no effect ($P > 0.05$) on essential AA (EAA) or branched-chain AA (BCAA). Although a higher milk protein synthesis was observed for AB infusion, the mammary extraction rate was not higher ($P > 0.05$) than for AP infusion. Across infusion site, AP and AB infusions increased plasma concentration of EAA and BCAA, but compared with GB infusion, the mammary extraction rates tended ($P < 0.10$) to be lower. It is concluded that abomasal nutrient infusion increases loss of MFN and that the gastrointestinal metabolism influences the nutrients available for milk synthesis. Our conclusion is that when glucose was infused, AA limited a further milk protein synthesis, but when AA was infused, glucose or energy substrate might have been the limiting factor. Our results verify that glucogenic substrates are limiting when cows are in negative energy balance.

Keywords: amino acid infusion, dairy cows, early lactation, glucose

Introduction

Milk protein response to dietary supplementation has been addressed in numerous reviews (DePeters and Cant, 1992; Chamberlain and Yeo, 2003). The response depends on a number of factors, including stage of lactation, genetic capacity, basal protein and energy status, amount of amino acid (AA) and energy substrates supplied as well as their interactions. Production and infusion experiments that have evaluated these factors have shown variable success in affecting both milk protein production and content. The low and variable response of metabolisable protein supply on milk protein production (Aikman *et al*., 2002; Raggio *et al*., 2004; Schei *et al*., 2005) may be due to the fact that some AA are utilised as an energy source, some AA are used as a glucogenic precursors and also, increased AA supplementation may affect hormones regulating lactogenic metabolism (Reynolds *et al*., 1994). Further improvement in prediction of dietary effects on milk protein production requires a better understanding of factors

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affecting intermediary metabolism of glucogenic substrates and AA. Lately, attention has been given to the gastro-intestinal tissues and the liver metabolism. Their high rate of protein turn-over and other energetically expensive processes may affect the nutrient supply to the mammary gland (Reynolds, 2002). This might imply that AA that could be available for milk protein synthesis is used as energy substrate in the gastro-intestinal tract or in other tissues. Thivierge *et al.* (2002) discussed this by comparing separate experiments of intravenous and abomasal AA infusions and concluded that milk protein yield would be higher with intravenous than with abomasal infusion. However, in their own study when comparing abomasal and jugular AA infusion, only small differences in milk yield and protein responses were observed in cows on restricted feed intake and low protein diets (Thivierge *et al.*, 2002). They concluded that the size of the effect of the gastro-intestinal and liver energy and protein turn-over on nutrient delivery to the mammary gland was still not clear. In a similar study, Aikman *et al.* (2002) found no effect of mesenteric compared with abomasal AA infusion on milk protein response. Therefore, our hypothesis is that compared with intravenous AA infusion, increased intestinal AA supply will only marginally increase the milk protein production and increase the urine nitrogen (N) excretion due to an increased metabolism of these AA in the intestine or the liver.

It has been suggested that increased intestinal starch digestion may enhance milk protein production by sparing AA from being used in the gut and liver (Nocek and Tamminga, 1991). Post-ruminal infusion studies have shown that increased starch supply reduces urinary N excretion and increase N body retention (Volden and Harstad, 2002). Small intestinal starch digestion may also provide glucose for absorption and can increase the glucose supply to the mammary gland directly and therefore reduce liver gluconeogenesis. However, it may also allow greater glucose oxidation by peripheral tissues (Reynolds *et al.*, 2001) and thus reduce glucose delivery to the mammary gland. The effects of intestinal glucose or starch supply on milk protein synthesis have proved to be highly variable (Hurtaud *et al.*, 1998 and 2000; Reynolds *et al.*, 2001) and our understanding of the fate of glucose liberated during starch digestion in the small intestine is rather limiting. As far as we are aware, no direct comparison of post-ruminal starch and intravenous glucose infusions has been done and our hypothesis is that intravenous glucose supplementation will give similar milk protein response as increased intestinal starch supply. Therefore, we want to compare directly, within the same animals, the effect of abomasal starch or intravenous glucose delivery on mammary glucose supply. Moreover, we also hypothesise that when infusing an abomasal AA mixture versus starch the essential AA (EAA) supply to the mammary gland will be higher and thus improve the milk protein production. Cows in negative energy balance have a high need for glucose because they mobilise fat as energy and have limited body reserves of glucogenic compounds. A combination of low energy supply and high metabolisable protein supply has shown to stimulate milk production (Ørskov *et al.*, 1981; Schei *et al.*, 2005). However, it is not clear if this response is solely explained by increased AA supply or that part of the AA has been used as source for glucose and thus improved glucogenic status of the animal. Therefore, the present study was designed to use cows in negative energy balance.

With this background we have performed an experiment where we wanted to evaluate multiple comparisons of starch, glucose or AA infused in the abomasum or intravenously on production responses, N utilisation and plasma metabolites in early lactation. The objectives were to compare the effects of (1) abomasal starch *versus* intravenous glucose infusion, (2) abomasal *versus* intravenous AA infusion, (3) abomasal starch *versus* AA infusion, (4) intravenous glucose *versus* amino acid infusion.

Material and methods

Animals and basal diets

Eight Norwegian Red cows were fitted with ruminal cannulae on average 49 days before expected calving date. The cows were from two different genetic groups and were in their second or third (two cows) lactation. The experiment was conducted in early lactation, starting 54 ± 4 (mean ± S.E.) days *post partum* with a mean daily milk yield of 33.4 ± 1.7 kg. Cows were housed in individual tie stalls and milked twice daily at 0630 and 1530 h. Cows were fed a basal ration consisting of concentrate and grass silage in the ratio 55:45 on dry matter (DM) basis and at a fixed rate of 95% of net energy lactation (NEL) requirements (Ekern, 1991) based on 10-day milk yields prior to the start of the experiment to ensure constant DM intake (DMI). The concentrate mixture consisted mainly of barley and oats (Table 1) and was formulated to have low metabolisable protein content (Table 2). The grass silage was first cut and pre-wilted; it consisted of a mixture of timothy, meadow fescue and red clover, ensiled with a formic acid based additive ('GrasAAT', Hydro Agri, Oslo, Norway). Silage was stored in tower silos until trial start, then packed in plastic bags and frozen until use. The concentrate mixture was fed from parturition, and silage was fed from 2 weeks before

Table 1 *Ingredients of the concentrate mixture (g/kg) used in the basal diet*

Ingredient	g/kg
Barley	564
Oats	200
Soya-bean meal	120
Molasses	50
Rapeseed	30
Limestone powder	14
Vitamins and minerals	22

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Table 2 *Chemical composition (g/kg dry matter (DM)), protein values (g/kg DM), and energy values (MJ/kg DM) of the concentrate mixture and the grass silage*

	Concentrate	Silage
Dry matter (g/kg)	883	228
Crude protein	170	141
Neutral-detergent fibre	204	602
Crude fat	46	42
Starch	440	–
Ash	71	63
RDP[†]	88	–
RUP[‡]	82	–
AAT[§]	105	69
NEL[¶]	7.97	5.73

[†] Rumen degradable protein, calculated at a ruminal outflow rate of 8% per h.
[‡] Rumen undegradable protein, calculated at a ruminal outflow rate of 8% per h.
[§] Amino acids absorbed in the small intestine.
[¶] Net energy lactation (Van Es, 1975 and 1978; Ekern, 1991).

experimental start. One week before the first infusion period, DM feed allowance was reduced to the experimental level. Feed was offered manually three times daily, at 0600, 1400 and 2200 h at equal amounts for the first two offers, but for practical reasons the amount was reduced to half at 2200 h. Feed refusals were removed daily before morning feeding and recorded. Cows had free access to water and trace mineralised salt blocks.

Experimental procedures and treatments

Two restrictedly run 4 × 4 Latin squares were designed. Periods of 14 days were used, where days 1 to 7 served as an adaptation and resting period, and days 8 to 14 were for infusions. Measurements and sample collections were performed from days 12 to 14. The restriction of the design was to avoid two consecutively intravenous treatments on the same animal. Infusions were done in four cows simultaneously. The cows within square were randomly allocated to the first infusion treatment. When the first four cows ended an infusion period and went over to the resting period of 7 days, the other four cows started the infusion period. The treatments were two isoenergetic substrates of starch and glucose and two equal AA mixtures infused into the abomasum or jugular vein. The AA infusion levels were chosen to provide similar metabolisable energy (ME) as the carbohydrate (CHO) infusions. Treatments were: (1) starch in the abomasum (SP), (2) glucose in the blood (GB), (3) AA in the abomasum (AP), and (4) AA in the blood (AB). Pure wheat starch (*Tritici amylum*) and glucosum anhydricum from Norsk Medisinaldepot (Oslo, Norway) were used as starch and glucose supplements. Starch, and not glucose, was infused in the abomasum to simulate a more real feeding since glucose available for absorption enters the intestine as starch. Hydrolysed wheat starch was used because it has close to 100% digestibility in the small intestine. The AA mixture contained 16 L-amino acids from AppliChem (Darmstadt, Germany) and Sigma Chemical (aspargine only; St Louis, MO) with an overall composition similar to the AA profile in duodenal digesta on a grass silage-based diet (Volden, 1999; Table 3). Daily infusion of starch, glucose and AA were 400, 446 and 400 g, respectively, corresponding to 23 g of infusion per kg DMI. The daily substrate infusion was equivalent to 4.68 and 4.01 MJ ME for starch/glucose and AA, respectively (Boisen and Verstegen, 2000). ME values of starch/glucose and AA were 3.51 and 3.01 MJ, respectively. The abomasal infusions of starch and AA were dissolved in 16 and 12 l of tap water, respectively. Starch portions were bulked in a 100-l tank with a mixer for continuous stirring to avoid sedimentation. The AA infused into the abomasum was dissolved in temperate tap (35°C) water to avoid precipitations. Daily solutions of starch and AA were infused using the same peristaltic pump with an infusion rate of 11.1 and 8.3 ml/min, respectively. Total daily volumes of glucose and AA infusions in the jugular vein were 8 l, which provided an infusion rate of 5.6 ml/min. Intravenous glucose and AA solutions were sterilised before use. The following procedures were performed: Batches of seven daily portions of glucose and AA were dissolved in 14 and 35 l of pyrogen-free sterile water, respectively. The solutions were filtered through a vacuum filter, 0.2 μm pore size (Pall Norge AS, Hønefoss), into sterile bottles of concentrated daily portions of 2 and 5 l and then frozen at −18°C until use. Before use, the solutions were thawed and dissolved to 8 l in ultra-purified sterile pyrogen-free water with temperature of about 40°C and pH in the AA solution was adjusted to 7.4 using HCl. Abomasal infusions were done with a tube inserted through the rumen

Table 3 *Amino acid (AA) profile (% of AA) and daily amounts (g/day) of AA infused*

	AA	
	Profile	g/day
Ala	6.3	25.6
Arg	5.2	20.9
Asp[†]	11.0	44.7
Cys	1.6	6.5
Glu	13.9	56.2
Gly	9.2	37.3
His	2.3	9.2
Ile	5.5	22.3
Leu	8.1	33.0
Lys[‡]	9.1	37.0
Met	2.1	8.4
Phe	4.8	19.4
Pro	4.4	17.9
Ser	5.1	20.7
Thr	5.1	20.6
Val	6.2	25.2
Total	100.0	404.9

[†] Aspargine monohydrate.
[‡] Lysine monohydride.

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cannulae and the reticulo-omasal orifice. Prior to the experiment, cobalt EDTA was infused for 5 days to check the possibility of ruminal backflux of solutions, but no increase in cobalt was observed in the rumen. The equipment was checked twice daily to ensure correct placement in the abomasum. The blood infusions were done through the jugular vein, and the tubing was inserted under local anesthesia of 1 ml Lidocain (Adrenalin 5 μg/ml).

Measurements and sample collections

Milk yields were recorded daily and milk samples were taken at each milking. Daily milk samples were bulked in proportion to total volume, and bronopol (2-bromo-2-nitropropan-1,3-diol) was added as a preservative. The samples were stored at 2 to 4°C until analysed for fat, protein and lactose. Milk yields and chemical composition for days 4 to 6 were used as an adaptation period to infusion and 11 to 13 were used to compare effects of infusions. Silage samples were collected at the time the silage was packed in plastic bags and stored frozen. Two composite samples of the silage and one composite sample of the concentrate mixture were analysed for chemical composition. To measure total tract organic matter (OM) digestibility and N balance, total faeces and urine were collected separately for three consecutive days, starting on day 11. From daily amounts of faeces and urine, 10% was stored at −20°C. After finishing each collection period, the 3 days' samples within cow were mixed and 10% of the combined samples were stored frozen (−20°C) until analyses. Sulphuric acid was added to urine samples when necessary to keep pH below 4. Blood samples from the jugular vein, mammary vein and coccygeal vein (10 ml in EDTA tubes) were drawn on days 7 and 14 in each period at 0500, 0800 and 1200 h. For those cows having intravenous infusions, blood samples were taken from the opposite jugular vein of the infusion site. Jugular blood was used for plasma urea and hormone analyses, and blood from the tail vein was used for determination of other plasma metabolites. Blood from the tail vein is considered to represent arterial blood as metabolism in the tail is assumed to be insignificant in relation to whole body metabolism (Vanhatalo *et al.*, 2003b). Samples were immediately put on ice and then centrifuged at 500 **g** for 20 min. Plasma was stored at −80°C until analysis of glucose, non-esterified fatty acids (NEFA), urea, free AA and hormones (insulin, glucagon, IGF-1, GH). For AA analysis, plasma samples were pooled over the daily sampling times to provide one sample per cow. Body temperature was taken twice daily to reveal possible infections at an early stage; no antibiotic was added to the intravenous infusion solutions.

Analytical procedures

Samples of silage and faeces were freeze-dried and ground through a 1.0-mm screen prior to analyses. Content of DM, ash and Kjeldahl-nitrogen (N) in feeds, faeces and urine (N only) were determined according to Association of Official Analytical Chemists (1980). Nitrogen in faeces and urine were analysed in fresh samples. Feeds and faeces were analysed for neutral-detergent fibre (NDF) (Van Soest *et al.*, 1991), crude fat (Volden *et al.*, 1999) and starch (McCleary *et al.*, 1994). NEL in the silage was measured by Infralyser 500 (Bran Luebbe, Germany) calibrated on sheep *in vivo* digestibility data, and NEL and amino acids absorbed in the small intestine (AAT) in the concentrate and silage (AAT only) was calculated from tabulated values after methods developed by Van Es (1975 and 1978), Ekern (1991) and Madsen *et al.* (1995). Nucleic acid bases (NAB) in faeces were determined using HPLC as described by Volden *et al.* (1999). However, in accordance with the findings of Makkar and Becker (1999), the method was further modified by reducing the concentration of $HClO_4$ to 2 mol/l, lowering the hydrolysis temperature to 95°C, and removal of excess $HClO_4$ by precipitation with KOH. Allopurinol was used as internal standard. The sum of all nucleic acid bases (adenine, cytosine, guanine, thymine and uracil) was used in the calculation of the microbial N in faeces. Individual milk samples were analysed for fat, protein and lactose with an infrared milk analyser (MilkoScan, Foss Electric, Hillerød, Denmark). Energy-corrected milk (ECM) and N in milk were calculated from chemical composition according to the method of Sjaunja *et al.* (1990). Plasma NEFA, urea and glucose were analysed on Cobas Mira S spectrophotometer (F. Hoffmann – La Roche & Co, Switzerland). Plasma urea was analysed using enzymatic urease/GLDH, kit no. 07 0385 6, NEFA was measured by enzymatic colorimetric procedure (kit no 994–75 409), and glucose by using hexokinase/kit no A11A 0116. For glucagon analysis, 1 ml plasma was added 70 μl bovine aprotinin (Sigma-Aldrich, Norway) as a preservative. Glucagon was analysed by a radio-immunoassay using a double antibody glucagon procedure (DPC, Los Angeles, USA). Non-competitive time-resolved immunofluorometric assays (TR-IFMA) of the sandwich type was used to determine insulin, BGH and IGF-1 as previously described by Løvendahl and Purup (2002), Løvendahl *et al.* (2003) and Frystyk *et al.* (1995), respectively. The content of AA in milk and free AA in plasma were analysed by ion exchange chromatography using a Biochrom 20 amino acid analyser (Biochrom Ltd, Cambridge, UK). Separation of total AA was achieved on a sodium high-performance column using sodium-based eluents (Oxidised Hydrolysate Chemical Kit, Biochem Ltd), whereas separation of free AA was achieved on a lithium high-performance column using lithium-based effluents (Physiological Fluid Chemical Kit, Biochrom Ltd). Peak detection was performed with UV absorbance at 440 nm and 570 nm after post-column derivatisation with ninhydrin. Data were analysed against appropriate external standards (Sigma Chemical, St Louis, Mo., USA) using the Chromeleon® Chromatography Management Software (Dionex Ltd, Surrey, UK). The milk samples were oxidised with a performic acid/phenol mixture, hydrolysed with 6 mol/l HCl for 23 h, pH adjusted to 2.200, diluted with a 0.2 mol/l sodium citrate loading buffer, pH 2.2 (Biochrom Ltd) and micro-filtrated (0.2 μm Spartan membrane filter,

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Schleicher & Schuell, Dassel, Germany) prior to injection (40 μl). Norleucine was used as an internal standard. Plasma (300 μl) was deproteinised by mixing with 30 μl of 35% sulphosalicylic acid. The mixture was incubated at 4°C for 30 min and centrifuged at 16 000 **g** for 15 min (Biofuge Fresco, Heraeus Instruments, Kendro Laboratory Products GmbH, Hanau, Germany). Of the supernatants, 200 μl were diluted with 200 μl 0.2 mol/l lithium citrate loading buffer, pH 2.2 (Biochrom Ltd) and micro-filtrated (0.2 μm Spartan membrane filter, Schleicher & Schuell) prior to injection (30 μl). S-2-aminoethyl-1-cysteine was used as an internal standard. The samples were analysed immediately or stored in a refrigerator (4°C) for a maximum of 3 days.

Animal care

All cows were cared for according to laws and regulations controlling experiments in live animals in Norway (i.e. the Animal Protection Act of 20 December, 1974, and the Animal Protection Ordinance Concerning Experiments in Animals of 15 January, 1996).

Calculations and statistical analysis

Intake of ME was calculated from total tract digestibility of organic matter using the formulas in the Dutch NEL system (Van Es, 1975 and 1978). Infusions of abomasal substrates were added to the intake when calculating the digestibility, ME intakes, and energy balance but infusions are not included when total DMI is presented. Intravenously infused substrates were also added to the calculated ME values. Gross energy value of 15.6 MJ/kg was used for glucose, and the sum of gross energy values of individual AA in the mixture, calculated to 20.6 MJ/kg, was used for the AA mixture (Boisen and Verstegen, 2000). Animal maintenance requirements were estimated by using individual body weights as described by Van Es (1975). The NEL balance (MJ/day) as a percentage of milk NEL was calculated as: 100 × [(ME intake − ME maintenance) × 0.67 − milk NEL]/milk NEL. Energy efficiency was calculated as: 100 × milk NEL/(ME intake − ME maintenance). Nitrogen balance was calculated as N intake minus N secretion and excretion in milk, urine and faeces. Mammary extraction of plasma metabolites and AA was calculated as arteriovenous (AV) difference divided by arterial concentration. Data used in the statistical analyses were averages from the last 3 days of each treatment period. The effects of treatment on feed intake, digestibility, milk yield, milk chemical production and composition, and N balances were run using the following statistical model with the MIXED procedure of Statistical Analysis Systems Institute (1999):

$$Y_{ijkl} = \mu\alpha_i(\delta_k) + \chi_j + \delta_k + A_l(\delta_k) + \chi_j\delta_k + \varepsilon_{ijkl}$$

where: Y_{ijkl} = dependent variable; μ = overall mean; α_i = fixed effect of infusion period i within genetic group k { $i = 1,..,4$}; χ_j = fixed effect of treatment j {$j = 1,..,4$}; δ_k = fixed effect of genetic group k {$k = 1, 2$}; $A_l(\delta_k)$ = random effect of cow l within genetic group k {$l = 1,..,4$}, $A(\delta_l) \sim N(0, \sigma^2_A)$; $\chi_j\delta_k$ = interaction between treatment and genetic group; ε_{ijkl} = random residual variation, $\epsilon \sim N(0, \sigma^2)$. The experimental variation of period across square was assumed controlled and similar. When data on plasma metabolites and hormones were tested, a fixed effect of sampling time and its interactions with treatment were included in the model. All determinations of glucagon from the first period are missing because of lack of preservatives. The model was tested for residual effects, but no effect was detected, and it was therefore removed from the model. Pearson correlation coefficient based on daily means of individual cow samples from each period was run between plasma urea and urinary N excretion. All results are presented as least square means with standard error of the mean. Pdiff-statement was used to test multiple comparisons between treatment means. In the adjustments of the differences of LSMeans the ADJUST = TUKEY and DIFF = ALL option was used. Differences were considered statistically significant when $P \leq 0.05$, and trends were considered to exist when $0.05 < P \leq 0.10$.

Results

Feed intake and total tract digestibility

Infusion levels, feed intake and total tract apparent digestibility of OM and crude protein (CP) are presented in Table 4. The amount of substrates infused was lower than planned and this will affect the calculated nutrient supply. Daily ME intake was not affected by treatments ($P > 0.10$) but as planned, total CP intake was higher in AP and AB infusions than in SP and GB infusions, on average 331 g higher. Treatments did not differ ($P > 0.10$) in OM digestibility, but the digestibility of CP was 3.8%-units higher ($P < 0.05$) when starch was infused than when other nutrients were infused. Calculated energy balances showed that the cows were underfed by, on average, 15.1 MJ NEL but no difference ($P < 0.05$) was found between treatments.

Milk yield, composition and efficiency

Milk yield, milk composition and milk efficiency are presented in Table 5. In general, the production responses were mostly affected by infusion site (abomasal *v.* blood; measured as individual treatment comparisons), whereas infusion substrates (CHO *v.* AA; measured as individual treatment comparisons) were of minor importance. Compared with the AP infusion, the AB infusion was higher ($P < 0.05$) or tended to ($P < 0.10$) be higher in milk yield, ECM, protein and lactose yields, and protein concentration, with numerical values of 1.8 and 2.2 kg, 83 and 82 g and 1.1 g/kg, respectively. The GB infusion had 55 g and 1 g/kg higher ($P < 0.05$) protein yield and protein concentration than the SP infusion. No differences ($P > 0.05$) were found between GB and AB infusions, or between SP and

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Table 4 *Means of daily intake and digestibility of nutrients by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in early lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Infusion (g)	378[j]	365[jk]	341[kl]	333[l]	9.0	*
Intake (kg dry matter)						
Total	17.1	17.3	17.0	17.3	0.39	
Concentrate	9.6	9.6	9.6	9.6	–	–
Silage	7.5	7.7	7.4	7.7	0.39	
Crude protein[‡]	2.723[k]	2.734[k]	3.037[j]	3.081[j]	0.0570	***
Starch intake[‡]	4.678[j]	4.290[k]	4.300[k]	4.302[k]	0.0085	***
Metabolisable energy (MJ)	170	171	168	172	4.3	
Energy balance[§] (%)	−12.4	−13.2	−15.8	−16.1	4.68	
Digestibility						
Organic matter (%)	68.5	68.1	68.8	67.5	0.75	
Crude protein (%)	66.5[k]	69.4[j]	71.2[j]	70.2[j]	0.95	*

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] SP = starch infused in abomasum; GB = glucose infused in blood; AP = amino acids infused in abomasum; AB = amino acids infused in blood.
[‡] Included infused solutions.
[§] 100 × [(ME intake − ME maintenance) × 0.67 − milk NEL]/milk NEL.

AP infusion except in lactose concentration which tended to differ ($P < 0.10$) between treatments.

N balance

Nitrogen balance data are presented in Table 6. The N intake was in accordance with dietary CP intake and infusion treatments. A numerical, but not significantly ($P > 0.05$) higher total faecal N loss was observed for SP and AP treatments compared with GB and AB treatments, respectively. In percentage of N intake, the faecal N loss was higher ($P < 0.05$) by the SP infusion than for GB, AP and AB infusions, with respectively values of 3.0, 4.8 and 7.0% units. Total faecal N loss was also 4% units higher ($P < 0.05$) for GB infusion than for AB infusion. The AP and AB infusions had, on average, 47 g (7.2% units) higher ($P < 0.05$) urinary N excretions than the SP and GB infusions. The SP infusion had 0.92 mmol/l lower ($P < 0.05$) plasma urea than the other treatments. Based on individual cow measurements, a low correlation was found between plasma urea and excretion of total N ($r = 0.33$, $n = 64$, $P < 0.05$) or N concentration ($r = 0.27$, $n = 64$, $P < 0.05$) in the urine. When N secretion in milk was calculated as a proportion of total N intake, the GB infusion had the highest and AP the lowest N output. The sum of N output in

Table 5 *Means of daily milk yield, milk composition and energy efficiency by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in early lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Yield						
Milk (kg)	30.6	31.5	30.4	32.2	1.50	‖
ECM[‡] (kg)	28.4	29.7	28.3	30.5	1.53	‖
Protein (g)	897[k]	952[j]	897[k]	980[j]	39.3	*
Fat (g)	1096	1152	1098	1192	74.6	
Lactose (g)	1481	1515	1453	1535	66.8	‖
Milk composition (g/kg)						
Protein	29.3[l]	30.3[jk]	29.5[kl]	30.6[j]	0.50	*
Fat	35.5	36.2	36.4	36.9	1.26	
Lactose	48.2	48.1	47.8	47.7	0.59	‖
Milk energy (MJ)	88.8	92.9	88.4	95.3	4.79	‖
Energy efficiency[§] (%)	79.4	80.2	81.0	82.5	4.31	

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] SP = starch infused in abomasum; GB = glucose infused in blood; AP = amino acids infused in abomasum; AB = amino acids infused in blood.
[‡] Energy corrected milk.
[§] 100 × milk energy/(ME intake − ME maintenance).
[‖] Approaching significance ($P < 0.10$).

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Table 6 *Means of daily nitrogen (N) intake and utilisation, plasma urea and faecal nucleic acid bases by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in early lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
N intake (g)	436[k]	437[k]	486[j]	493[j]	9.1	***
N output (g)						
Faeces	146[j]	134[jk]	140[jk]	131[k]	4.2	**
Urine	95[k]	103[k]	143[j]	149[j]	5.1	***
Milk	141[k]	149[j]	141[k]	154[j]	6.2	*
Balance	52	53	56	58	7.5	
Plasma urea (mmol/l)	3.96[k]	4.85[j]	4.64[j]	5.16[j]	0.23	**
N output (% of intake)						
Faeces	33.6[j]	30.6[k]	28.8[kl]	26.6[l]	0.94	**
Urine	21.7[k]	23.8[k]	29.4[j]	30.4[j]	1.33	***
Milk	32.4[k]	34.0[j]	29.0[l]	31.1[k]	1.20	***
Balance[‡]	12.0	11.8	11.4	11.6	1.53	
NAB[§] (% of N)	6.30	6.32	6.07	6.54	0.23	

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] SP = starch infused in abomasum; GB = glucose infused in blood; AP = amino acids infused in abomasum; AB = amino acids infused in blood.
[‡] Calculated as: balance = intake -milk-faeces-urine.
[§] Nucleic acid bases.

milk, urine and faeces was similar for all treatments with an average of 88.3%. No difference ($P > 0.10$) between treatments was found in faecal NAB excretion, which indicates similar microbial hind-gut fermentation.

Plasma metabolites

Plasma metabolites, hormones and extraction rates of plasma metabolites are presented in Tables 7, 8 and 9. Plasma glucose and extraction rates of glucose in the mammary gland were not affected ($P > 0.1$) by treatments (Table 7). Infusion of glucose into the blood by 365 g daily did not increase ($P > 0.05$) plasma glucose or insulin concentrations above that of the other treatments, although glucose concentration by the GB tended ($P < 0.1$) to be higher than the SP and AB treatments. Higher ($P < 0.05$) arterial NEFA concentration was observed in the AB treatment, and the extraction rate of NEFA was positive for this treatment, but negative for the other treatments. Plasma glucagon concentration was lower ($P < 0.05$) for GB infusion and IGF-1 was higher ($P < 0.05$) by intravenous infusions (both GB and AB) than with abomasal infusions (SP and AP). The plasma concentration of total AA (TAA) with AP infusion tended to be ($P < 0.10$) higher than with the other treatments (Table 8). Treatments AP and AB had higher ($P < 0.05$) plasma concentrations of EAA and branched-chain amino acids (BCAA) than the SP and GB

Table 7 *Plasma metabolites and mammary extractions of metabolites by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in early lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Glucose (mmol/l)						
Arterial	3.28	3.42	3.37	3.28	0.074	
Extraction (%)	22.9	24.6	26.5	26.2	2.25	
NEFA[‡] (mmol/l)						
Arterial	0.185[k]	0.170[k]	0.190[k]	0.222[j]	0.013	**
Extraction (%)	−2.9[j]	−11.8[k]	−5.0[jk]	2.5[j]	3.90	**
Hormones						
Insulin (pmol/l)	71.1	89.8	77.1	75.5	11.4	
Glucagon (ng/ml)	56.3[j]	48.4[k]	56.1[j]	60.7[j]	3.52	**
IGF-1 (ng/ml)	63.6[l]	93.0[j]	71.8[k]	89.2[j]	9.67	***
BGH (ng/ml)	2.25	1.99	2.31	2.08	0.53	

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] SP = starch infused in abomasum; GB = glucose infused in blood; AP = amino acids infused in abomasum; AB = amino acids infused in blood.
[‡] Non esterified fatty acids.

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Table 8 *Mean arterial plasma amino acid concentrations (μmol/l) by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in early lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Essential						
Arg	78.8	93.2	110.3	70.9	11.94	[§]
His	57.7	62.9	74.2	77.9	7.68	
Ile	177	163	193	212	15.1	
Leu	150[k]	156[k]	201[jk]	232[j]	17.8	*
Lys	112	103	127	110	10.0	
Met	21.4	24.6	24.0	19.5	2.94	
Phe	56.6	57.9	64.1	69.1	3.94	
Thr	149[j]	134[jk]	159[j]	111[k]	10.6	*
Val	338[k]	315[k]	439[j]	449[j]	25.0	**
Non-essential						
Ala	297[j]	291[j]	285[j]	223[k]	17.9	*
Asn	75.9	66.2	95.1	72.8	8.35	
Asp	11.4	13.9	11.5	9.3	2.35	
Cys	24.9[k]	19.1[l]	23.6[kl]	30.4[j]	1.91	**
Glu	72.2	83.1	74.6	58.7	6.10	[§]
Gln	342[k]	422[j]	372[jk]	317[k]	20.4	*
Gly	385	388	431	352	20.5	
Pro	119[k]	95[k]	149[j]	116[k]	10.1	*
Ser	128[j]	121[j]	136[j]	98[k]	7.1	*
Tyr	58.6	53.2	50.1	46.7	4.31	
Orn	56.8[k]	52.8[k]	70.2[j]	55.6[k]	4.13	**
Cit	93	102	111	105	8.0	
EAA[‡]	1140[kl]	1110[l]	1392[j]	1351[jk]	70	*
NEAA[‡]	1513[j]	1553[j]	1628[j]	1289[k]	56	**
BCAA[‡]	664[k]	634[k]	833[j]	893[j]	54	*
TAA[‡]	2653	2662	3020	2640	100	[§]

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] SP = starch infused in abomasum; GB = glucose infused in blood; AP = amino acids infused in abomasum; AB = amino acids infused in blood.
[‡] EAA = essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val); NEAA = non-essential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr); BCAA = branched-chain AA (Ile, Leu, and Val); TAA = EAA + NEAA.
[§] Approaching significance ($P < 0.10$).

Table 9 *Mean extraction rate (%) of plasma amino acid by the mammary gland by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in early lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Essential						
Arg	6.1	67.4	54.4	40.5	16.5	[§]
His	46.6	52.7	25.9	35.2	6.66	[§]
Ile	24.3	38.8	21.1	18.4	7.65	
Leu	34.5	55.2	40.5	32.4	9.42	
Lys	44.0	55.4	41.5	43.2	7.98	
Met	30.4	68.0	32.1	54.7	18.1	
Phe	38.8[jk]	53.8[j]	44.3[jk]	28.8[k]	5.28	*
Thr	24.1	25.4	23.2	10.4	7.88	
Val	20.3	22.4	15.5	8.8	4.92	
Non-essential						
Ala	9.2[j]	13.4[j]	1.6[jk]	−6.4[k]	4.39	*
Asn	31.0	15.0	15.4	6.7	8.34	
Asp	42.8	19.0	22.4	11.8	12.2	
Cys	−7.8	−32.9	−12.2	4.3	16.9	
Glu	65.8	71.5	69.7	67.2	5.56	
Gln	25.6[jk]	33.3[j]	23.3[jk]	14.3[k]	3.98	*
Gly	0.5	4.6	1.2	−3.4	3.39	
Pro	−4.2	−10.0	0.6	1.5	9.10	
Ser	13.7	20.5	15.8	1.5	8.29	
Tyr	42.3	37.6	53.2	41.6	8.01	
Orn	43.3	52.0	44.9	43.4	5.31	
Cit	5.3	−6.6	−3.7	−2.4	9.34	
EAA[‡]	27.9	41.5	29.2	22.5	5.27	[§]
NEAA[‡]	15.8[j]	19.7[j]	14.5[jk]	5.3[k]	3.22	*
BCAA[‡]	25.5	35.3	22.4	16.4	6.24	
TAA[‡]	21.5	28.9	21.4	14.0	3.91	[§]

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] SP = starch infused in abomasum; GB = glucose infused in blood; AP = amino acids infused in abomasum; AB = amino acids infused in blood.
[‡] EAA = essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val); NEAA = non-essential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr); BCAA = branched-chain AA (Ile, Leu, and Val); TAA = EAA + NEAA.
[§] Approaching significance ($P < 0.10$).

infusions, and the concentration of non-essential AA (NEAA) was lower ($P < 0.05$) for AB infusion than with the other treatments. Of the EAA, Leu was higher on the AB treatment whereas Val was higher by both AP and AB treatments than by the SP and GB treatments. The AB infusion had lower plasma Thr ($P < 0.05$) and Arg ($P < 0.10$) concentrations than the AP treatment. Of the NEAA, plasma concentrations of Ala and Ser were lower ($P < 0.05$) and Cys was higher ($P < 0.05$) for AB infusion than by the other treatments. Moreover, the AP treatment had lower ($P < 0.05$) Pro and Orn, whereas Gln concentration was higher ($P < 0.05$) for GB infusion. The extraction rates differed ($P < 0.05$) between treatments in NEAA and tended to ($P < 0.10$) differ in TAA and EAA concentrations (Table 9). Highest extraction rate was found on the GB treatment and lowest on the AB treatment. The extraction rate of EAA for the GB infusion was 49, 42 and 84% higher than for the SP, AP and AB infusions, respectively.

Discussion

Planning of this experiment was done on the basis of abomasal nutrient flow and what can be achieved in practical diets. Hydrolysed wheat starch was chosen because it has a high intestinal digestibility, whereas the profile of the AA mixture was similar to what has been observed with grass silage-based diets. Choosing an AA profile other than casein that is more realistic to a practical feeding situation is important (Chamberlain and Yeo, 2003). DePeters and Cant (1992) and Schei *et al.* (2005) suggested that milk responses to additional nutrient supply depend on animal energy and nutrient balances. The production experiment of Schei *et al.* (2005) showed that increased metabolisable protein supply, when cows were in negative energy balance, increased milk protein production. However, it was not clear if the positive response was explained by solely an increased AA supply or a combination with increased

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glucose supply from increased gluconeogenesis. Therefore, the present experiment was designed to compare increased AA and glucose supplementation to cows in negative energy balance. The infusion periods lasted for 6 days and thus the duration should be suitable for achieving milk production responses since infusion responses largely occur within 1 to 3 days (Metcalf *et al*., 1996; Reynolds *et al*., 1994). Moreover, Knowlton *et al.* (1998) showed by summarising literature data that short-term (<1 week) infusion of glucose increased milk yield, whereas longer infusion indicated no prolonged responses. Using periods of 14 days, of which the first 7 days were with no infusion and the next 3 days were adapting to the infused nutrients before the start of treatment measurement, was sufficient to avoid possible carry-over effects from previous treatment. The restriction of the order of the treatments; avoiding two subsequent intravenous infusions, were done across substrates so there should be no systematic order of the substrates. Several studies have demonstrated a positive effect on milk production of infusing nutrients into the gastrointestinal tract or blood (Metcalf *et al*., 1996; Hurtaud *et al*., 2000; Reynolds *et al*., 2001; Aikman *et al*., 2002). However, limited data are available comparing both post-ruminal and intravenous AA and glucose supplementation in the same experiment and animals.

Comparison of infusion sites within substrate type

Changing infusion site from abomasal (SP and AP) to intravenous administration (GB and AB) gave higher daily milk yield (1.4 kg), milk protein yield (69 g) and protein content (1.1 g/kg). These results indicate that increased supply of glucose and AA in the blood increased the nutrient supply to the mammary gland and that the supply of glucose and AA limited milk production. Therefore, our results suggest that starch and AA supplied via the abomasum did not reach the mammary gland although it clearly affected intermediary metabolism shown by differences in plasma urea concentrations, urinary N excretion and plasma AA concentrations.

Abomasal starch versus intravenous glucose infusions. No problem with soft faeces and diarrhoea were observed during starch infusions, and the dosage used here was lower than in other post-ruminal glucose or starch infusion studies (Hurtaud *et al*., 1998 and 2000; Reynolds *et al*., 2001). In a typical Norwegian dairy cow diet, which mainly consists of grass silage and a barley/oats based concentrate mixture when cows are fed 14 to 18 kg DM, abomasal infusion of 257 to 376 g of starch will increase the post-ruminal supply by approximately 100% (Volden, 1999). The total tract digestibility of starch was close to 100%, but despite small amounts of starch infused it can not be excluded that the small intestinal digestibility was somewhat lower. However, no indication of higher microbial activity, measured as NAB excretion, was observed. This does not totally exclude faecal microbial growth differences between treatments since NAB measured in faeces could also be remnants of ruminal synthesis. But since cows were fed the same amount of feed, differences in ruminal microbial protein synthesis among infusion treatments is not expected. Starch has to undergo enzymatic activity for hydrolysis which also requires energy for enzyme synthesis and export to the lumen, and this might have reduced the availability of AA and energy compared with glucose infused into blood. The SP infusion resulted in lower apparent digestibility of CP than did GB infusion. In addition to hind gut fermentation this difference could also be explained by a higher loss of endogenous protein due to increased intestinal digesta flow with abomasal infusion. Thomsen (1979) reviewed data on true protein digestibility and excretion of metabolic faecal nitrogen (MFN) in ruminants. The reported values of 84 to 96% true digestibility and 4.3 to 7.2 g MFN per kg DMI. Raggio *et al.* (2004) measured N balances in cows fed increasing amounts of dietary protein, and from their data the excretion of MFN can be estimated to 7.3 to 7.8 g/kg DMI assuming that 90% of dietary protein is truly digested at the faecal level. Using this assumption for the present study, the loss of MFN with starch infusion is calculated to 6.0 g/kg DMI. The corresponding figure with intravenous glucose infusion is 5.2 g/kg DMI. Shifting N excretion from urine to faeces would not decrease AA availability per se, whereas an increased endogenous protein loss would. The higher AA availability through glucose blood infusion gave higher milk protein yield and protein concentration. However, the slightly higher feed intake on GB treatment would also contribute to a numerical higher milk yield. Higher AA availability on blood infusion numerically tended to increase urinary N loss and increased plasma urea level. The intention of infusing CHO substrate was to increase the glucogenic status and spare AA for use as glucose or energy substrates and thereby increase the availability of AA for protein synthesis in the mammary gland. Moreover, this will increase the arterial concentrations of glucogenic AA (Lemosquet *et al*., 2004). Substrates infused into the abomasum have to undergo digestion and absorption and pass the portal-drained viscera (PDV) and the liver before entering the peripheral blood. These processes may reduce the amount available for milk protein synthesis compared with intravenous infusion since these organs have high energy requirements (Reynolds, 2002). No differences were found in plasma EAA, NEAA, BCAA and TAA between SP and GB infusions. Decreased plasma BCAA and EAA concentrations have been found in some trials with glucose infusion (Kim *et al*., 2001; Huhtanen *et al*., 2002) but not always (Kim *et al*., 2000; Rulquin *et al*., 2004). The variable effect is probably related to the response in milk protein yield. Limiting AA for milk protein synthesis can be identified when the plasma concentration is low and the mammary extraction rate is high (Guinard and Rulquin, 1994). In the SP treatment, extraction rates were generally low and it was highest for His. However, the relatively high arterial plasma His concentration indicates

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that His did not limit milk protein synthesis on the SP treatment. The extraction rate of His was rather high on the GB treatment, but still lower than the average value of 59% as found when His has been identified as the first limiting AA (Vanhatalo *et al*., 1999). The higher plasma Gln concentration for GB infusion suggests a sparing effect of this AA from gluconeogenesis.

Abomasal v. *intravenous AA infusions*. Compared with the dietary metabolisable protein intake (the basal diet), abomasal infusion of 400 g AA increased the metabolisable AA supply by more than 20%, which has been shown to increase daily milk yield by 2.8 kg and milk protein production by 104 g in early lactating cows on low energy supply (Schei *et al*., 2005). Volden (1999) observed a 1.8-kg increase in milk yield with 190 g increased duodenal AA supply in early lactating cows. In the present experiment, abomasal AA infusion resulted in a lower milk protein response than intravenous infusion. The abomasal infused AA have been absorbed, as shown by higher urinary N excretion, plasma urea and AA values, but it has not been used for milk protein synthesis. Our results indicate that abomasal infusions have increased the total protein loss and thus affected the AA supply for milk protein production. The faecal N loss was lower for blood infusion, but the apparent digestibility (calculated on basis of enteric protein intake) tended to be higher for abomasal infusion due to a higher protein intake. Assuming again a 90% true digestibility of enteric protein and amino acids, the loss of MFN with the AP treatment can be estimated as 5.4 g/kg DMI. Thus, the MFN with abomasal AA infusion seems to be somewhat higher than with intravenous infusion (5.0 g/kg DMI). The higher AA availability (digested + infused) with blood infusion gave a higher milk protein yield, and also a higher lactose and milk yield, probably due to increased gluconeogenesis from AA. However, a numerical higher feed intake with the AB treatment would also contribute to the higher milk yield.

The lower milk protein response in the AP treatment than the AB treatment could also have been due to an interaction between AA used in the small intestine and the infused AA profile. The AA reaching the duodenum in a common grass silage-based diet can be limiting in specific AA. Infusion of individual AA, as His (Vanhatalo *et al*., 1999), Met (Pisulewski *et al*., 1996; Robinson *et al*., 2000) and Lys (Robinson *et al*., 2000) has increased the milk protein production, which indicates that these AA have been limiting milk protein synthesis. Compared with in His limiting diets (Thivierge *et al*., 2002), the present plasma His concentrations were high, and combined with low extraction rates, this suggests that His was not limiting on the AP or AB treatments. Using National Research Council (2001) to calculate individual AA supply (including infused AA and assuming 100% intestinal digestibility) indicate that neither Lys nor Met were limiting AA in the present study. This corresponds well to the high plasma concentrations observed for Lys. Hovewer, Met concentrations were clearly lower than what have been observed in high-yielding cows with possible Met limitation (Pisulewski *et al*., 1996).

When infusing into the blood the AA availability for intermediary metabolism is 100% while infusing into the gut it can be limited by digestion and absorption. The abomasal infusions have to cross the splanchnic tissues and are first modified by the liver (Reynolds *et al*., 1994) before it goes further to the mammary gland or other tissues. Thus, abomasal infusion will probably reduce the AA available for milk production compared with intravenous infusions. It has been shown that there is a high rate of energy and protein turnover in the lumen wall and the PDV (Cant *et al*., 1996; Reynolds, 2002) and that there is a substantial metabolism of EAA during absorption by intestinal enterocytes (MacRae *et al*., 1997). Elevated luminal nutrient supply has shown to influence activity which promotes tissue growth (Cant *et al*., 1996). This is also demonstrated by infusion of glucose into the jejunum of rats, which stimulated mucosal protein synthesis (Weber *et al*., 1989). Reynolds *et al*. (2004) found that changes in gastro-intestinal and liver mass during transition are apparently consequences of changes in DMI and nutrient supply and not initiated by lactation per se. They also found that the type of diet had little effect on visceral mass. The idea of giving intravenous infusions is to avoid the first-pass extraction by the splanchnic tissues. However, 80% of AA sequestered in the gut tissue arises from the arterial supply to the gut (Seal and Parker, 2000) and, therefore, the gut tissue competes with the mammary gland for absorbed nutrients. This could partly explain the low recovery in milk protein also observed by intravenous infusions (Aikman *et al*., 2002) but it should indicate only a small reduction of response to abomasal infusion compared with intravenous infusion, which was not observed in the present study.

The loss of individual AA during absorption accounts for 36 to 100% (Tagari and Bergman, 1978). The highest consumption is found for Glu and Asp, which relates to their preferential use as energy substrates in intestinal tissues (Reynolds *et al*., 1994). However, the net flux of many AA is perhaps affected more by their uptake in the PDV from the blood (Reynolds, 2002). The relative rates of AA metabolism in PDV vary with requirements, nutrient supply (Reynolds, 2002) and physiological status, but the effect on milk protein production is not clear (Thivierge *et al*., 2002; Aikman *et al*., 2002). In our study, abomasal AA infusion resulted in higher plasma NEAA concentrations, and tended to be higher in TAA concentration, than did intravenous infusion, but the EAA and BCAA concentrations were unaffected. These results are in contrast to those of Thivierge *et al*. (2002), who used a similar AA mixture. They found that jugular infusion tended to increase EAA and BCAA plasma concentrations compared with abomasal infusion in cows 84 days in milk, but no differences were found in concentrations of NEAA. In their study, the basal protein level was low, but the energy balance of the cows was positive. This may suggest a higher supply of energy-yielding substrates than in the present study, which could result

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in a different fate of the infused AA. Our results indicate that there was a higher use of AA for glucose production leading to a higher milk lactose yield on the AB treatment than on the AP treatment. This agrees with the lower plasma concentrations of Ala, which is perhaps the most important glucogenic AA (Reynolds *et al*., 1994).

Reduced NEAA in plasma was also found in an earlier trial with abomasal casein infusion on a grass silage-based diet in which glucose was assumed to be a limiting factor (Miettinen and Huhtanen, 1997). In spite of the high content of Gln and Glu in the AA mixture infused, plasma concentrations of these AA did not increase compared with the SP and GB treatments. Gln and Glu did not differ between AP and AB infusion, which suggest a high use of these AA regardless of infusion site. As mentioned above, a high rate of Glu disappearance during intestinal absorption means that Glu and Gln from intravenous infusion have a different fate. These two AA are involved in a large number of metabolic activities in the body, and their utilisation as energy sources have been demonstrated by reduced plasma concentrations during energy deficiencies (Meijer *et al*., 1995). Glu and Gln are also important precursors for glucose synthesis (Black *et al*., 1990) and when given intravenously, they could have contributed to the higher milk lactose yield. Danfær *et al*. (1995) reported from the literature a wide range of estimates (2 to 40%) of the proportion of glucose derived from AA, and they found in their own study with lactating goats that portally infused AA could contribute to 36% of the hepatic glucose production. They concluded that the contribution of AA to glucose synthesis was regulated to a great extent by the availability of substrates. Gln has also been suggested to be a potential limiting AA for milk protein synthesis in high yielding dairy cows, and the mammary extraction rate may be close to 100% (Meijer *et al*., 1995). Insufficient supply of Gln to the mammary gland will cost carbon skeletons derived primarily from glucose, and when milk protein synthesis is stimulated by infusion of protein, the plasma NEAA concentration will decrease (Meijer *et al*., 1995). However, the current understanding of the major role of Gln in the nutrient partitioning that occurs during lactation is not clear (Lobley *et al*., 2001).

Higher plasma NEAA on the AP than the AB infusion was related to higher concentrations of Ala, Pro and Ser, and the low extraction ratios of these AA suggest an excessive supply from infusion in relation to the requirement on the AP treatment. Pro and Ser are also known to be glucogenic, although not to the same extent as Glu, Gln, Ala, Asp (Black *et al*., 1990). Higher plasma NEAA concentration for AP, and similar plasma urea and urinary N excretion as for AB infusion means that these AA have been utilised anabolically in other peripheral tissues than the mammary gland. Higher endogenous protein excretion might have occurred on the abomasal infusions, and this would suggest a higher protein turnover and AA requirement at the intestinal level.

Comparison of substrate type within infusion site

When comparing CHO and AA infusions within infusion site no differences in milk production were found, except for milk lactose concentration. Plasma urea, urinary N excretion and N balance were however affected by substrate type.

Abomasal starch versus AA infusions. The apparent protein digestibility was lower on SP infusion than on AP infusion probably because the infused AA had higher intestinal digestibility than AA of dietary and microbial protein origin. The higher AA availability on the AP treatment increased plasma EAA and BCAA but it also resulted in increased urinary N excretion and plasma urea concentration. The lack of difference in milk responses between the two treatments does not support our hypothesis that post-ruminal AA supply to cows in negative energy balance would increase the EAA delivery to the mammary gland and thus improve milk protein synthesis compared with starch supply. The starch infusion did not affect milk fat concentration which is in line with earlier studies, when small amounts of glucose were infused (Kim *et al*., 2000; Vanhatalo *et al*., 2003a).

It has been shown by Volden (1999) that a diet consisting of grass silage and barley/oat based concentrate gives a low intestinal starch supply, which in turn could limit the glucose supply to the cows (Huhtanen *et al*., 2002). Increased ruminal escape of starch can improve N utilisation in PDV and liver, because the unfermented starch may directly supply glucose to PDV and spare use of glucogenic AA in the liver (Meijer *et al*., 1997). However, post-ruminal infusions of starch or glucose have shown minor effects on plasma AA (Vanhatalo *et al*., 2003b; Rulquin *et al*., 2004) maybe because energy and/or protein have been supplied close to or above requirements. Infusion of an AA mixture will provide both NEAA and EAA, and if NEAA are preferred as fuel in the gastro-intestinal tract and liver (Reynolds *et al*., 1994), the supply of EAA to the mammary gland might be improved. This was hypothesised in our study and we therefore expected increased milk protein content as a response to an improved EAA supply (Metcalf *et al*., 1996). However, this did not happen. Plasma concentrations of EAA and BCAA were higher on the AP than on the SP treatment. Although not always significant, numerically higher concentrations of many of the individual AA were observed with the AP infusion than the SP infusion, but only minor effects were found on mammary extraction rates. The higher plasma concentration of EAA and BCAA in AP than in SP did not result in increased milk protein synthesis as found in other trials (Reynolds, 2002; Vanhatalo *et al*., 2003a). Higher plasma concentrations of Arg and Orn indicate a higher AA deamination on the AP treatment as supported by a higher plasma urea concentration and an increased urinary N excretion. The plasma concentration of Gln was not increased by abomasal AA infusion compared with starch infusion, which reflect a high rate of utilisation in the PDV during absorption as discussed above. It has been

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shown that glucose and Gln are equally important as energy substrates in mucosal cells of rat small intestine (Fleming *et al*., 1997) but *in vitro* studies have shown that Gln is not as important as glucose as energy source for enterocytes of lactating cows (Okine *et al*., 1995).

Intravenous glucose v. AA infusions. The higher AA availability in the AA infusion increased urinary N excretion compared with glucose infusion. Numerical lower energy balance in the AB than in the GB infusion is a result of a higher mobilisation of AA in the AB treatment or a higher partition of nutrients towards the body in the GB treatment. The positive effect of infusing glucose or AA into the blood on milk yield indicates that the cow's glycogenic status, as well as the AA supplied limited milk protein production. These results are in line with observations found by Vanhatalo *et al.* (2003a) where abomasal infusion of 300 g of casein or glucose to cows in early lactation increased milk and protein yield in similar amount, suggesting that glucose alone increased milk protein production by sparing AA from hepatic utilisation and that these cows suffered from both limited AA and glucose supply. However, in the present study, the low supply of metabolisable protein from the basal diet and reduced infusion amounts in the AP and AB infusions compared with SP and GB infusion, combined with similar responses may suggest that AA were more limited for milk protein synthesis than did glucose. Most comparisons of CHO and AA infusions have been done using casein or profile of casein as the AA substrate (Kim *et al*., 2000; Vanhatalo *et al*., 2003a and b). With the exception of Vanhatalo *et al.* (2003a), these trials showed a positive effect of casein compared with glucose, suggesting that AA were more limiting than glucose.

Hepatic metabolism of glucogenic AA into glucose synthesis is to a great extent regulated by the availability of substrates (Danfær *et al*., 1995). When glucose supply is limited, particularly in early lactation, the use of AA as glucose precursors may be high to prevent the shortfall of glucose (Danfær *et al*., 1995). The infusion of glucose could spare almost twice its own weight of glucogenic AA (Krebs, 1964). In our study, the type of intravenous substrate affected plasma concentration of BCAA, EAA and NEAA. The extraction rate of EAA for GB infusion was high and for AB infusion low compared with what has been reported by others (Vanhatalo *et al*., 2003a and b). Extraction rates of NEAA were lower than that of EAA and varied more between the individual AA, which agrees with results from Guinard and Rulquin (1994). The ratio between plasma EAA and NEAA concentration has been used to evaluate the adequacy of AA supply as this ratio should increase when the protein balance is improved (Bergen *et al*., 1973). On the GB treatment, this ratio was 0.71 and the corresponding value for the AB treatment was 1.04. According to this approach, the protein balance was improved for AB infusion compared with GB infusion. These ratios are lower than those observed by Thivierge *et al.* (2002) who compared abomasal and jugular infusions. But they are higher or close to corresponding values where glucose supply was considered as limiting (Miettinen and Huhtanen, 1997; Vanhatalo *et al*., 2003a).

Intravenous infusion of the AA mixture provided precursors both in terms of EAA for milk protein synthesis and NEAA for gluconeogenesis in the liver. The higher level of EAA and the lower level of NEAA in plasma observed on the AB treatment are in agreement with results from abomasal casein infusion studies (Miettinen and Huhtanen, 1997; Vanhatalo *et al*., 2003b). Decreased plasma concentrations of Ala, Gln, Gly and Ser confirm the role of these particular AA as substrates for glucose synthesis (Black *et al*., 1990) and correspond well to the reduced NEAA concentrations found by Vanhatalo *et al.* (2003b). The results confirm the importance of NEAA as carbon sources for liver glucose synthesis, but their utilisation depends on an adequate and balanced supply of EAA (Reynolds, 2002).

Conclusions

This study demonstrate that, compared with post-ruminal starch and AA infusion, intravenous infusion of glucose or AA daily have a positive effect on protein yield and concentration to cows in negative energy balance. These findings suggest higher losses of MFN by abomasal infusions, and that the abomasally infused nutrients did not reach the mammary gland and thus resulted in lower milk production. Intravenous infusion of glucose did not increase plasma glucose or insulin concentrations above that of abomasal starch infusion. Compared with abomasal starch infusion, intravenous glucose infusion had minor effect on plasma AA. Abomasal AA infusion increased plasma NEAA concentration compared with intravenous AA infusion, but infusion site of AA had no effect on EAA or BCAA. Although a higher milk protein synthesis was observed by intravenous AA infusion, the mammary extraction rate was not higher than by abomasal AA infusion. These results suggest that some of the AA infused in the abomasum have been used in the gastrointestinal tract and/or the liver. Lower plasma NEAA concentrations, in particular Ala, by intravenous AA infusion suggest a higher gluconeogenesis of these AA. Infusion of AA increased plasma concentration of EAA and BCAA, but compared with blood glucose infusion, the mammary extraction rate tended to be lower. Our results suggest that when glucose was infused, AA limited a further milk protein synthesis, but when AA was infused, glucose or energy substrate might have been the limiting factor. Gastro-intestinal metabolism influences the nutrients available for milk synthesis and should therefore be taken into account when developing new feed evaluation systems for dairy cows.

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