

## Effects of intravenous infusion of amino acids and glucose on the yield and concentration of milk protein in dairy cows

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**SUMMARY.** To test the hypothesis that the availability of glucose or its precursors can influence the response of milk protein concentration to the intravenous infusion of amino acids, five cows were used in a  $5 \times 5$  Latin square design with period lengths of 7 d. The five treatments were the basal diet of grass silage *ad lib.* plus 5 kg/d of a cereal-based supplement containing feather meal (Basal); Basal plus 4 g/d histidine, 8 g/d methionine and 26 g/d lysine (4H); Basal plus 8 g/d histidine, 8 g/d methionine and 26 g/d lysine (8H); and these two amino acid mixtures together with 600 g/d of glucose (4HG and 8HG respectively). Earlier experiments with this basal diet had shown that histidine was first-limiting for secretion of milk protein, followed by methionine and lysine. The yield of milk protein was increased progressively with the amount of histidine infused. The efficiency of transfer of histidine into milk protein was 0.42 for the 4H and 4HG and 0.35 for the 8H and 8HG treatments, and the concentration of milk protein was increased over Basal by all infusion treatments. However, milk protein concentrations were higher, and lactose concentrations in the milk were lower, in the absence of added glucose. Concentrations of insulin in blood plasma were not affected by treatment. It is concluded that, with the treatments without added glucose, a shortage of glucose prevented an increase in lactose secretion, and hence limited the increase in milk yield, leading to an increased concentration of protein in the milk.

**KEYWORDS:** Milk protein, diet, glucose, amino acid, dairy cow nutrition, milk yield.

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The influence of nutrition of the dairy cow on the concentration of protein in the milk is poorly defined. It is well established that an increase in the dietary intake of metabolizable energy (ME) from carbohydrate, particularly starch, leads to an increase in the concentration of milk protein (Emery, 1978; Coulon & Rémond, 1991) and that dietary inclusions of fat can reduce the milk protein concentration (Wu & Huber, 1994). However, responses to changes in the intake of protein itself are variable and unpredictable (Thomas & Chamberlain, 1984). Attempts to aid interpretation of responses by infusing nutrients direct into the postruminal gut have met with limited success. Although infusion of casein usually increased the yield of milk protein, changes in the concentration of milk protein were unpredictable (Clark, 1975; Choung & Chamberlain, 1993, 1995*a*). Similarly, infusion of glucose or starch

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gave inconsistent responses in the yield and concentration of milk protein (Thomas & Chamberlain, 1984; Reynolds *et al.* 1997).

Abomasal or intravascular infusions of the first-limiting amino acid, or groups of amino acids containing the first-limiting acid, have often led to increases in the concentration and yield of milk protein (Fisher, 1972; Choung & Chamberlain, 1995*b*; Metcalf *et al.* 1996; Kim *et al.* 1999) but, again, sometimes the response has been confined to an increase in the yield of milk protein (Vanhatalo *et al.* 1999; Kim *et al.* 2000). It is tempting to argue that the inconsistent responses might occur because of variations in the availability of glucose or its precursors. A shortage of glucose for lactose synthesis might prevent an increase in milk yield such that the concentration, as well as the yield, of milk protein would increase. On the other hand, the shortfall in glucose could be met from the amino acid pool, so limiting the use of amino acid for milk protein synthesis, such that the yield of milk protein increases proportionately less than milk volume, leading to a fall in the concentration of milk protein.

Hence, even from such a simple hypothesis, it is difficult to predict the overall effect on the concentration of milk protein. The aim in this experiment was to throw light on the subject by exploring the effects of the availability of glucose or its precursors on the response of milk production to intravenous infusion of a mixture of amino acids containing two levels of the first-limiting acid.

#### MATERIALS AND METHODS

##### *Animals and treatments*

Five Friesian cows in their second to fifth lactations were used. They were all between five and six weeks into their lactations at the start of the experiment and weighed, on average, 625 kg. The animals were housed individually in metabolism stalls and milked each day at 08.00 and 16.00. They were given access *ad lib.* to grass silage and received 5 kg/d (4.2 kg dry matter (DM)) of a pelleted mixture of rolled barley, feather meal and molassed sugar beet pulp (0.50:0.25:0.25, on a fresh weight basis). The silage was well preserved with a pH of 3.79 and contained (g/kg DM) total N, 21.6 (of which ammonia N constituted 128 g/kg N); lactic acid, 114; acetic acid, 14; butyric acid, 2; ethanol, 25; water-soluble carbohydrate, 17. The concentrate cube contained (g/kg DM) total N, 57.5; neutral-detergent fibre, 248; starch, 266; water-soluble carbohydrate, 76. The diet was calculated (AFRC, 1992) to supply ME about 10% in excess of requirement. Feed was offered in two equal meals and silage on offer was renewed each day at 08.00 and 16.00; intakes were measured daily and the amount of silage offered was adjusted to ensure refusal of ~ 15% of that offered.

The experiment was designed as a 5 × 5 Latin square with five treatments and five experimental periods each of 7 d. All animals received the basal diet for at least 21 d before the start of the experiment. The five treatments were the basal diet alone (Basal) and four infusion treatments supplying 4 g/d of histidine (4H) and 8 g/d of histidine (8H) without and with 600 g/d of glucose (4HG and 8HG). All the amino acid infusions also supplied 8 g/d of methionine and 26 g/d of lysine, the ratio of lysine to methionine being that found in milk protein (Kaufman, 1980). Amino acids (pharmaceutical grade; Forum Chemicals, Redhill RH1 6YS) were dissolved in 0.5 l saline (9 g NaCl/l), adjusted to pH 7.4, filtered (0.45 μm cellulose nitrate; Whatman, Maidstone ME16 0LS) and autoclaved at 121 °C for 15 min. Glucose solutions were made up separately by dissolving in 1.5 l of saline, filtering and autoclaving as for the amino acid solutions. The glucose solutions were added to the amino acid solutions

before infusing into the animals. An equivalent volume of autoclaved saline was added to the treatments without glucose, such that the infused volume with all treatments, except for Basal for which no infusion was given, was 2 l/d. Solutions were infused continuously during 23 h each day into a jugular vein through an indwelling polyethylene catheter (14 g × 8 cm; Arrow International, Reading, PA 19605, USA) using a volumetric infusion pump (Smith & Nephew Medicals, Hull HU3 2BN). Catheters were inserted on the first day of each infusion period. Milk yield was measured daily and milk composition was determined on a representative, composite sample from the last four consecutive milkings in each period. Blood samples were taken from the jugular catheter at 11.00, 12.30, 14.00 and 15.30 on the last day of each period. The infusion was stopped and the catheter flushed with sterile citrate saline before withdrawing the blood sample. Samples were taken into heparinized tubes and centrifuged immediately to separate plasma, which was stored at  $-20^{\circ}\text{C}$ .

#### *Chemical analysis*

Chemical analysis of feeds, milk and blood plasma was as described by Choung & Chamberlain (1993). Insulin was determined by radioimmunoassay using a commercial kit (Euro/DPC, Caernarfon, LL55 4EL).

#### *Statistical analysis*

For statistical analysis, mean values for feed intake and milk yield were taken for the last four days of each experimental period. At the beginning of the first period, one of the animals became lame and her feed intake dropped. She was treated and her intake recovered by the start of the second period. The results for this animal for the first period were excluded from the statistical analysis. The results were analysed by the mixed model procedure of Genstat 5 (Rothamsted Experimental Station) with cow and period as random effects and histidine infusion treatment and glucose infusion treatment as fixed effects in the model. The ANOVA model used was:

$$Y = \text{Mean} + \text{Cow} + \text{Period} + \text{Control} + \text{Control.Histidine} + \text{Control.Glucose} \\ + \text{Interaction} + \text{Error}$$

Control has two levels, histidine infusion has two levels and glucose infusion has two levels. The treatment effects tested were 'Control' for a difference between Basal and infusion treatments (CON), 'Histidine' for the difference between the two doses of histidine (HIS), 'Glucose' for the difference between histidine and histidine plus glucose (GLU) and 'Interaction' for histidine interaction by glucose (INT).

## RESULTS

Feed intake was not affected by treatment (Table 1). Milk yield was increased ( $P < 0.05$ ) over Basal by the two infusions containing glucose but not by the amino acid without added glucose. The yield of milk protein was increased ( $P < 0.05$ ) progressively with each dose of histidine, the inclusion of glucose having no effect. All infusion treatments increased ( $P < 0.05$ ) the concentration of milk protein over Basal but, for the histidine treatments without glucose, protein concentration was increased ( $P < 0.05$ ) progressively with level of histidine such that the value for 8H was greater ( $P < 0.05$ ) than for all the other infusions. The yield of fat was not affected by treatment but the concentration of fat was reduced ( $P < 0.05$ ) relative to Basal by 8HG treatment. None of the treatments affected the yield of lactose compared with Basal but the lactose output was lower ( $P < 0.05$ ) for 8H than for

Table 1. *Feed intake and milk production of cows consuming a basal diet of grass silage and a supplement containing feather meal and given intravenous infusions of amino acids without (4H,8H) or with (4HG, 8HG) added glucose*

	Treatment†					SED	P value‡			
	Basal	4H	8H	4HG	8HG		CON	GLU	HIS	INT
Silage intake (kg dry matter/d)	14.2	14.4	14.8	14.2	14.3	0.32	0.372	0.132	0.248	0.448
Supplement intake (kg dry matter/d)	4.2	4.2	4.2	4.2	4.2	—	—	—	—	—
Milk yield (kg/d)	28.7 <sup>a</sup>	29.4 <sup>ab</sup>	29.5 <sup>ab</sup>	29.7 <sup>b</sup>	30.3 <sup>b</sup>	0.41	0.011	0.102	0.217	0.420
Fat (g/kg)	40.1 <sup>b</sup>	39.3 <sup>ab</sup>	39.3 <sup>ab</sup>	38.5 <sup>ab</sup>	37.2 <sup>a</sup>	1.00	0.078	0.066	0.339	0.394
(g/d)	1152	1152	1152	1135	1119	28.6	0.581	0.249	0.711	0.695
Protein (N × 6.38) (g/kg)	28.5 <sup>a</sup>	30.0 <sup>b</sup>	31.4 <sup>c</sup>	29.7 <sup>b</sup>	30.3 <sup>b</sup>	0.43	< 0.001	0.040	0.006	0.189
(g/d)	814 <sup>a</sup>	878 <sup>b</sup>	921 <sup>c</sup>	878 <sup>b</sup>	916 <sup>c</sup>	14.6	< 0.001	0.787	0.002	0.794
Lactose (g/kg)	46.7 <sup>c</sup>	45.7 <sup>b</sup>	44.9 <sup>a</sup>	46.2 <sup>bc</sup>	46.0 <sup>b</sup>	0.28	< 0.001	0.002	0.022	0.177
(g/d)	1347 <sup>ab</sup>	1345 <sup>ab</sup>	1329 <sup>a</sup>	1375 <sup>ab</sup>	1397 <sup>b</sup>	25.3	0.486	0.020	0.850	0.308

† See text for details of treatments.

‡ Statistical significance by *F*-test (see text for details).

<sup>a,b,c</sup> Means in the same row without a common superscript were significantly different:  $P < 0.05$  (*t*-test).

Table 2. *The concentrations of glucose, urea and insulin in the blood plasma of cows consuming a basal diet of grass silage and a supplement containing feather meal and given intravenous infusions of amino acids without (4H,8H) or with (4HG, 8HG) added glucose*

	Treatment†					SED	P value‡			
	Basal	4H	8H	4HG	8HG		CON	GLU	HIS	INT
Glucose (mg/l)	641 <sup>ab</sup>	663 <sup>abc</sup>	622 <sup>a</sup>	725 <sup>c</sup>	691 <sup>bc</sup>	30.4	0.180	0.011	0.108	0.859
Urea (mg/l)	154 <sup>b</sup>	138 <sup>ab</sup>	131 <sup>a</sup>	131 <sup>a</sup>	130 <sup>a</sup>	8.4	0.009	0.551	0.507	0.579
Insulin (ng/ml)	0.16	0.17	0.17	0.18	0.19	0.016	0.138	0.262	0.841	0.380

† See text for details of treatments.

‡ Statistical significance by *F*-test (see text for details).

<sup>a,b,c</sup> Means in the same row without a common superscript were significantly different:  $P < 0.05$  (*t*-test).

8HG. The concentration of lactose was decreased progressively ( $P < 0.05$ ) with histidine dose in the absence of added glucose but, in the presence of added glucose, the reduction over Basal was significant ( $P < 0.05$ ) only for 8HG and, even then, the effect was much less ( $P < 0.05$ ) than for 8H.

Only the 4HG infusion affected ( $P < 0.05$ ) the Basal concentration of glucose in the blood plasma (Table 2). The concentration of blood urea was reduced relative to Basal ( $P < 0.05$ ) by all treatments except 4H for which the reduction was not significant ( $0.10 < P > 0.05$ ). The concentration of insulin in blood was not affected by treatment.

The concentrations of the infused amino acids (His, Met, Lys) in blood plasma were increased over Basal by all the infusions ( $P < 0.05$ ) but, for histidine, the increase in concentration was seen only for the higher level of infusion (Table 3). Inclusion of glucose lowered (at least  $P < 0.05$ ) the plasma concentrations of branched-chain amino acids (BCAA; valine, leucine, isoleucine). Concentrations of arginine and ornithine were increased ( $P < 0.05$ ) over Basal by the 8H treatment.

Table 3. The concentrations ( $\mu\text{mol/l}$ ) of free amino acids in the blood plasma of cows receiving a basal diet of grass silage and a supplement containing feather meal without and with intravenous infusions of amino acids without (4H,8H) or with (4HG, 8HG) added glucose

	Treatment†					SED	P value‡			
	Basal	4H	8H	4HG	8HG		CON	GLU	HIS	INT
Histidine	11 <sup>a</sup>	13 <sup>a</sup>	27 <sup>b</sup>	14 <sup>a</sup>	25 <sup>b</sup>	2.5	< 0.001	0.908	< 0.001	0.428
Threonine	147	145	135	163	162	12.9	0.693	0.030	0.613	0.642
Arginine	62 <sup>a</sup>	69 <sup>ab</sup>	79 <sup>b</sup>	64 <sup>a</sup>	64 <sup>a</sup>	5.9	0.191	0.028	0.232	0.257
Tryptophan	34	34	33	32	34	2.7	0.507	0.673	0.762	0.453
Methionine	12 <sup>a</sup>	22 <sup>b</sup>	21 <sup>b</sup>	20 <sup>b</sup>	22 <sup>b</sup>	1.9	< 0.001	0.502	0.512	0.339
Valine	237 <sup>bc</sup>	225 <sup>abc</sup>	241 <sup>c</sup>	189 <sup>a</sup>	192 <sup>ab</sup>	21.0	0.156	0.014	0.552	0.683
Phenylalanine	42	41	41	37	38	3.1	0.251	0.144	0.744	0.762
Isoleucine	94 <sup>bc</sup>	100 <sup>bc</sup>	102 <sup>c</sup>	73 <sup>a</sup>	82 <sup>ab</sup>	8.7	0.449	0.002	0.367	0.623
Leucine	93 <sup>b</sup>	88 <sup>b</sup>	90 <sup>b</sup>	66 <sup>a</sup>	66 <sup>a</sup>	7.7	0.027	0.002	0.857	0.848
Lysine	46 <sup>a</sup>	73 <sup>bc</sup>	77 <sup>c</sup>	61 <sup>b</sup>	66 <sup>bc</sup>	6.5	< 0.001	0.024	0.371	0.909
Aspartic acid	4 <sup>a</sup>	4 <sup>a</sup>	9 <sup>b</sup>	3 <sup>a</sup>	5 <sup>ab</sup>	2.1	0.618	0.138	0.087	0.262
Glutamic acid	38 <sup>a</sup>	40 <sup>ab</sup>	44 <sup>bc</sup>	45 <sup>bc</sup>	47 <sup>c</sup>	2.4	0.005	0.042	0.081	0.713
Serine	144	135	132	150	135	8.1	0.390	0.138	0.139	0.363
Glycine	329 <sup>a</sup>	348 <sup>a</sup>	323 <sup>a</sup>	452 <sup>b</sup>	364 <sup>ab</sup>	45.9	0.266	0.045	0.108	0.357
Alanine	132	152	156	152	158	13.5	0.061	0.907	0.608	0.974
Tyrosine	69	65	62	64	58	5.5	0.141	0.496	0.270	0.666
Asparagine	44	46	46	43	46	4.0	0.612	0.658	0.580	0.672
Taurine	36	34	29	35	29	4.1	0.198	0.841	0.074	0.961
Glutamine	172	188	162	193	186	16.1	0.450	0.238	0.165	0.419
Ornithine	28 <sup>a</sup>	31 <sup>ab</sup>	35 <sup>b</sup>	28 <sup>a</sup>	30 <sup>a</sup>	2.0	0.132	0.007	0.064	0.559
Total AA	1772	1850	1841	1881	1807	103.6	0.390	0.983	0.585	0.663

† See text for details of treatments.

‡ Statistical significance by *F*-test (see text).

<sup>a,b,c</sup> Means in the same row without a common superscript were significantly different:  $P < 0.05$  (*t*-test).

## DISCUSSION

The experimental conditions were contrived to maximize the likelihood of exposing an influence of the availability of glucose or its precursors on the response to infusion of the amino acids limiting milk protein synthesis. We chose the particular basal diet because experiments in this laboratory clearly identified histidine as the first-limiting amino acid for milk protein production, and methionine and lysine as the next-limiting acids with this diet (Choung & Chamberlain, 1995*a, b*; Kim *et al.* 1999). Also, the amounts of histidine infused gave linear increases in the yield of milk protein when given with the amounts of methionine and lysine used here (Kim, 1999). The responses in the yield of milk protein to the infusion of the two levels of histidine in the present experiment were similar to those seen in the earlier experiments. It has been suggested that dairy cows consuming grass silage diets can be short of glucose to meet the demands of lactose synthesis (Huhtanen *et al.* 1997). This hypothesis was tested (Kim *et al.* 2000) and glucose supply was shown not to be first-limiting for milk production. However, the possibility remains that, if glucose were the second-limiting nutrient, its shortage might prevent full expression of the response of milk output to provision of amino acids, as suggested by Vanhatalo *et al.* (1999).

Our aim with the glucose infusion was to raise the plasma concentration of glucose but to avoid an increase in plasma insulin concentration, which would complicate the interpretation of the results, since it has been claimed that insulin can regulate the concentration of protein in milk (Griinari *et al.* 1997; Mackle *et al.* 1999).

From published work (Hurtaud *et al.* 1998), the amount of glucose infused was thought unlikely to increase plasma concentrations of insulin. Another concern was to keep the amount of glucose infused relatively small so as to have minimal effect on the total ME supply because an increase in ME supply, in itself, can lead to an increased concentration of milk protein (Coulon & Rémond, 1991).

It was thought at the outset that a shortage of glucose would affect the response to amino acid infusion in one of two ways. Either a greater use of amino acids for gluconeogenesis, at the expense of milk protein synthesis, would lower the potential response of milk protein yield but lactose output, and hence milk yield, would increase by a proportionately greater amount, leading to a fall in the concentration of milk protein. Or, milk protein synthesis would take priority in amino acid use, at the expense of glucose production, leading to an increased concentration of protein in the milk because the shortage of glucose would allow little, if any, increase in lactose output, and hence little increase in milk yield. The results tended to follow the latter pattern, suggesting that the increased concentration of milk protein with the treatments without added glucose was caused by a shortage of glucose. Preventing the shortfall by including glucose in the infusions, led to an increase in lactose output ( $P = 0.002$ ) and milk yield ( $P = 0.102$ ), and a corresponding fall in milk protein concentration ( $P = 0.04$ ) (Table 1). It is not known why the alternative pattern of response referred to above did not occur, despite appearing equally likely in theory, although it is worth noting that, to our knowledge, there is no clear demonstration of this pattern of response in published work. The efficiency of transfer of infused histidine into milk protein was unaffected by the presence of glucose in the infusate, indicating that the use of histidine for gluconeogenesis was not influenced by the availability of glucose. Assuming milk protein contains 26 g histidine/kg (Kaufman, 1980), the efficiency of transfer was 0.42 and 0.35 for the 4H and 8H treatments, respectively, with or without glucose; very similar values were found previously for this diet (Kim, 1999).

In experiments in which amino acids were infused intravascularly and glucose flux measured (Danfaer *et al.* 1995), an increased use of amino acids for milk protein synthesis went hand in hand with an increased use of amino acids in gluconeogenesis. Because glucose flux was not measured, any comments on the use of amino acids for gluconeogenesis in the present experiments must be speculative. All that can be said is that there were no clear signs of effects of glucose infusion on gluconeogenesis. Glucose infusion lowered plasma concentrations of arginine and ornithine (Table 3) but did not affect blood urea concentrations, despite lowering concentrations of BCAA. However, it is worth making the point that if the main glucose precursor, propionate, was in short supply with this diet (Huhtanen *et al.* 1997), it is conceivable that amino acids were already heavily used for gluconeogenesis. Diversion of amino acids to milk protein synthesis, in response to the increased supply of histidine, might then reduce the availability of glucose precursors.

Lowering plasma concentrations of glucose to  $< 500$  mg/l by infusion of insulin reduces secretion of lactose, leading to a corresponding fall in milk yield (Rook & Thomas, 1983). In these circumstances, the concentration of protein in the milk can rise markedly (Rook & Thomas, 1983; Thomas *et al.* 1987). The present results imply that glucose was in short supply for lactose synthesis despite the fact that plasma concentrations of glucose, at  $> 600$  mg/l, were well above the level usually associated with rate-limiting effects on lactose synthesis ( $< 500$  mg/l; Rook & Thomas, 1983). However, Huhtanen *et al.* (1993) suggested that lowered concentrations of lactose in the milk might indicate limitations on glucose supply to the



mammary gland. Moreover, reductions in lactose concentration in milk at similar plasma glucose concentrations to those in the present experiment were seen when the availability of glucose precursors was reduced by substituting butyrate for propionate in intraruminal infusions (Huhtanen *et al.* 1993; Miettinen & Huhtanen, 1996). This raises the question of whether increased concentrations of milk protein in response to intravascular infusions of amino acids are always accompanied by reduced concentrations of lactose in the milk. A glance at recently published results rules this out, however, because increases in milk protein concentration have sometimes (e.g. Metcalf *et al.* 1996), but not always (e.g. Kim *et al.* 2000), been accompanied by falls in lactose concentration.

In conclusion, the present results show that the availability of glucose or its precursors can affect the response to intravascular infusion of limiting amino acids, such that a shortage of glucose can lead to an increase in the concentration of milk protein. Furthermore, the changes in milk protein concentration occurred in the absence of any change in circulating levels of insulin. Of course, this is only one mechanism whereby increases in amino acid supply might lead to increases in the concentration of milk protein. Further research will be needed to establish the overall relevance of these findings to practice.

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