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animal

Post-ruminal or intravenous infusions of carbohydrates or amino acids to dairy cows 2. Late lactation

I. Schei[1,2], A. Danfær[3], L. T. Mydland[1] and H. Volden[1†]

[1]*Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, PO Box 5003, N-1432Ås, Norway;* [2]*TINE BA, PO Box 58, N-1430Ås, Norway;* [3]*Danish Institute of Agricultural Sciences, Research Centre Foulum, PO Box 50, DK-8830 Tjele, Denmark*

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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 (N) utilisation, plasma metabolites and mammary extraction rate of dairy cows in late 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 165 ± 4 days (mean ± s.e.) post partum *(milk yield 22.5 ± 1.1 kg) Daily amounts of nutrients infused were 257, 283, 233, and 260 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 a dry-matter (DM) basis), where total dry-matter intake (DMI) was 13.3 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. Responses to intravenous infusions (GB or AB) were similar to those in early lactation, but more pronounced. Compared with SP infusion, GB infusion increased (P < 0.05) milk yield, energy-corrected milk (ECM), protein and lactose yield by 1.4 and 0.9 kg, 38 and 59 g, respectively. The AB infusion had 1.4 and 1.3 kg, 51, 52 and 50 g higher (P < 0.05) milk yield, ECM, protein, fat and lactose yields than the AP infusion, respectively. N balance data indicated higher losses of metabolic faecal nitrogen (MFN) by abomasal than by intravenous infusions, but the catabolism of AA was lower than in early lactation indicated by no difference (P < 0.05) in urinary N excretion between treatments. Intravenous AA infusion increased plasma glucose and insulin above that of intravenous glucose infusion. The treatment effects on plasma insulin concentrations were higher in late than in early lactation, suggesting a higher sensitivity in late lactation even at similar negative energy balance. Compared with the SP infusion, GB infusion showed lower (P < 0.05) concentrations of essential AA (EAA) and branched-chain AA (BCAA) resulting in a higher AA utilisation because of a higher milk protein production. AP infusion increased (P < 0.05) plasma non-essential AA concentration compared with AB infusion, but infusion site of AA had no effect (P > 0.05) on plasma EAA or BCAA. It is concluded that it is the nutrient supply and not the lactation stage per se that is important for the response in milk production. Nevertheless, stage of lactation affects the N metabolism and the response in plasma hormone concentrations even when cows are in negative energy balance in both lactation stages.*

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

Introduction

Milk protein response to supplemental metabolizable amino acids (AA) depends on stage of lactation (Aikman *et al*., 2002). However, it is not clear if this is a lactation stage effect *per se* or an interaction with animal energy status. Therefore, we wanted to compare directly, within the same animals and similar energy balance at two lactation stages, the effect of abomasal or intravenous carbohydrate (CHO) or AA delivery on milk production response, nitrogen (N) utilisation and plasma metabolites. In our accompanying paper (Schei *et al*., 2007), we presented the responses obtained in early lactation starting 54 (±4) days *post partum*. In this paper we will present a replicate of the experiment using the same animals and the same basal diet in late lactation, starting 165 (±4) days *post partum*. The objectives of this study were to evaluate multiple comparisons of starch, glucose or AA infused in the abomasum or intravenously on production responses, N utilisation and plasma metabolites in late lactation. The experimental

† E-mail: harald.volden@umb.no

comparisons were: (1) abomasal starch *versus* intravenous glucose infusion, (2) abomasal *versus* intravenous AA infusion, (3) abomasal starch *versus* AA infusion, and (4) intravenous glucose *versus* AA infusion. The early lactation study was conducted with animals in negative energy balance and therefore same energy balance was used in late lactation. Similar energy balances were important since it may have effects on milk responses (Hanigan *et al.*, 1998; Schei *et al.*, 2005) and also makes it more comparable with early lactation responses.

Material and methods

Animals and basal diets

Eight Norwegian Red cows fitted with ruminal cannulae were used. The cows were the same as used in the early lactation experiment presented in our accompanying paper (Schei *et al.*, 2007). The experiment was conducted in late lactation starting 165 ± 4 (mean ± s.e.) days *post partum* (milk yield 22.5 ± 1.1 kg). The cows were fed the same basal ration consisting of concentrate and grass silage in the same ratio 55 : 45 and using the same feeding management as in the early lactation experiment (Schei *et al.*, 2007). Silage was stored in tower silos until start of the early lactation experiment, then packed in plastic bags and frozen until use also for the late lactation trial, to ensure that the same feed was used in both lactation stages. In the period between the early and late lactation experiment, the cows were fed ad libitium silage and one week before the first infusion period, dry matter (DM) feed allowance was reduced to the experimental level, restricted to 95% of pre-experimental net energy (NEL) requirements. For all cows, daily DM intake (DMI) was set to 7 kg concentrate DM and 6.3 kg silage DM. Ingredients and chemical composition of the basal diet are presented by Schei *et al.* (2007).

Experimental procedures and treatments

This experiment was replicate of the early lactation experiment and has thoroughly been described by Schei *et al.* (2007). Two 4 × 4 Latin squares were designed with the following treatments: (1) starch in the abomasum (SP), (2) glucose in the blood (GB), (3) amino acids in the abomasum (AP), and (4) amino acids in the blood (AB). Pure wheat starch (*Tritici amylum*) and glucosum anhydricum were used as starch and glucose supplements. The amino acid mixture contained 16 L-amino acids with an overall composition as presented by Schei *et al.* (2007) but daily infusion of starch, glucose and AA were reduced to 300, 335 and 300 g, respectively, corresponding to 23 g of infusion per kg DMI. The procedures for the infusion management and the infusion rates were equal as in the early lactation stage experiments (Schei *et al.*, 2007). Because some AA were precipitated after freezing and heavily dissolved, the procedures for preparing the AA were changed in the late stage of lactation. Therefore, the AA solutions were not frozen, but prepared daily. When dissolved in 8 l of ultra-purified sterile pyrogen-free water, pH was adjusted and then the solution was sterilised through the vacuum filters into sterile bottles ready for use.

Measurements, sample collection and analytical procedures

Measurements, sample collection and analytical procedures have been described by Schei *et al.* (2007).

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

All calculations have been described by Schei *et al.* (2007). Data used in the statistical analyses were averages from the last 3 d of each treatment period. The effects of treatment on feed intake, digestibility, milk yield, milk chemical production and composition, N balances and plasma metabolites were run using the same statistical model with the MIXED procedure of Statistical Analysis Systems Institute (1999) as presented in our corresponding paper (Schei *et al.*, 2007). The model was tested for residual effects, but no effect was detected, and it was therefore removed from the model. Results are presented as least square means with standard error of the mean (s.e.). Pdiff-statement was used to identify significant differences between means. 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 digestibility of organic matter (OM) and crude protein (CP) are presented in Table 1. The amount of substrates infused was lower than planned and this affected the calculated nutrient supply. Total metabolisable energy (ME) intake was lower ($P < 0.05$) in the SP infusion compared with the other treatments because of a lower CP digestibility. As planned, total CP intake was higher in AP and AB infusions than in SP and GB infusions, on average 237 g higher. The OM digestibility was higher on AP infusion than on the SP and GB infusions. The digestibility of CP was, on average, 3.6% units lower ($P < 0.05$) when starch was infused than when the other nutrients were infusions. Calculated energy balances showed that the cows were underfed by, on average, 11.8 MJ NEL. Higher ($P < 0.05$) energy balance was observed for the AP infusion than for the other treatments, mainly because of a higher OM digestibility.

Milk yield, composition and efficiency

Milk yield, milk composition and milk efficiency are presented in Table 2. In general, the production responses

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Table 1 *Means of daily intake and digestibility of nutrients by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in late lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Infusion (g)	257[k]	283[j]	233[l]	260[k]	6.2	***
Intake (kg dry matter)						
Total	13.3	13.3	13.2	13.2	0.23	
Concentrate	7.0	7.0	7.0	7.0	–	–
Silage	6.3	6.3	6.2	6.2	0.22	
Crude protein[‡]	2.092[k]	2.095[k]	2.320[j]	2.341[j]	0.0321	***
Starch[‡]	3.385[j]	3.128[k]	3.128[k]	3.128[k]	0.0046	***
Metabolisable energy (MJ)	134[k]	137[j]	137[j]	138[j]	2.4	‖
Energy balance[§] (%)	−16.5[j]	−17.2[j]	−12.6[k]	−17.7[j]	4.14	*
Digestibility						
Organic matter (%)	70.2[k]	70.7[k]	72.1[j]	71.2[jk]	0.52	*
Crude protein (%)	67.9[k]	70.9[j]	72.4[j]	71.3[j]	0.72	**

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] Treatments; 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.
[‖] Approaching significant ($P < 0.10$).

were highly affected by infusion site (abomasal versus blood; measured as individual treatment comparisons), whereas infusion substrates (CHO *v.* AA; measured as individual treatment comparisons) were of minor importance. Treatments differed ($P < 0.05$) for all variables except milk composition (protein, fat and lactose concentrations) which tended to differ ($P < 0.10$). The GB and AB infusions were higher ($P < 0.05$) in milk yield, energy corrected milk, protein and lactose yields than the SP and AP infusions, in average, 1.4 and 1.1 kg, 45 and 55 g higher, respectively. Moreover, milk fat yield was 52 g higher ($P < 0.05$) on AB treatment compared with the AP treatment. The GB tended to ($P < 0.10$) be lower in milk fat concentration than the other treatments, and also tended to have lower ($P < 0.10$) protein concentration compared with the AB infusion. The ME efficiency for milk production was lower ($P < 0.05$) for the AP infusion than for the other treatments. No differences ($P > 0.05$) were found between GB and AB infusions, or between SP and AP infusion except in lactose concentration which tended to differ ($P < 0.10$) between treatments.

Table 2 *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 late lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Yield						
Milk (kg)	21.3[k]	22.7[j]	21.2[k]	22.6[j]	1.16	**
ECM[‡] (kg)	20.6[k]	21.5[j]	20.6[k]	21.9[j]	1.28	**
Protein (g)	651[l]	689[jk]	657[kl]	708[j]	34.4	*
Fat (g)	822[k]	847[jk]	822[k]	874[j]	59.5	**
Lactose (g)	1004[k]	1063[j]	995[k]	1045[j]	60.3	**
Milk composition (g/kg)						
Protein	30.6	30.5	31.1	31.4	0.63	‖
Fat	38.4	37.3	38.7	38.5	1.53	‖
Lactose	47.0	46.8	46.8	46.3	0.73	‖
Milk energy (MJ)	64.3[k]	67.2[j]	64.3[k]	68.3[j]	3.99	**
Energy efficiency[§] (%)	81.8[j]	82.5[j]	78.8[k]	82.6[j]	3.43	*

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] Treatments; 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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N balance

The N balance data are presented in Table 3. The N intake was in accordance with dietary CP intake and infusion treatments. The SP and AP infusion had 11 and 8 g/day higher ($P < 0.05$) faecal N losses by than the GB and AB infusion, respectively. In percentage of N intake, the SP infusion had 3% units higher ($P < 0.05$) faecal N loss compared with the GB infusion. The corresponding difference between the AP and AB treatments were 2.1% unit higher ($P < 0.05$) in AP than in AB infusion. In percentage of N intake, the SP infusion showed 4.5%-units higher ($P < 0.05$) faecal N loss than the AP infusion, and the GB infusion was 3.6% units higher than the AB infusion. No difference was found between treatments in urinary N excretion measured either as g/day or in % of N intake. 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 N balance was lower ($P < 0.05$) for SP infusion than for AP and AB infusions. All treatments differed ($P < 0.05$) from each other in plasma urea concentration and the lowest values were found in the SP treatment and the highest for the AB infusion.

Plasma metabolites

Plasma metabolites, hormones and mammary extraction rates of plasma metabolites are presented in Tables 4, 5 and 6. Infusion of glucose into the blood by 283 g daily in late lactation did not increase ($P < 0.05$) plasma glucose or insulin concentrations above that of the other treatments (Table 4). However, the AB infusion had higher ($P < 0.05$) plasma concentration of both glucose and insulin than the other treatments. Plasma non-esterified fatty acid (NEFA) concentrations were higher ($P < 0.05$) on treatment AP and AB than on treatment SP and GB. The extraction rates of glucose and NEFA were not affected ($P > 0.10$) by treatments (Table 4). The GB infusion increased IGF-1 concentration compared with the other treatments. BGH tended to differ ($P < 0.10$) between treatments, whereas glucagon was unaffected ($P > 0.10$). The plasma level of total AA (TAA) was not affected ($P > 0.10$) by treatments, but essential AA (EAA), non-essential AA (NEAA) and branched-chain AA (BCAA) differed ($P < 0.05$) between treatments. Compared with the SP and GB infusions, the AB and AP infusions had higher ($P < 0.05$) plasma concentrations of EAA and BCAA, and the plasma concentrations of EAA and BCAA were lower ($P < 0.05$) on the GB infusion than on the SP infusion. The plasma NEAA concentration was lower ($P < 0.05$) for AB infusion compared with the other treatments. Of the individual EAA, His, Leu and Val were higher ($P < 0.05$) on treatments AP and AB, Ile, Leu and Val was lower ($P < 0.05$) on treatment GB, and Lys was higher ($P < 0.05$) on treatment AP compared with the other treatments. The most important effects on the NEAA were the lower ($P < 0.05$) plasma Ala concentration on the AB infusion and higher ($P < 0.05$) Gln and Ser concentration on the GB treatment compared with the other treatments. The mammary extraction rates of the TAA, EAA and NEAA were not affected ($P < 0.05$) by treatments, but the extraction rate of the BCAA was higher on the GB infusion than on the other treatments. Of the individual AA, extraction rate of His was lower by the AB infusion than for SP and GB infusion, and extraction rates of Ile and Leu also differed ($P < 0.05$) between treatments. For these AA, lowest extraction rate was observed on the AP treatment and highest on the AB and GB treatments.

Discussion

Nutritional responses on milk production are related to the animal physiological state, which is dependent on stage of

Table 3 *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 late lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
N intake (g)	335^{k}	335^{k}	371^{j}	375^{j}	5.1	***
N output (g)						
Faeces	108^{j}	97^{kl}	103^{jk}	95^{l}	3.0	*
Urine	116	110	126	123	6.9	
Milk	102^{l}	108^{jk}	103^{kl}	111^{j}	5.4	*
Balance	9^{k}	20^{jk}	39^{j}	45^{j}	8.4	*
Plasma urea (mmol/l)	3.56^{m}	4.22^{l}	5.07^{k}	5.50^{j}	0.14	***
N output (% of intake)						
Faeces	32.1^{j}	29.1^{k}	27.6^{k}	25.5^{l}	0.69	***
Urine	34.5	32.8	34.1	33.1	1.94	
Milk	30.4^{k}	32.1^{j}	27.7^{l}	29.5^{k}	1.22	***
Balance[‡]	3.0	6.0	10.6	12.0	2.46	[§]

[j,k,l,m] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] Treatments; 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.
[§] Approaching significance ($P < 0.10$).

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Table 4 *Plasma metabolites and mammary extraction rates of plasma metabolites by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in late lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Glucose (mmol/l)						
Arterial	3.37[k]	3.42[k]	3.37[k]	3.56[j]	0.066	**
Extraction (%)	24.2	25.8	25.2	25.4	0.83	
NEFA[‡] (mmol/l)						
Arterial	0.185[k]	0.185[k]	0.198[j]	0.200[j]	0.005	*
Extraction (%)	−14.7	−13.7	−9.3	−8.4	3.67	
Hormones						
Insulin (pmol/l)	78.6[k]	100.0[k]	81.4[k]	127.5[j]	11.1	***
Glucagon (ng/ml)	40.9	42.8	44.0	45.0	2.86	
IGF-1 (ng/ml)	66.2[k]	81.0[j]	64.7[k]	68.3[k]	7.64	***
BGH (ng/ml)	1.14	0.79	1.29	1.07	0.221	[§]

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] Treatments; 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.
[§] Approaching significance ($P < 0.10$).

Table 5 *Mean arterial plasma amino acid concentrations (μmol/L) by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in late lactation*

	Treatments[†]					
	SP	GB	AP	AB	s.e.	Significance
Essential						
Arg	96.5[jk]	74.3[kl]	106.6[j]	69.3[l]	9.44	*
His	36.8[k]	33.7[k]	56.0[j]	59.0[j]	5.70	***
Ile	165[j]	140[k]	180[j]	172[j]	9.9	**
Leu	130[l]	111[m]	147[k]	173[j]	8.6	***
Lys	107[k]	106[k]	133[j]	118[k]	9.6	**
Met	24.9	25.1	23.4	22.6	1.47	
Phe	53.7[k]	48.1[k]	52.1[k]	60.4[j]	2.59	*
Thr	121	126	134	119	6.2	[§]
Val	263[k]	222[l]	321[j]	327[j]	16.7	***
Non-essential						
Ala	329[j]	322[jk]	304[k]	275[l]	14.5	**
Asn	68.8[k]	78.9[j]	79.3[j]	82.6[j]	3.86	**
Asp	8.6	8.3	8.5	7.8	0.50	
Cys	18.1	18.1	20.4	19.7	1.26	
Glu	82.7	82.8	70.2	72.7	5.20	[§]
Gln	359[k]	401[j]	339[kl]	307[l]	17.2	**
Gly	394[jk]	441[j]	399[jk]	358[k]	20.2	*
Pro	130	121	130	130	7.1	
Ser	122[k]	134[j]	113[kl]	111[l]	6.1	***
Tyr	54.3[j]	48.0[jk]	39.9[k]	41.5[k]	3.30	*
Orn	54.4[k]	51.0[k]	66.4[j]	55.0[k]	4.88	***
Cit	90[k]	89[k]	104[j]	96[jk]	4.6	*
EAA[‡]	998[k]	887[l]	1152[j]	1120[j]	49	***
NEAA[‡]	1566[jk]	1656[j]	1504[k]	1406[l]	52	***
BCAA[‡]	558[k]	474[l]	648[j]	672[j]	32	***
TAA[‡]	2564	2543	2656	2526	70	
Plasma urea (mmol/l)	3.56[m]	4.22[l]	5.07[k]	5.50[j]	0.14	***

[j,k,l,m] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] Treatments; 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 ($P < 0.10$).

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Table 6 *Mean extraction rate (%) of plasma amino acids by the mammary gland by dairy cattle infused with carbohydrates or amino acids into the abomasum or blood in late lactation*

	Treatments[†]					
Item	SP	GB	AP	AB	s.e.	Significance
Essential						
Arg	25.2	12.3	28.9	23.5	9.42	
His	49.0[j]	49.2[j]	34.7[jk]	22.2[k]	6.07	*
Ile	31.3[kl]	35.2[jk]	27.7[l]	36.2[j]	2.63	**
Leu	42.0[kl]	49.5[j]	40.1[l]	47.9[jk]	3.00	*
Lys	55.3	56.7	52.5	58.7	2.44	
Met	55.4	54.3	63.2	67.6	6.07	
Phe	44.4	44.7	51.6	42.6	3.31	
Thr	28.1	29.7	25.7	28.9	3.27	
Val	24.7	28.6	22.5	22.6	2.54	
Non-essential						
Ala	8.5	9.9	10.0	−0.4	3.84	
Asn	23.4	26.5	23.4	26.3	5.10	
Asp	18.0	34.2	36.1	35.3	8.53	
Cys	7.8	5.0	8.4	1.7	4.57	
Glu	75.4	72.8	78.2	72.7	2.28	
Gln	25.2	18.6	22.5	21.6	2.68	
Gly	3.9	6.4	6.0	3.7	2.56	
Pro	7.5	5.1	5.7	−2.3	10.1	
Ser	11.2	17.7	18.5	14.4	4.68	
Tyr	42.9	36.7	57.8	59.4	6.02	[§]
Orn	49.7	50.5	48.4	51.2	3.22	
Cit	7.9	4.9	10.1	6.9	2.99	
EAA[‡]	36.9	38.7	34.7	35.0	2.47	
NEAA[‡]	17.1	16.2	17.7	14.5	2.24	
BCAA[‡]	33.4[k]	38.9[j]	29.9[k]	32.3[k]	2.38	*
TAA[‡]	24.9	23.9	25.0	23.6	2.23	

[j,k,l] Means within the same row and lactation stage with different superscripts differ ($P < 0.05$).
[†] Treatments; 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 ($P < 0.10$).

lactation and nutrient balances. Stage of lactation may have an effect on nutrient partitioning between milk and body tissues. However, it can be questioned whether the physiological status is more related to nutrient supply than stage of lactation *per se* (Kirkland and Gordon, 2001). In the present experiment, similar energy balances in early and late lactation make it possible to compare the nutritional effects on the animal performance. The response to absorbed protein is dependent on the energy status of the animal (Hanigan *et al.*, 1998), where glucogenic AA might be oxidised or used as glucose precursors. For cows in negative energy balance higher AA availability will increase gluconeogenesis and thus lactose synthesis and thereby milk and protein yield, but have little effect on protein content as found in a Norwegian study (Schei *et al.*, 2005). The negative energy balance in the present study might explain the general low milk protein content, since there is a positive correlation between energy intake and milk protein content (Coulon and Rémond, 1991; Rigout *et al.*, 2003). However, for cows in positive energy balance, higher glucose availability has been shown to increase tissue energy balance and glucose oxidation, but have little effect on milk or protein yield (Reynolds *et al.*, 1994). Total tract digestibility of OM and CP were 2.8 and 1.3% units higher in late lactation than in early lactation, respectively, which corresponds well to observations that increased feeding level decreases the digestibility of the same diet (Volden, 1999). Moreover, the percentage of urinary N excretion was 7.3 units higher in late lactation than in early lactation in spite of a lower feeding level, which also corresponds well to Volden (1999). Responses in milk yield and milk protein production were similar in early and late lactation. The higher AA availability due to AA infusions increased urinary N excretion in early lactation and plasma urea in late lactation. Similar ME, and reversed urinary N and plasma urea in early and late lactation might indicate a different fate of the available AA with only minor effect on milk production. Lower fat concentration in the GB treatment in late lactation could be a dilution effect of increased yield resulting from an increase in glucose availability for lactose synthesis without concomitant increases in milk fat synthesis (Gaynor *et al.*, 1995) which has been observed in other studies with glucose (Fisher *et al.*, 1966; Hurtaud *et al.*, 1998) or starch infusions (Reynolds *et al.*, 2001). The responses in milk yield expressed in percentage were marginally higher in late than in early lactation (4.6 *v.* 6.6%). This is interesting since the infusion amounts in late lactation were lower than in early lactation. The N balance showed that the recovery of N in milk, urine and faeces were higher in late (92.1%) than in early lactation (88.3%), and therefore, the body protein retention was also higher in early lactation. This could be a result of a higher metabolic activity in the gastrointestinal tissues in early lactation as discussed in our corresponding paper (Schei *et al.*, 2007). Using the same assumption as calculated by Raggio *et al.* (2004) as we did in the early lactation experiment (Schei *et al.*, 2007) the loss of metabolic faecal nitrogen (MFN) with starch infusion was calculated to 5.6 g/kg DMI. The corresponding figures with intravenous glucose infusion were 4.8 g/kg DMI. Comparing these calculations with the corresponding values for early lactation (Schei *et al.*, 2007), demonstrate that there is a higher loss of MFN in early than in late lactation. This indicates an increased protein turn-over and tissue growth in the gut. This could explain the higher retention in early lactation.

The AB infusion increased plasma concentration of both glucose and insulin above that found for GB infusion. These observations were not found in early lactation (Schei *et al.*, 2007). This is difficult to explain, but Kim *et al.* (2000) also reported higher insulin concentration by intravenous NEAA compared with glucose infusion. Data on blood metabolite concentrations are often difficult to interpret in terms of nutrient fluxes, but the observed glucose

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concentrations on treatments GB and AB indicate that gluconeogenesis was reduced by glucose infusion more strongly in late than in early lactation. In both lactational stages, the plasma level of EAA was higher on treatment AB than on treatment GB, whereas the opposite was the case for the concentration of NEAA. This suggest that gluconeogenesis from AA was higher with AB than with GB infusion as NEAA is preferred to EAA as glucogenic substrates in cows (Black *et al*., 1968). On the other hand, the insulin concentration in late lactation was higher on treatment AB than on treatment GB, and insulin is known to inhibit gluconeogenesis and to increase the uptake of glucose and AA in muscle cells (Sjaastad *et al*., 2003). Moreover, these results suggest that insulin concentration is hardly affected by nutrient supply in early lactation. The higher insulin level for AB infusion in late lactation can be explained by the higher glucose concentration since insulin is more sensitive to glucose than to AA (Lemosquet *et al*., 1997). Individual AA like Arg and Leu may stimulate insulin more than other AA, and there is a synergistic effect on insulin release between glucose and AA (Lemosquet *et al*., 1997), which may have been important in our experiment. Abomasal AA infusion did not have the same effect on insulin as intravenous AA infusion, probably as a result of lower blood glucose level due to a lower gluconeogenesis. Higher arterial glucose concentrations with jugular compared with abomasal AA infusion was also observed by Thivierge *et al.* (2002) in cows 84 days in milk, but the arterial insulin concentrations did not differ. However, a higher insulin response may be expected with blood infusion of AA because the pancreas in this case would be exposed to the infused AA before these are taken up by the liver. Infusion of glucose in the blood increased IGF-1 concentrations in both early (Schei *et al*., 2007) and late lactation, which is in line with other studies (Rigout *et al*., 2002; Lemosquet *et al*., 2004). This is related to the parallel increase in milk yield and milk protein yield (Lemosquet *et al*., 2004). Low circulating concentrations of IGF-1 in early lactation have been associated with negative energy balance (Spicer *et al*., 1990; Vicini *et al*., 1991) and with negative protein balance when energy balance is not already limiting (Ronge *et al*., 1988).

Conclusions

This study demonstrated that, compared with post-ruminal starch and AA infusion, intravenous glucose or AA infusion in late lactation had a positive effect on milk production to cows in negative energy balance. These results suggest that the mammary nutrient supply were lower when the substrates were given abomasaly. The lower nutrient supply is explained by a higher loss of MFN and use of these substrates in the intestinal tract or other tissue than the mammary gland. Higher urinary N losses by AA infusions in early than in late lactation indicate a higher AA catabolism in early than in late lactation. In late lactation the milk response to glucose infusion was higher than in early lactation when compared with abomasal infusion of starch and AA. Compared with abomasal starch infusion, intravenous glucose infusion had minor effect on plasma AA concentration in early lactation, but in late lactation, the concentrations of EAA and BCAA were lower, because of a higher utilisation for milk protein production. Intravenous AA infusion increased plasma glucose and insulin above that of intravenous glucose infusion. The treatment effects on plasma insulin concentrations were higher in late than in early lactation, suggesting a higher sensitivity in late lactation even at similar negative energy balance. It is concluded that it is the nutrient supply and not the lactation stage per se that is important for the milk production response. The lactation stage is important for the response in plasma hormone concentrations.

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