Effects of Casein and Glucose on Responses of Cows Fed Diets Based on Restrictively Fermented Grass Silage

A. Vanhatalo, T. Varvikko, and P. Huhtanen

Animal Production Research, MTT, Agrifood Research Finland,
FIN-31600 Jokioinen, Finland

ABSTRACT

This study was conducted to investigate whether well fed dairy cows given restrictively fermented grass silage diet will respond to incremental glucose and amino acid supply at early stage of lactation. Four rumen-cannulated Finnish Ayrshire cows were used in a 4 × 4 Latin square experiment with 14-d periods. The cows were fed good quality restrictively fermented grass silage ensiled with a formic acid additive for ad libitum intake. A concentrate mixture consisting of barley (85%) and solvent extracted rapeseed meal (11.4%) was given at a rate of 9 kg/d. The four treatments were continuous abomasal infusions of water (control), casein 300 g/d, glucose 300 g/d, and casein 300 g/d + glucose 300 g/d. The infusions had only minor effects on feed intake, diet digestibility, or rumen fermentation pattern. Both casein and glucose infusions increased milk, protein and lactose yields the effects being partly additive on the combined infusion. Infused casein increased milk protein and urea as well as plasma urea concentrations. Both casein and glucose tended to increase plasma glucose concentration. Casein increased arterial plasma concentrations of essential amino acids (EAA), branched-chain AA (BCAA), and total AA (TAA). Both casein and glucose, although glucose usually less than casein, increased arteriovenous differences of EAA, nonessential AA, BCAA, and TAA. Extraction efficiencies of AA were higher for glucose than for casein. Mammary plasma flow was at highest on the control diet, but reduced owing to infused nutrients, the reduction being less with combined rather than separate infusions of casein and glucose. Based on the partly additive increases in milk production parameters and changes in plasma metabolites, it is suggested that glucose alone increased milk protein yield by sparing AA from hepatic utilization, while casein increased both supply of AA and glucose. It was concluded that cows at early stage of lactation fed diets comprising of restrictively fermented

grass silage and a cereal-based concentrate suffer from both limited AA and glucose supplies.

(Key words: grass silage, dairy cow, glucose, amino acids)

Abbreviation key: AV = arteriovenous, BCAA = branched-chain AA, C = casein treatment, CG = casein and glucose treatment, EAA = essential AA, G = glucose treatment, ME = metabolizable energy, NEAA = nonessential AA, TAA = total AA.

INTRODUCTION

Rumen fermentation of cows fed large amounts of restrictively fermented grass silage is characterized by a high efficiency of microbial protein synthesis but with a low production of propionate in the rumen (Huhtanen, 1998). Recent studies show that protein supplementation of grass silage-cereal diets increases RUP but does not affect microbial flow, which still comprises majority of the protein flow entering to the small intestine (Ahvenjärvi et al., 1999; Korhonen et al., 2002). Therefore, AA supply of cows fed grass silage diets is highly determined by the features of microbial protein, which is, however, a not quite optimal protein source for milk production. As microbial protein is poor in His relative to milk protein (Clark et al., 1992), it is not surprising that His is often obtained as the first limiting AA in milk production on grass silage-based diets (Vanhatalo et al., 1999, Kim et al., 1999; Korhonen et al., 2000). It is also well known that a supplementary AA supply in the form of postruminally infused casein gives a consistent increase in milk production of grass silage-fed cows (Choung and Chamberlain, 1993a; Huhtanen et al., 1997; Vanhatalo et al., 2003).

On the other hand, given that ruminal propionate production is low and grass silage diet is poor in postruminal starch, the glucose supply of the cows may be limited with these diets (Huhtanen, 1998; Hurtaud et al., 2000). It has been suggested that in this dietary situation, part of the AA in protein entering the lower tract may be utilized for gluconeogenesis decreasing mammary AA availability for milk protein synthesis (Huhtanen, 1998; Vanhatalo et al., 2003). Hepatic me-

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Corresponding author: A. Vanhatalo; e-mail: aila.vanhatalo@mtt.fi.

tabolism of propionate and glucogenic AA into glucose synthesis is regulated to a great extent by the availability of these substrates (Danfær, 1995). However, increasing glucose supply either by ruminal propionate infusions or postruminal glucose infusion has given inconsistent results. Sometimes they have increased yields of milk, protein, and lactose (Huhtanen et al., 1998; Huhtanen et al. 2002) but not always (Kim et al., 2000; Vanhatalo et al., 2003). Sufficient glucose supply from the basal diet may explain the lack of responses to glucogenic substrates (Kim et al., 2000), but it is also possible that some other nutrient but glucose, such as first-limiting AA, has primarily limited milk production (Vanhatalo et al., 2003). This is supported by the recent finding that by increasing the supply of glucose efficiency of utilizing of the first-limiting AA, His, could be improved (Huhtanen et al., 2002). Therefore, question arises whether the milk production responses on grass silage-based diets are primarily restricted by limited supply of glucose or by limited supply of AA, or by both glucose and AA.

Thus, this experiment was designed to study if glucose supply of dairy cows fed restrictively fermented grass silage supplemented with a cereal-based concentrate is sufficient and if responses to AA in terms of casein are restricted by limited supply of glucose. The infusion trial was conducted with cows at early stage of lactation to evaluate if the negligible response to glucose was related to the low production level of cows in our recent experiment (Vanhatalo et al., 2003). Casein and glucose were infused both separately and in combination into the abomasum of cows to look at the effects of the infusates on milk production and plasma metabolites. The control diet was formulated to fulfil the requirements of nutrient supply in terms of total supply of metabolizable AA and energy (Tuori et al., 2000).

MATERIALS AND METHODS

Cows and Management

Four Finnish Ayrshire cows at second, fourth, or fifth lactation averaging 54 DIM (SD = 9.7) were used in the experiment. The BW of cows was 534 kg (SD = 64.4) at the beginning, and 550 kg (SD = 60.2) at the end of the experiment. They were fitted with rumen cannulas, and infusion catheters were introduced into their abomasum as described previously (Varvikko et al. 1999). The cows were kept in individual stanchions and milked twice daily at 0700 and 1630 h. They had a free access to restrictively fermented grass silage to allow refusals of 10% of the total consumption. The cows had also free access to water and salt block. The concentrate mixture given to the cows at a rate of 9 kg/d on a fresh weight

basis consisted of barley (85%), solvent-extracted rapeseed meal (11.4%) (Rehuraio Oy, Raisio, Finland), mineral mixture (2.8%) (Viher Hertta-Minera Muro, Suomen Rehu Oy, Helsinki, Finland), calcium carbonate (0.6%), and sodium chloride (0.2%). Adjustment of concentrate supplementation in the basal diet was done in order to avoid a shortage of RDP and also to avoid an excessive supply of RUP, because it could possibly mask the differences between the treatments. Grass silage was prepared from primary growth of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward. The grass was cut with a disc-mower and harvested after a wilting period of 4 to 6 h using a precision-chop forage harvester. It was ensiled into a bunker silo of 50-tonne capacity using formic acid-based additive (5 L/tonne of grass; in kg of additive: 800 g of formic acid and 20 g of orthophosphoric acid).

Experimental Procedures and Chemical Analyses

The experiment was designed as a 4 × 4 Latin square with 14-d experimental periods, of which the latter 5 d formed the collection period. The four treatments were continuous abomasal infusions of 1) water (Control), 2) casein 300 g/d (C), 3) glucose 300 g/d (G), and 4) casein 300 g/d + glucose 300 g/d (CG). Casein was given as sodium caseinate (Kainuun Osuusmeijeri, Sotkamo, Finland) and glucose as dextrose (Xyrofin Ltd, Helsinki, Finland). Both casein and glucose were dissolved in 6 L of water, and the solution was infused continuously via a peristaltic pump as described previously (Varvikko et al., 1999). Infusion equipment was checked daily during the experiment to ensure correct placement in the abomasum. Feed intake and milk yield of cows were recorded daily but data from collection period only were used in statistical analyses. Feeds were sampled for chemical analyses during each collection period. Milk samples were taken on four consecutive milkings on each collection period. In addition, a separate milk sample from evening and morning milking was taken for urea determination. Rumen fluid was sampled four times and blood from the mammary and tail vein three times during the feeding cycle on the last day of each experimental period. Both of these samples were taken just before the morning feeding and at 3-h intervals thereafter. In order to evaluate energy status of the cows, total digestibility of diets was measured using acid-insoluble ash as an internal marker. Fecal grab samples were collected for 5 d for this purpose. Sampling of feed, milk, blood, rumen fluid, and feces have been described in more detail previously (Varvikko et al., 1999).

The chemical composition of feeds and feces were analysed as described earlier (Varvikko et al., 1999).

The procedures for VFA of grass silage and rumen fluid, and plasma glucose, NEFA and BHBA, as well as plasma AA and urea were described in detail in our previous papers (Varvikko et al., 1999; Vanhatalo et al., 2003). Plasma insulin was colorimetrically analyzed with Phadeseph Insulin RIA kit (cat. no. 10-9169-01, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Plasma samples were reduced to period pools for each cow before the AA analyses. Milk fat, protein, lactose, and urea contents were analyzed as described previously (Varvikko et al., 1999).

Calculations and Statistical Analyses

Mammary extraction and uptake of blood metabolites and AA and plasma flow were calculated as described earlier (Vanhatalo et al., 1999). In brief, mammary extraction efficiency of blood metabolites and AA were calculated as arteriovenous (AV) difference divided by arterial concentration. Mammary plasma flow was estimated in reference to Phe and Tyr output in milk protein by application of Fick principle assuming that Phe and Tyr are incorporated directly into milk protein without no further metabolism or synthesis of these two AA.

Data were analyzed by the ANOVA for Latin square: cow (d.f. 3), period (d.f. 3), treatment (d.f. 3), and the residual effects (d.f. 6) were the sources of variation. The general linear models procedure of SAS (1996) was used. The data for rumen fermentation and blood metabolites except for plasma AA were analyzed by the ANOVA using split-plot approach model including cow, period, treatment, whole-plot error, time, interaction between time and the treatment, and subplot error. However, as there were not any significant ($P > 0.05$) treatment $\times$ time effects, data were averaged over time for all rumen fermentation and blood metabolite parameters. Treatment effects were further separated into a single d.f. comparison by orthogonal contrasts to evaluate effects of: 1) casein (control + G vs. C + CG), 2) glucose (control + C vs. G + CG), and 3) an interaction between casein and glucose (control + CG vs. C + G).

RESULTS

Characteristics of the Basal Diet

The chemical composition of grass silage and concentrate used in the experiment are presented in Table 1. Grass silage fed was of high quality both in terms of nutrient content and fermentation characteristics. The in vitro digestibility of silage OM was high, and the high content of water soluble carbohydrates and low contents of fermentation acids and ammonia N in grass silage indicated restricted fermentation in the silo. Feed

Table 1. Chemical composition of feedstuffs.

	Grass silage	Concentrate ¹
pH	4.12	
DM, %	29.2	88.1
	(% of DM)	
Ash	8.4	6.4
CP	17.8	15.3
NDF	52.7	22.9
Water soluble carbohydrates	8.5	
Lactic acid	3.8	
Acetic acid	1.6	
Propionic acid	0.03	
Butyric acid	0	
Ammonia N, % of N	3.5	
Soluble N, % of N	67.5	
In vitro cellulase digestibility, % of OM	77.3	

¹Concentrate was a mixture of barley (85%), rapeseed meal (11.4%) and minerals (3.6%). The mineral mixture contained 16% of Ca, 6.4% of P, 9% of Na and 8% of Mg, 150,000 IU of vitamin A/kg, 100,000 IU of vitamin D/kg, and 500 mg of vitamin E/kg. It also contained trace elements as follows (per kg): 500 mg of Cu, 10 mg of Se, 3000 mg of Zn, 8 mg of Mo, 10 mg of Co, 1000 mg of Mn, and 100 mg of I.

intake, rumen fermentation, and diet digestibility are shown in Table 2. The grass silage DMI was high. However, cows refused some of the concentrates on each treatment probably owing to the high quality grass silage freely available. Both casein ($P = 0.11$) and glucose ($P = 0.08$) infusions tended to reduce silage intake. Rumen fermentation parameters or digestibility of nutrients were not significantly ($P > 0.05$) affected by the treatments, except for the slightly reduced NDF digestibility of the diet when glucose alone was infused ($P < 0.05$ for C $\times$ G interaction). Digestibility of OM and CP tended ($P \geq 0.07$) to increase owing to infusion of casein.

Milk Production Responses

Milk production data are shown in Table 3. Both casein ($P = 0.06$) and glucose ($P = 0.07$) infusions increased milk yield. Increases in milk yields due to casein and glucose were partly additive (C and G alone +1.8 kg/d, C and G together +2.8 kg/d). Casein infusion increased ($P < 0.01$) milk protein content (31.9 vs. 30.6 g/kg), and tended ($P = 0.10$) to decrease milk fat content (37.0 versus 40.0 g/kg), whereas glucose infusion did not affect ($P > 0.10$) these parameters. Infusion of casein increased ($P < 0.01$) milk urea concentration (28.8 vs. 26.1 mg/dl) and infusion of glucose tended ($P = 0.08$) to decrease it (26.7 versus 28.2 mg/dl). Increases in milk protein yields due to casein ($P < 0.01$) and glucose ($P = 0.10$) infusions were also partly additive (+117, +74, and 138 g/d for C, G, and CG, respectively). Infusion of casein tended to increase ($P = 0.10$), and infusion of glucose increased ($P < 0.05$) milk lactose yield.

Table 2. Least squares means for feed intake, rumen fermentation, and digestibility of diets.

	Nutrients infused postruminally ¹				SEM	P-value		
	Control	C	G	CG		C	G	C × G
DMI, kg/d								
Silage	13.0	12.0	12.0	11.7	0.32	0.11	0.08	0.39
Concentrate	6.8	7.3	7.5	6.9				
Total ²	19.8	19.6	19.8	19.2	0.37	0.38	0.54	0.54
ME intake, MJ/d ³	222	223	217	229	4.3	0.18	0.91	0.21
Rumen fermentation								
pH	6.26	6.10	6.04	6.07	0.068	0.39	0.10	0.22
NH ₃ -N, mmol/L	9.23	9.97	8.72	8.37	0.796	0.81	0.24	0.52
VFA, mmol/L	120	126	123	128	3.5	0.18	0.49	0.87
Molar proportion, mmol/100 mmol								
Acetate	65.5	64.5	64.1	64.0	0.49	0.30	0.10	0.42
Propionate	18.4	18.7	19.2	19.3	0.36	0.56	0.10	0.79
Butyrate	11.4	11.9	11.7	11.9	0.34	0.28	0.74	0.73
Digestibility, %								
OM	73.2	73.4	71.7	75.3	0.86	0.06	0.82	0.10
CP	68.9	70.7	67.6	71.9	1.44	0.07	0.99	0.39
NDF	65.9	65.1	63.6	66.7	0.79	0.21	0.68	0.05

¹Control = No infusion of nutrients; C = 300 g/d of casein infused postruminally; G = 300 g/d of glucose infused postruminally; CG = 300 g/d of casein and 300 g/d of glucose infused postruminally.

²Total DMI including infused nutrients.

³Metabolizable energy (ME) intake of cows as calculated on the basis of digestibility of OM measured and including infused nutrients.

Plasma Energy Metabolites and AA

Mammary plasma flow, arterial concentrations, calculated AV differences, extractions, and mammary uptakes of plasma energy-yielding metabolites as well as insulin concentration are given in Table 4. Combined infusion of nutrients reduced plasma flow less than infusion of these nutrients separately as indicated by an interaction between casein and glucose infusions in mammary plasma flows expressed as L/d ($P = 0.05$) or L/kg of milk ($P = 0.07$). Both casein and glucose infusions tended ($P = 0.11$) to increase arterial glucose concentra-

tion, but neither of them affected ($P > 0.05$) insulin or NEFA concentrations. Infusion of glucose decreased ($P < 0.01$) arterial concentration of BHBA. Infusion of glucose was also associated to the decreases in mammary AV difference of glucose ($P = 0.10$) and BHBA ($P < 0.05$), extraction of glucose ($P = 0.06$), and uptake of BHBA ($P = 0.07$). A significant interaction ($P = 0.02$) between casein and glucose infusions in mammary extraction of BHBA indicated that infusion of casein alone increased extraction but in the presence of glucose infusion decreased it.

Table 3. Least squares means for milk yield and milk components.

	Nutrients infused postruminally ¹				SEM	P-value		
	Control	C	G	CG		C	G	C × G
Yield, kg/d								
Milk	32.3	34.1	34.1	35.1	0.61	0.06	0.07	0.55
ECM ²	31.3	33.0	33.4	32.9	0.56	0.35	0.12	0.10
Milk composition, g/kg								
Protein	30.3	31.9	30.8	31.8	0.34	0.01	0.68	0.41
Lactose	48.3	48.3	48.8	48.8	0.19	0.32	0.16	0.32
Fat	40.2	38.1	39.8	35.9	1.56	0.10	0.44	0.58
Urea, mg/dl	26.8	29.5	25.3	28.0	0.74	0.01	0.08	0.99
Yield, g/d								
Protein	971	1088	1045	1109	24.5	0.01	0.10	0.32
Lactose	1558	1645	1659	1693	31.2	0.10	0.05	0.42
Fat	1266	1288	1347	1249	37.0	0.34	0.60	0.16

¹Control = No infusion of nutrients; C = 300 g/d of casein infused postruminally; G = 300 g/d of glucose infused postruminally; CG = 300 g/d of casein and 300 g/d of glucose infused postruminally.

²ECM = Energy-corrected milk; calculated according to Sjaunja et al. (1990).

Table 4. Least squares means for arterial plasma insulin concentration, mammary plasma flow, arterial plasma concentrations, extractions, arteriovenous (AV) differences, and mammary uptake of energy metabolites.

	Nutrients infused postruminally ¹				SEM	P-value		
	Control	C	G C	G		C	G	C × G
Insulin, mU/L	7.9	8.3	8.4	7.2	0.92	0.63	0.77	0.42
Mammary plasma flow								
L/d	20,592	14,360	14,028	17,415	2,022.5	0.51	0.42	0.05
L/kg of milk	668	426	411	504	75.2	0.36	0.28	0.07
Glucose, mmol/L								
Arterial	3.21	3.23	3.23	3.33	0.030	0.11	0.10	0.24
Difference	0.90	0.96	0.88	0.85	0.032	0.70	0.10	0.25
Extraction, %	28.5	29.8	27.5	25.8	1.38	0.85	0.06	0.21
Uptake, g/kg milk	101.1	70.9	63.7	73.6	10.64	0.38	0.15	0.11
NEFA, mmol/L								
Arterial	0.16	0.13	0.13	0.14	0.016	0.78	0.66	0.51
Difference	0.01	0.01	-0.01	0.01	0.011	0.81	0.77	0.63
Extraction, %	-11.8	-7.8	-28.2	-8.6	6.11	0.46	0.59	0.62
Uptake, g/kg milk	-0.33	0.75	-0.70	0.84	2.534	0.62	0.96	0.93
BHBA, mmol/L								
Arterial	0.99	0.99	0.77	0.73	0.033	0.77	0.01	0.81
Difference	0.32	0.42	0.30	0.27	0.027	0.37	0.05	0.09
Extraction	31.4	43.3	39.7	36.3	3.27	0.12	0.81	0.02
Uptake, g/kg milk	22.1	19.0	12.5	13.5	3.44	0.78	0.07	0.58

¹Control = No infusion of nutrients; C = 300 g/d of casein infused postruminally; G = 300 g/d of glucose infused postruminally; CG = 300 g/d of casein and 300 g/d of glucose infused postruminally.

Arterial plasma AA and urea concentrations are given in Table 5. Infusion of casein increased ($P \geq 0.06$) plasma urea, total AA (TAA), essential AA (EAA), and branched-chain AA (BCAA) concentrations, but had no effect on nonessential AA (NEAA) concentration. Of the individual AA, infusion of casein increased ($P \geq 0.02$) concentration of His, Ile, Leu, Lys, Trp, Val, Cys, Orn, and Pro, whereas infusion of glucose increased ($P \geq 0.02$) that of Asp and Gly. As for Orn, casein infusion alone increased concentration of Orn, the increase being, however, less in the presence of glucose infusion ($P < 0.05$ for interaction C × G).

Arteriovenous differences of AA by the mammary gland are presented in Table 6. Significant interactions ($P \geq 0.03$) between casein and glucose in AV differences of TAA, EAA and BCAA indicated that casein increased the response variables mentioned but the increase was compromised by simultaneous infusion of glucose. The same was true ($P < 0.03$) for AV differences of His, Leu, Lys, Met, Val, and Orn. Significant interactions between casein and glucose ($P \geq 0.05$) in AV differences of NEAA indicated that both of the infusions increased the response variable mentioned, but the effect was more pronounced when nutrients were infused separately rather than in combination. The same was found ($P < 0.001$) for AV difference of Phe.

Mammary extractions of AA are presented in Table 7. Mammary extraction of BCAA increased with glucose infusion, but the increase was compromised with simultaneous infusion of casein ($P < 0.01$ for C × G interac-

tion). Casein infusion decreased mammary extraction of His, especially in the presence of glucose infusion ($P < 0.04$ for C × G interaction). Mammary uptakes of AA (data not shown) were not affected by the nutrient infusions ($P > 0.05$) with the exception that infusion of glucose significantly ($P < 0.02$) reduced uptake of Arg. Uptakes of TAA were 29.8, 34.6, 32.4, and 36.0 g/kg of milk (SEM 2.40) for control, C, G, and CG treatments, respectively.

DISCUSSION

Nutrient Supply

Assuming metabolizable energy (ME) concentration of the control diet on the basis of the digestibility of OM measured the basal diet supplied about 101% of the ME and 104% of the amount of total AA required for the observed milk production (Tuori et al., 2000). As found earlier (Varvikko et al., 1999; Vanhatalo et al., 1999; Huhtanen et al. 2002), the nutrients infused did not significantly ($P > 0.05$) affect total DMI, rumen fermentation, or diet digestibility. The rumen fermentation pattern was in the range of that reported for diets comprising high amounts of restrictively fermented grass silage and a cereal-based concentrate (Huhtanen, 1998). However, the present proportion of propionate in the rumen was clearly higher and the proportion of butyrate lower as compared with the reported average values. Ammonia concentration in the rumen was high because of grass silage high in CP.

Table 5. Least squares means for arterial plasma AA and urea concentration ($\mu\text{mol/L}$).

AA ²	Nutrients infused postruminally ¹				SEM	P-value		
	Control	C	G	CG		C	G	C $\times$ G
Arg	68	79	60	72	6.5	0.12	0.28	0.91
His	27	52	31	51	2.8	0.001	0.63	0.42
Ile	108	142	104	122	5.6	0.001	0.07	0.21
Leu	104	147	105	133	7.2	0.001	0.36	0.33
Lys	87	126	100	114	8.3	0.02	0.97	0.17
Met	22	27	26	27	2.5	0.27	0.37	0.50
Phe	45	52	48	51	3.1	0.15	0.76	0.57
Thr	116	134	132	136	10.0	0.31	0.38	0.51
Trp	27	30	29	31	1.0	0.05	0.20	0.65
Val	203	280	204	253	11.7	0.001	0.30	0.27
Ala	227	250	260	254	17.1	0.64	0.33	0.41
Asn	58	68	63	67	5.7	0.27	0.74	0.60
Asp	5.4	6.2	6.5	6.8	0.25	0.09	0.02	0.29
Cit	87	98	102	101	7.1	0.51	0.25	0.46
Cys	21	27	25	27	1.2	0.02	0.17	0.14
Gln	242	244	276	258	18.4	0.67	0.24	0.60
Glu	50	52	51	50	2.2	0.79	0.87	0.49
Gly	307	297	334	327	6.9	0.29	0.01	0.82
3Methis	3.3	3.4	3.3	3.8	0.32	0.47	0.57	0.62
Orn	43	64	50	56	2.9	0.001	0.86	0.05
Pro	80	125	91	122	9.1	0.01	0.66	0.44
Ser	117	114	120	121	9.1	0.89	0.62	0.87
Tyr	47	60	52	59	5.6	0.13	0.76	0.68
Tau	33	39	45	39	2.7	0.99	0.07	0.07
BCAA	415	570	412	507	24.1	0.001	0.23	0.26
EAA	806	1069	837	990	51.3	0.01	0.66	0.32
NEAA	1155	1244	1280	1291	67.6	0.49	0.25	0.58
TAA	1961	2313	2117	2280	110.7	0.06	0.60	0.43
Urea	4804	5582	4954	5331	224.7	0.04	0.83	0.41

¹Control = No infusion of nutrients; C = 300 g/d of casein infused postruminally; G = 300 g/d of glucose infused postruminally; CG = 300 g/d of casein and 300 g/d of glucose infused postruminally.

²BCAA = Branched-chain AA (Val, Ile, and Leu). EAA = Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val). NEAA = Nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr). TAA = EAA + NEAA.

Lactation Responses

The small increase in ME intake owing to casein and glucose infusions was compromised with a minor decrease in grass silage intake so that total DMI or ME intake did not change between the treatments (Table 2). Thus, the responses obtained in the present study can be related to the changes in the supply of infused nutrients. Infusions of casein and glucose either separately or in combination clearly increased yields of milk, protein and lactose in the present study. This indicates that neither the supply of AA nor glucose on the control diet was sufficient for potential milk production despite cows being near energy balance and calculated adequacy in total supply of AA. The colimitation of glucose and AA in the present dietary conditions may be more closely emphasized by comparing production responses of the combined and separate infusions of casein and glucose. With combined infusion increases in the yields of milk protein (+138 g/d) and lactose (+135 g/d) were nearly equal, whereas respective increases with separate casein infusion were +117 g/d for protein and +74

g/d for lactose, and with separate glucose infusion +87 g/d for protein and +101 g/d for lactose. It can be calculated that the separate casein infusion accounted for 85% of the achieved protein response (117/138 g/d) and 64% of the achieved lactose response (87/135 g/d), whereas the separate glucose infusion accounted for 54% of the protein and 75% of the lactose responses, respectively. The greater response of protein than lactose produced with casein suggests that insufficient glucose supply possibly limited production response with casein alone treatment, whereas the greater lactose than protein response produced with glucose suggests that insufficient AA supply limited further production response with glucose alone treatment. Either way, increased lactose yield associated with the separate casein infusion puts forward that part of the casein AA were used for glucogenic purposes, whereas increased protein yield associated with separate glucose infusion may be explained with improved utilization of AA from the basal diet in the presence of glucose by sparing AA from utilization for gluconeogenesis.

Table 6. Least squares means for mammary arteriovenous difference ($\mu\text{mol/L}$) of plasma AA.

AA ²	Nutrients infused postruminally ¹				SEM	P-value		
	Control	C	G	CG		C	G	C $\times$ G
Arg	30.9	36.4	20.2	26.2	5.82	0.36	0.12	0.97
His	10.2	18.4	14.5	14.4	0.65	0.001	0.89	0.001
Ile	32.9	55.2	48.6	54.0	2.45	0.001	0.03	0.01
Leu	49.2	76.7	66.6	68.2	3.64	0.01	0.27	0.01
Lys	42.8	64.3	56.4	58.0	1.88	0.001	0.10	0.001
Met	11.3	15.7	15.1	14.1	0.86	0.10	0.25	0.02
Phe	16.0	25.8	23.9	23.0	1.08	0.02	0.05	0.001
Thr	18.6	34.7	32.4	33.8	4.37	0.09	0.19	0.15
Trp	0.8	2.6	4.6	4.2	1.18	0.58	0.06	0.40
Val	41.4	75.1	67.8	67.0	5.51	0.02	0.15	0.03
Ala	23.0	36.9	55.2	41.0	9.34	0.99	0.10	0.18
Asn	10.3	21.8	18.3	20.4	2.10	0.02	0.17	0.06
Asp	2.7	3.3	3.0	3.0	0.48	0.51	0.94	0.56
Cit	-3.0	0.9	7.6	5.2	5.45	0.90	0.22	0.58
Cys	-0.3	2.4	2.4	1.7	0.74	0.24	0.23	0.07
Gln	36.9	66.8	66.6	60.1	6.68	0.13	0.14	0.03
Glu	38.1	43.6	41.6	38.6	2.17	0.59	0.74	0.10
Gly	-16.4	20.3	24.6	17.8	10.31	0.20	0.11	0.08
3Methis	-0.6	0.1	-0.3	0.2	0.22	0.04	0.38	0.53
Orn	17.6	25.8	22.4	21.9	1.28	0.02	0.74	0.01
Pro	7.8	23.2	16.8	23.8	3.09	0.01	0.17	0.23
Ser	-5.4	19.3	16.1	18.2	5.78	0.06	0.13	0.10
Tyr	15.2	24.0	23.0	22.4	2.26	0.12	0.21	0.08
Tau	-0.6	3.4	3.4	3.0	0.97	0.11	0.11	0.06
BCAA	123.5	207.0	182.9	189.2	10.48	0.01	0.09	0.01
EAA	254.1	404.9	350.1	362.8	20.69	0.01	0.24	0.02
NEAA	111.9	261.4	267.4	247.0	35.10	0.12	0.09	0.05
TAA	366.0	666.3	617.5	609.8	52.97	0.03	0.12	0.03

¹Control = No infusion of nutrients; C = 300 g/d of casein infused postruminally; G = 300 g/d of glucose infused postruminally; CG = 300 g/d of casein and 300 g/d of glucose infused postruminally.

²BCAA = Branched-chain AA (Val, Ile, and Leu). EAA = Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val). NEAA = Nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr). TAA = EAA + NEAA.

As also found in the present study, abomasal infusions of casein have repeatedly increased milk protein concentration, yields of milk, and protein on grass silage-based diets irrespective of the stage of lactation or silage fermentation type (Choung and Chamberlain, 1993a; Huhtanen et al., 1997; Vanhatalo et al., 2003). However, the response mechanism to casein have depended on silage fermentation type extensively fermented silage benefiting from increased supply of AA and restrictively fermented silage in addition to AA also more indirectly from increased supply of glucose (Huhtanen et al., 1997; Vanhatalo et al., 2003). Increased lactose yield and milk urea concentration with separate casein infusion in the present study are in line with this explanation.

Opposite to casein infusions production responses to glucose infusions on grass silage based diets have been inconsistent. Calculation of the supply of glucose according to stoichiometric principles described elsewhere (Huhtanen, 1998) proposed that supply of glucose on the control diet was about 90% of that required for the observed milk production in the present study. The infusions of 300 g/d of glucose corresponded well

with the calculated deficit of 285 g/d of glucose and led to positive milk production responses being in good agreement with recent data (Hurtaud et al., 2000; Rigout et al., 2002). In contrast, glucose was not primarily limiting milk production of cows at midlactation (Kim et al., 2000; Vanhatalo et al., 2003). However, recent reports on significant production responses to intravascularly (Kim et al., 2001) or abomasally (Huhtanen et al., 2002) infused glucose, especially in the presence of His infusion, indicate that glucose alone was furthermore limited by lack of first-limiting AA in the diet. Therefore, reasons for the inconsistent responses to glucose infusions may lie not only in the stage of lactation (Vanhatalo et al., 2003) but also in the adequacy of other nutrients such as AA on the basal diet.

In contrast to earlier study (Huhtanen et al., 1997) milk fat concentration tended to decrease with casein infusion, probably reflecting the dilution of fat into the greater amount of milk produced with casein infusion. That glucose infusion did not affect milk fat concentration is in line with earlier studies, where relatively small amounts of glucose were infused (Kim et al., 2000; Huhtanen et al., 2002; Vanhatalo et al., 2003). Also,

Table 7. Least squares means for extraction (arteriovenous difference/arterial concentration) of plasma AA by the mammary gland (%).

AA ²	Nutrients infused postruminally ¹				SEM	P-value		
	Control	C	G	CG		C	G	C × G
Arg	45.1	47.0	28.7	33.2	8.82	0.73	0.14	0.89
His	45.6	40.2	56.3	30.9	3.91	0.01	0.87	0.04
Ile	31.0	38.6	46.2	44.2	1.58	0.13	0.001	0.03
Leu	47.6	52.4	62.9	50.9	3.36	0.32	0.09	0.05
Lys	50.1	50.5	56.2	50.9	4.20	0.58	0.46	0.52
Met	55.6	59.3	58.7	52.8	7.28	0.89	0.83	0.53
Phe	37.1	49.3	50.2	46.1	3.75	0.32	0.23	0.07
Thr	17.4	25.5	24.6	24.5	3.37	0.27	0.39	0.27
Trp	3.5	8.6	15.7	13.5	3.81	0.71	0.07	0.38
Val	20.5	26.4	33.0	26.0	2.04	0.81	0.02	0.02
Ala	10.4	14.4	21.1	16.1	3.39	0.88	0.12	0.23
Asn	19.5	30.6	28.2	30.4	3.72	0.12	0.30	0.28
Asp	48.7	54.0	45.8	44.8	7.55	0.79	0.45	0.69
Cit	-3.6	-8.3	5.6	5.2	8.15	0.76	0.21	0.80
Cys	-1.8	8.6	9.8	6.1	3.67	0.40	0.26	0.10
Gln	17.5	27.7	24.2	24.0	2.65	0.11	0.58	0.10
Glu	76.9	83.0	80.5	77.0	2.50	0.62	0.65	0.10
Gly	-4.9	6.6	7.3	5.3	2.97	0.16	0.11	0.06
3Methis	-19.1	0.0	-9.5	3.8	6.86	0.06	0.37	0.69
Orn	41.0	39.4	45.6	39.4	3.31	0.28	0.51	0.51
Pro	9.6	17.6	18.2	19.5	2.88	0.16	0.11	0.29
Ser	0.9	15.7	14.1	16.2	3.46	0.05	0.09	0.12
Tyr	35.6	39.4	44.6	38.6	5.48	0.85	0.48	0.41
Tau	-2.3	7.2	7.7	7.3	3.02	0.19	0.15	0.15
BCAA	30.0	35.9	43.9	36.9	1.82	0.80	0.01	0.01
EAA	31.8	37.4	41.3	36.2	2.46	0.84	0.14	0.07
NEAA	10.7	20.6	20.8	19.1	2.62	0.17	0.15	0.07
TAA	19.3	28.3	28.9	26.5	2.44	0.22	0.16	0.06

¹Control = No infusion of nutrients; C = 300 g/d of casein infused postruminally; G = 300 g/d of glucose infused postruminally; CG = 300 g/d of casein and 300 g/d of glucose infused postruminally.

²BCAA = Branched-chain AA (Val, Ile, and Leu). EAA = Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val). NEAA = Nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr). TAA = EAA + NEAA.

unchanged NEFA and insulin concentrations due to glucose infusions (Table 4) were in agreement with unchanged milk fat content.

N Utilization

The apparent N utilization (milk N/feed N) were 29.0, 31.0, 31.9, and 32.7% for control, C, G, and CG treatments, respectively, proposing that infusion of casein and glucose both improved N utilization, though glucose more than casein (+2.3 vs. +1.4% units). Higher N utilization with glucose than casein increment was in accordance with the reduced milk urea concentration with glucose infusion (28.2 vs. 26.7 mg/dl), suggesting that a greater proportion of AA with glucose than casein was partitioned into milk or tissue synthesis. For casein infusion increases in arterial plasma and milk urea concentrations (4.9 vs. 5.5 mM; 26.1 vs. 28.8 mg/dl, respectively), may be a consequence of the use of plasma AA for hepatic glucose synthesis. A tendency for increased plasma glucose concentration with infused casein supports this explanation. It should be noted, how-

ever, that present improved N utilization with incremental casein is rather exceptional. Generally, in spite of the positive production responses to protein supplement incremental N source in the diet impairs N utilization.

The marginal increases in milk protein yields for casein infusions (C and CG treatments) were 0.44 and 0.52 g/g, being in the range of values reported for casein (see Choung and Chamberlain 1993a). The clearly higher transfer rate for casein in the presence of infused glucose is parallel with our recent study in which efficiency of utilization of the first-limiting AA, His, increased from 11 to 24% with increased supply of glucose (Huhtanen et al., 2002). The positive interaction between increased AA and glucose supply in milk production has also been obtained in production trials. Cows given incremental AA supply in terms of rapeseed meal and incremental gluconeogenic precursors in terms of wet distillers' solubles increased milk protein yield by 55 and 52 g/d when given these supplements separately, but by 99 g/d when given them simultaneously (Huhtanen et al., 1995). A similar tendency was found when

supplementing restrictively fermented grass silage diet with incremental rapeseed meal and propylene glycol (Jaakkola et al., 1996). However, the same concept in another experiment (Shingfield et al., 2002) failed to result in a positive interaction. Lack of interaction was also found when supplementing corn silage-based diet with casein and propionic acid (Hurtaud et al., 1993). Differences in the lactation stage of cows in various experiments may explain contrasting results. It seems that positive responses to incremental AA in terms of casein (Hurtaud et al., 1993; Choung and Chamberlain 1993a, 1999b) or rapeseed meal (Saarisalo et al., 2002) can be expected even with well-fed cows at late stage of lactation producing less than 20 kg of milk per day, whereas positive responses to glucose supplement (present study; Hurtaud et al., 2000; Rigout et al., 2002) or interactions with AA and glucose supplements (present study; Huhtanen et al., 1995; Jaakkola et al. 1996) may rather be expected with cows at early stage of lactation producing more than 25 to 30 kg of milk per day. The positive responses related to increased AA supply irrespective of the production level may be due to qualitative improvements in amino acid profile of NAN entering to the intestines (Korhonen et al., 2002), whereas the positive responses to incremental glucose supply may be related to quantitatively increased glucose need especially at the early stage of lactation.

Plasma Metabolites and AA

Unchanged plasma insulin and NEFA concentrations as well as slight increases in arterial glucose due to infused nutrients were in line with other studies in which relatively small amounts of casein and glucose were infused (Kim et al., 2000; Vanhatalo et al., 2003). That arterial NEFA was relatively low lends support to the interpretation that cows, despite being in early stage of lactation, were near energy balance without any apparent need for mobilizing extra energy from body fat stores. As reviewed by Rigout et al. (2002) increases in insulin concentration is known to inhibit gluconeogenesis. Based on the unchanged insulin concentration and relatively small amounts of casein and glucose infused, it is probable that incremental nutrients did not decrease hepatic production of glucose in the present study. Even combined infusion of casein and glucose could not provide more than approximately 447 g/d of incremental glucose assuming a glucose yield of 55 g from 100 g of AA (Krebs, 1964). However, decreased AV differences and extraction efficiencies of glucose due to added glucose refer to sufficient glucose supply on the CG treatment.

In spite of the production responses obtained, the relationship between glucose AV difference and arterial

glucose concentration ($r^2 = 0.34$, $n = 16$) was relatively weak. This suggests that mammary glucose uptake is not necessarily dependent on arterial concentration, in contrast to previous findings with restrictively fermented grass silage (Miettinen and Huhtanen, 1997). The stable insulin concentration together with the higher plasma glucose concentration on the control diet in the present study may explain this. Decreased BHBA with glucose infusions may arise from the increased glucose supply. As reviewed by Ørskov et al. (1999), exogenous glucose has been attributed to antiketo-genic effects.

Plasma AA concentration of cows fed grass silage-cereal diets have been characterized with a low concentration of His (Vanhatalo et al., 1999; Kim et al., 2000; Korhonen et al., 2000). Increased plasma His as well as increased concentrations of other EAA with infused casein (Table 5) indicated increased supply of these AA for the metabolism of mammary gland in agreement with previous studies (Miettinen and Huhtanen, 1997; Vanhatalo et al., 2003). However, opposite to our previous findings present infusion of casein did not decrease concentration of NEAA, which together with increased plasma urea have been attributed to increased utilization of NEAA for glucose synthesis (Miettinen and Huhtanen, 1997; Vanhatalo et al., 2003). A possible reason for this discrepancy was that the proportion of propionate in the rumen on the control diet was presently higher than previously providing higher glucose supply.

Despite the fact that infused glucose did not affect arterial concentrations of AA, it did, however, increase plasma AV differences of these AA, although usually less than did infusion of casein ($P \geq 0.05$ for $C \times G$ interaction). This can be explained by higher extraction efficiencies of AA with glucose than with casein infusion over the control treatment ($P \geq 0.07$ for $C \times G$ interaction). Thus, as evidenced by increased protein yield with glucose treatment, it seems that by infusing glucose on grass silage diet it was possible to spare part of AA supply from utilization for gluconeogenesis. An alternative explanation for the increased protein yield with glucose infusion might be that AA present in excess could be secreted in greater amounts due to an increase in their transport in secretory vesicles, which is dependent on the colocation of the transport of lactose and protein in the mammary gland (Hurtaud et al., 2000).

As discussed in our recent paper (Huhtanen et al., 2002) calculating mammary plasma flow by Fick principle based on uptake and output of Phe and Tyr, should provide useful data on mammary metabolism of plasma nutrients. The reduced plasma flows due to infused nutrients in the present study obviously reflect improved balance in supply of limiting nutrients over the basal diet to meet glucose and AA needs of mammary

gland for supporting the increased milk protein and lactose yields. The higher plasma flow with combined rather than separate infusions indicated, however, that as soon as availability of both of the limiting nutrients increased simultaneously a more optimal balance in supply of nutrients could be achieved by increasing mammary plasma flow. This was evidenced by further increased milk protein and lactose yields with the combined infusion of C and G. The ability of mammary gland to adapt to changes in nutrient supply by regulating mammary blood flow has been demonstrated earlier in several studies (Bequette et al., 2000; Mackle et al., 2000; Huhtanen et al., 2002; Rigout et al. 2002).

CONCLUSIONS

Cows at early stage of lactation fed restrictively fermented grass silage diets appear to suffer from both limited glucose and AA supplies as indicated by partly additive increases in protein, lactose and milk yields achieved with abomasal infusions of casein and glucose. The casein-only associated response accounted for 85% of the protein yield attained, with combined glucose and casein infusion indicating that the potential protein yield could not be gained by only increasing AA supply. Part of the casein AA were likely used for glucogenic purposes as evidenced by increased lactose yield but also by increases in plasma urea and glucose concentrations. However, the glucose-only associated responses accounted for 75% of the lactose yield attained, with combined infusion of glucose and casein indicating that the potential lactose yield could not be gained by only increasing glucose supply when inadequate AA supply limited further production response. These data demonstrate that supplementing grass silage diet by appropriate AA and glucose supply, efficiency of use of nutrients particularly that of N can be improved. Further studies are needed to quantify lactational and metabolic responses as a function of graded amounts of added glucose on diets based on abundant use of restrictively fermented grass silage.

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