Effects of Changes in Dietary Amino Acid Balance on Milk Yield and Mammary Function in Dairy Cows

J. -M. Yeo, C. H. Knight, and D. G. Chamberlain

Hannah Research Institute, Ayr, KA6 5HL, UK

ABSTRACT

Two experiments were conducted to determine whether longer-term deficiencies in the supply of limiting amino acids would be accompanied by a decline in mammary function (total DNA, cell proliferation rate and activities of key enzymes), and whether this would adversely affect the cow's ability to respond to a return to a nutritionally adequate diet. The first experiment was performed in early/mid lactation, and the second, using the same cows, was carried out in mid/late lactation. A control group of six cows were given a grass silage-cereal diet containing fish meal as the sole protein supplement (amino acid adequate) throughout the experiments, whereas another group of six cows in treatment received the control diet for 2 wk (lactation wk 5 and 6) and then were changed to a diet in which the fish meal was replaced by an equivalent amount of protein as feather meal (amino acid deficient) for 6 wk before returning to the fish meal diet for 4 wk (Experiment 1). After a rest period of 5 wk, the experimental procedure was repeated (Experiment 2). Although there was a fall in milk yield as lactation advanced, leading to lower milk yields in Experiment 2, the marked difference in milk yield between treatments was similar for the two stages of lactation (21% vs 16% in Experiment 1 and 2, respectively). In both experiments, the marked fall of milk yield in cows given the feather meal diet was completely recovered by a return to the fish meal diet. Despite the markedly lower milk yield with the amino acid-deficient diet, however, there was no clear evidence of corresponding changes in measurements of mammary function.

(**Key words:** dairy cow, amino acid, milk yield, mammary function)

Abbreviation key: ACC = acetyl CoA carboxylase, DOMD = digestible organic matter in the dry matter, EAA = essential AA, FAS = fatty acid synthetase, GT = galactosyltransferase, ME = metabolizable energy,

NEAA = nonessential AA, PCNA = proliferating cell nuclear antigen, TAA = total AA.

INTRODUCTION

Results of short-term experiments show that, in cows consuming a diet of grass silage and a cereal-based supplement containing feather meal as the sole protein supplement, milk yield is limited by a deficiency in the supply of specific AA (His, Met and Lys), and that milk yield increases markedly in response to intravenous infusions of the limiting AA (Kim et al., 1999; 2000). Furthermore, the response of milk production is seen within 24 hours of starting the infusion. Although changes in mammary function were not measured in those experiments, it would seem unlikely that such a rapid response would be accompanied by a change in mammary function in terms of cell numbers and enzyme activities. On the other hand, in longer term experiments, changes in mammary function appear to be correlated with changes in milk production, and it has been suggested that a gradual decrease in the number of mammary cells largely accounts for the decline in milk production with advancing lactation (Knight and Peaker, 1984; Capuco et al., 2001).

The apparent discrepancies raise the question, whether longer-term deficiencies in the supply of limiting AA would be accompanied by a decline in mammary function and whether this, in turn, would adversely affect the cow's ability to respond to a return to a nutritionally adequate diet. The two experiments reported here were designed to answer those questions. The first experiment was performed in early/mid lactation, and the second, using the same cows, was carried out in mid/late lactation. A control group were given a diet based on grass silage and cereal containing fish meal as the sole protein supplement throughout the experiments, whereas cows in the treatment group received the fish meal diet for 2 wk (lactation wk 5 and 6) and were then changed to a diet in which the fish meal was replaced by an equivalent amount of protein as feather meal for 6 wk before returning to the fish meal diet for 4 wk. After a rest period of 5 wk, the experimental procedure was repeated in the second experiment.

Received September 25, 2002.

Accepted December 19, 2002.

Corresponding author: D. G. Chamberlain; e-mail: chamberlaind@hri.sari.ac.uk.

Table 1. The chemical composition (% of DM, unless stated otherwise) of the fish meal cube, feather meal cube and sugar beet pulp used in the experiments.

	Fish meal cube	Feather meal cube	Sugar beet pulp
DM, %	86.9	88.4	87.2
Organic matter	87.8	91.7	88.2
Total N	4.73	4.82	1.79
Starch	27.7	26.6	0.5
Sugars	6.1	7.6	21.9
Neutral-detergent fiber	26.9	26.5	35.0
Acid-detergent fiber	6.9	8.6	20.0

MATERIALS AND METHODS

Cows and Their Management

The Home Office Inspectorate (United Kingdom) and the Ethical Review Committee of the Hannah Research Institute approved all animal handling and experimental procedures. Twelve Friesian cows in their 1st to 5th lactations were used in Experiments 1 and 2, and yielded on average about 28 kg/d before the start of Experiment 1. They were 3 to 8 and 15 to 24 wk into their lactation at the start of Experiments 1 and 2, respectively. Average BW of the cows was 571 kg (SD = 53) in Experiment 1 and 621 kg (SD = 56) in Experiment 2. The animals were housed individually in metabolism stalls with water freely accessible and were milked each day at 0700 h and 1500 h. Food was provided in two equal meals each day, immediately after each milking.

In both experiments cows were given a basal diet consisting of ad libitum access to grass silage and 3 kg/d of sugar beet pulp. The amount of silage offered was adjusted to ensure a daily refusal of around 15% of that offered. For both experiments, the silage was made from perennial ryegrass (*Lolium perenne*) cut at an early stage of growth and ensiled with the addition of an inoculum of *Lactobacillus plantarum* (Ecosyl; ICL pcl, Billingham, UK) at 10^6 cfu/tonne in a bunker silo of 70-tonne capacity. The silage DM was 23.8% and 22.1% with pH 3.9 and 3.8 in Experiment 1 and 2, respectively. The concentrations (% of DM) of other constituents were as follows: total nitrogen 2.42 and 2.09 of which 9.6% and 11.6% were present as ammonia in Experiment 1 and 2, respectively; lactic acid 12.5 and 11.4; water soluble carbohydrate 3.8 and 1.7; digestible organic matter in the dry matter (DOMD) 63.1 and 66.3 in Experiment 1 and 2, respectively. The chemical compositions of the supplements are shown in Table 1. For both experiments, forage accounted for about 65% of DMI.

Experimental Treatments and Design

Experiment 1. Experiment 1 was carried out between lactation wk 5 and 16. The experiment consisted of three periods. Cows were given a control diet consisting of the basal diet as described above plus 5 kg/d of a supplement containing fish meal (a pelleted mixture of 50% rolled barley, 30% fish meal and 20% citrus pulp on a fresh weight basis) for 2 wk in period 1 and split, on the basis of parity and milk yield, into two groups at the end of period 1. The control group continued on the control diet for the whole experiment. For the treatment group, the fish meal was replaced by an equivalent amount of RUP (values taken from Chamberlain et al., 1992) as 5 kg/d of a supplement containing feather meal (a pelleted mixture of 50% rolled barley, 25% feather meal and 25% citrus pulp on a fresh weight basis) for 6 wk in period 2, after which they received the control diet for 4 wk in period 3. Both concentrate cubes were formulated to supply vitamins and minerals to meet the requirements of lactating dairy cows (Agricultural Research Council, 1980).

Food intake and milk yield from each half-udder (two diagonally opposed quarters) were recorded daily. The composition of milk was determined on a representative, composite sample from four consecutive milkings just before the last day of each period. Samples of blood were taken from a tail vessel at 0930 h and 1400 h on the day before the end of each period. The volume of the empty udder was determined by the method of Knight and Dewhurst (1994) using a quick-setting polyurethane foam. Mammary tissue biopsies were obtained from one quarter (right hind or left front), following the procedure of Knight et al. (1992) except that a biopsy instrument (C. R. Bard Inc, Covington, USA) was used. Measurements of empty udder volume were made before the mammary tissue biopsy on the last day of each period. Immediately after its removal, a portion of mammary tissue was stored in liquid nitrogen for the determination of key enzyme activities and DNA content. A further portion of tissue was placed in fixative (4% formalin, pH 7.4) for determination of proliferating cell nuclear antigen (PCNA) as an indicator of cell proliferation.

Experiment 2. Cows were given a rest period of 5 wk after completion of Experiment 1. During the rest period, cows were given access to a bare grass field for a restricted time (around 6 h) during the day and each group was housed separately in metabolism cubicles for feeding a mixture of 89% silage, 3% fish meal cube as described above and 8% sugar beet pulp. Experiment 2 repeated the procedure used in Experiment 1 between lactation wk 22 and 32 in the same cows, with the

exception that, owing to a shortage of experimental silage, period 1 consisted of 1 wk instead of 2 wk.

Recording of food intake and milk yield and composition was as described for Experiment 1. As in Experiment 1, samples of blood and mammary tissue were taken, and the volume of the empty udder determined.

Chemical Analysis

Feeds, milk and blood were analysed as described previously (Kim et al., 1999). DOMD in silage was determined by the method of Morrison (1972). Mammary tissue samples were analysed for acetyl CoA carboxylase (ACC) (EC 6.4.1.2), fatty acid synthetase (FAS) and galactosyltransferase (GT) (EC 2.4.1.22) as described previously (Wilde et al., 1986), under conditions in which activity was linearly related to the amount of sample and incubation time. DNA concentration was measured in tissue homogenates by a fluorimetric method (Labarca and Paigen, 1980); total DNA was calculated by multiplying DNA concentration (mg/g) by udder weight (kg), calculated from udder volume and density measurements (Knight and Dewhurst, 1994). PCNA (Experiment 1) was analysed by immunohistochemistry using the Streptavidin-biotin staining procedure as described by DAKO (Cambridge, UK). Cells that stained positive for PCNA were quantified by using an image analysis system (Leica Q500MC, Nussloch, Germany) where at least 1000 nuclei per slide were counted, and PCNA was expressed as proportion of total cells.

Statistical Analysis

For statistical analysis, mean values for feed intake and milk yield were taken for the last 7 d of each experimental period. Treatment differences were compared for period 2 and 3 separately by analysis of covariance with the period 1 response as a covariate, using the directives of Genstat 5 (Lawes Agricultural Trust, Rothamsted, Herts, UK). One of the cows became lame during period 1 in Experiment 1 and was replaced before the start of period 2. In Experiment 1, one cow between lactation wk 10 and 12 and another cow in lactation wk 14 were unwell, and their milk yield and silage intake decreased. Although they recovered well during the rest of the experiment, the results for these animals for those lactation wk were omitted from the statistical analysis. In Experiment 2, one cow became unwell between lactation wk 23 and 26, and her silage intake and milk yield decreased. She recovered well during the rest of the experiment, but the results for this animal for those lactation wk were excluded from the statistical analysis.

Figure 1. Half-udder milk yield (kg/d) in Experiments 1 and 2. The control group (●) received fish meal throughout the experiments; the treatment group (○) received fish meal until wk 6 of lactation when they were changed to feather meal before returning to fish meal for 4 wk (Experiment 1). After a break of 5 wk, the experimental procedure was repeated using the same cows (Experiment 2).

RESULTS

Experiment 1

To present an overview of the results from the two experiments, milk yields from Experiments 1 and 2 are summarized in Figure 1. Milk yield from the half-udder from which the mammary biopsy was taken is not presented because milk yield was markedly decreased by the procedure, and recovery took approximately 2 wk. The mean rate of decline in milk yield in the control group was 1.9% and 1.6% per wk for Experiments 1 and 2, respectively. During period 1 in Experiments 1 and 2, when both groups were given the control diet, there were no significant differences in milk yield between control and treatment groups. In period 2, however, when the treatment group was transferred to the feather meal diet, the half-udder milk yield of the treatment group fell such that it was about 1 kg/d less than the control after 2 wk and about 2 kg/d less than the control after 6 wk. In Experiment 2, the half-udder yield of the treatment group was again about 1 kg/d less than

Table 2. Feed intake, half-udder milk production and body weight in Experiment 1.

	Period 1			Period 2			Period 3		
	Control ¹	Treatment	SED ²	Control	Treatment	SED	Control	Treatment	SED
DMI, kg/d									
Silage	12.3	12.0	0.78	12.8	11.2	0.48*	12.7	12.6	0.40
Total	19.2	19.1	0.78	19.8	18.1	0.48*	19.7	19.6	0.40
Milk yield, kg/d	14.7	14.4	0.88	13.0	10.3	0.94*	12.1	11.8	1.02
Protein, %	3.08	2.97	0.08	3.22	2.97	0.13	3.28	3.32	0.12
Protein, g/d	451	424	19.2	415	304	22.4**	395	392	27.9
Fat, %	3.56	3.92	0.27	3.70	4.31	0.20*	3.67	4.06	0.20
Fat, g/d	522	563	52.3	468	448	24.4	455	469	51.4
Lactose, %	4.87	4.94	0.08	4.74	4.66	0.10	4.67	4.60	0.11
Lactose, g/d	715	709	42.5	616	479	48.8*	569	540	53.6
Body weight, kg	607	575	29.7	605	610	15.9	607	617	17.4

¹The control group received fish meal throughout the experiments; the treatment group received fish meal in period 1 and then they were changed to feather meal in period 2 before returning to fish meal in period 3.

²SED, standard error of differences.

* $P \leq 0.05$.

** $P \leq 0.01$.

control by wk 2 but this time the difference between treatment and control had not increased by the end of the period. In both experiments, the fall of milk yield in the treatment group was completely recovered by a return to the control diet for 4 wk.

Results for feed intake, milk production and BW in Experiment 1 are shown in Table 2. When the fish meal diet was replaced by the feather meal diet in period 2, silage intake fell ($P < 0.05$). Yields of protein and lactose in the treatment group were reduced at the end of period 2 compared with those in the control group ($P < 0.01$ and $P < 0.05$, respectively). Changing the protein supplement in the diet did not affect milk fat yield but the concentration of milk fat was increased ($P < 0.05$) in period 2. The concentration of milk protein in the treatment group tended to be decreased ($P < 0.10$). When the treatment group returned to the control diet, after 1 wk, there were no significant differences in feed intake and milk production between treatments.

The concentrations of plasma free AA are shown in Table 3. The concentrations of Arg, Met, Lys, Glu and Asn were significantly lower ($P < 0.05$) in the treatment group than in the control group in period 2. Increases in the concentrations of Ser and Pro in the treatment group were evident at the end of period 2. There were no significant differences in the concentration of plasma AA between treatments in period 3.

Except that FAS activities and PCNA were lower ($P < 0.05$) in the treatment group than in the control group in period 1, no significant differences were found in mammary enzyme activities, PCNA, total DNA or udder volume between treatments in any period (Table 4).

Experiment 2

Results for feed intake, milk production and BW are shown in Table 5. There were no significant differences

in silage intake between treatments in any period. Yields of protein and lactose in the treatment group were significantly decreased ($P < 0.01$ and $P < 0.05$, respectively) at the end of period 2 compared with those in the control group. Although there was a tendency ($P < 0.10$) for the concentration of milk fat to increase with the feather meal diet at the end of period 2, no significant differences in milk fat yield between treatments were found throughout the experiment. BW was lower for the treatment group than for the control group in period 2. After the treatment group had been returned to the control diet for 1 wk, there were no significant differences in feed intake and milk production between treatments in period 3.

The concentrations of plasma free AA are shown in Table 6. Concentrations of Met and Lys were significantly lower in the treatment group than in the control group in period 2. The feather meal diet increased the concentrations of Val, Phe, Ser, Tyr and Pro at the end of period 2. There were no significant differences in the concentrations of plasma AA between treatments in period 3.

Activities of GT were lower ($P < 0.05$) and of FAS tended to be lower ($P < 0.10$) in the treatment group than in the control group in period 2 (Table 7). No significant differences were found for ACC activities, total DNA or udder volume between treatments in any period.

DISCUSSION

Although there was a fall in milk yield as lactation advanced, leading to lower milk yields in Experiment 2, the difference in milk yield between the two protein supplements was similar for the two stages of lactation (21% vs 16% in Experiment 1 and 2, respectively). The

Table 3. The concentrations ($\mu\text{mol/l}$) of plasma free AA in Experiment 1.

AA ³	Period 1			Period 2			Period 3		
	Control ¹	Treatment	SED ²	Control	Treatment	SED	Control	Treatment	SED
His	19	19	3.6	15	15	2.6	15	16	2.4
Thr	126	119	11.9	119	90	12.5	125	124	12.7
Arg	66	68	5.3	80	52	7.3**	86	81	14.1
Trp	37	38	2.2	35	28	3.0	41	40	3.3
Met	22	24	2.7	24	10	1.3***	26	25	2.4
Val	189	209	17.4	202	269	35.3	196	203	31.9
Phe	40	46	2.6	41	46	4.6	41	44	2.6
Ile	119	141	18.6	106	96	17.8	107	114	19.6
Leu	100	103	9.3	89	95	12.0	91	98	10.9
Lys	69	72	4.1	69	36	10.8*	75	74	11.3
Asp	11	9	1.0	0	0	0	0	0	0
Glu	71	74	3.1	55	42	4.3*	53	53	4.4
Ser	101	104	12.8	75	105	11.1*	90	99	6.8
Gly	458	437	62.6	442	474	71.3	367	405	42.5
Ala	193	160	24.9	166	142	17.1	174	202	13.9
Tyr	51	58	5.6	77	76	4.8	65	67	4.6
Asn	44	46	1.9	50	32	4.2**	52	54	4.1
Tau	44	35	2.9*	46	34	10.6	42	34	14.0
Glu	300	337	61.1	257	243	45.8	305	294	18.0
Orn	37	33	2.9	30	27	4.8	34	35	4.7
Pro	56	49	4.5	45	68	7.7*	59	73	9.2
EAA	786	839	50.0	775	740	80.7	799	822	96.6
NEAA	1365	1343	77.8	1252	1231	135.4	1249	1306	94.0
TAA	2152	2182	105.5	2029	1971	176.4	2051	2125	161.7

¹The control group received fish meal throughout the experiments; the treatment group received fish meal in period 1 and then they were changed to feather meal in period 2 before returning to fish meal in period 3.

²SED, standard error of differences.

³EAA = essential AA, NEAA = nonessential AA, TAA = total AA.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

differences in the yield of milk protein between the two protein supplements in Experiment 1 and 2 were 27 and 18%, respectively, which are similar to those obtained (15% to 24%) in the short-term studies in which the limiting AA were infused intravenously in cows consuming grass silage and a cereal-based supplement con-

taining feather meal (Choung and Chamberlain, 1995; Kim et al., 2000; 2001a). For both experiments, the marked decreases of milk yield in cows given the feather meal diet for 6 wk were completely recovered (within 2 wk) by a return to the fish meal diet and, thereafter, milk yield between treatments remained similar until

Table 4. Mammary enzyme activities, PCNA, total DNA and udder volume in Experiment 1.

	Period 1			Period 2			Period 3		
	Control ¹	Treatment	SED ²	Control	Treatment	SED	Control	Treatment	SED
Enzyme activities, $\mu\text{mol/min per mg DNA}$									
Acetyl CoA carboxylase	0.18	0.13	0.047	0.13	0.22	0.070	0.09	0.08	0.020
Fatty acid synthetase	0.85	0.41	0.167*	0.67	0.38	0.150	0.49	0.26	0.118
Galactosyltransferase	0.37	0.34	0.057	0.29	0.25	0.051	0.25	0.22	0.048
PCNA ³ , %	3.07	1.82	0.523*	0.41	0.79	0.285	1.84	1.35	1.359
Total DNA, g	30.6	27.8	7.86	33.3	33.5	7.20	30.5	27.0	3.44
Udder volume, l	12.3	12.1	1.96	13.0	12.9	1.27	13.7	11.8	1.08

¹The control group received fish meal throughout the experiments; the treatment group received fish meal in period 1 and then they were changed to feather meal in period 2 before returning to fish meal in period 3.

²SED, standard error of differences.

³Proliferating cell nuclear antigen.

* $P \leq 0.05$.

Table 5. Feed intake, milk production and body weight in Experiment 2.

	Period 1			Period 2			Period 3		
	Control ¹	Treatment	SED ²	Control	Treatment	SED	Control	Treatment	SED
DMI, g/d									
Silage	12.0	11.5	0.69	10.1	9.4	0.43	10.8	10.4	0.25
Total	18.9	18.5	0.69	17.1	16.4	0.43	17.7	17.4	0.25
Milk yield, kg/d	10.7	10.4	0.87	9.4	7.9	0.44*	9.1	9.8	0.39
Protein, %	3.58	3.41	0.14	3.56	3.43	0.09	3.71	3.62	0.09
Protein, g/d	381	355	25.0	333	273	17.8**	335	354	18.0
Fat, %	4.06	3.75	0.25	4.01	4.63	0.30	4.01	3.95	0.30
Fat, g/d	432	387	21.2	372	369	32.9	365	382	40.9
Lactose, %	4.66	4.67	0.08	4.65	4.67	0.07	4.64	4.62	0.05
Lactose, g/d	498	487	40.7	436	372	25.1*	423	451	19.9
Body weight, kg	617	587	34.2	618	598	7.5*	594	582	11.9

¹The control group received fish meal throughout the experiments; the treatment group received fish meal in period 1 and then they were changed to feather meal in period 2 before returning to fish meal in period 3.

²SED, standard error of differences.

* $P \leq 0.05$.

** $P \leq 0.01$.

the end of period 3, suggesting that the relatively long periods of reduced performance did not adversely affect the cow's ability to respond to a return to adequate AA nutrition. Oldham et al. (1979) reported that a low-protein diet decreased milk yield markedly, but their results suggested an over-recovery of milk yield, beyond the level of that in control group, on returning to adequate nutrition. The present experiments lend those results no support.

Because it can be argued that associated changes in feed intake are part and parcel of the animal's response to a change in AA nutrition, we decided to allow the cows ad libitum access to silage throughout the experiments. The drawback, however, is that differences in silage intake between the treatments can cloud the interpretation of the results because it might be argued that responses of milk production were the results of differences in energy intake. Nevertheless, although the feather meal diet decreased silage intake relative to the fish meal diet in Experiment 1, it was estimated that the cows consumed metabolizable energy (ME) in excess of their requirements for maintenance and milk production by approximately 35 and 27 MJ/d on the feather and the fish meal diet, respectively. Whilst it is recognized that such calculations can only be approximate, they indicate that the animals were in clear positive energy balance, which in turn would imply that milk production would be relatively insensitive to an increase in the intake of ME. Although an effect of ME intake on milk yield cannot be completely ruled out, our assumption that any effect was likely to be small is supported by the results of previous experiments with the feather meal diet, in which intravenously infused AA induced large increases in milk production with little or no effect on ME intake (Choung and Chamber-

lain, 1995; Kim et al., 1999; 2001a). Again, despite a large decrease in milk yield in cows given the feather meal diet in Experiment 2, silage intake did not differ between treatments.

The profile of AA in blood plasma in both experiments reflected the marked difference in the composition of AA between the two protein supplements, and the difference disappeared when both groups received the fish meal diet, except that the concentration of His remained similar between treatments in all periods. Analysis of plasma samples at the end of the second wk in period 2 (data not shown), however, showed a significant difference in the plasma concentration of His between treatments (19 vs 10 $\mu\text{mol/l}$ for control and treatment, respectively in Experiment 1; 24 vs 10 $\mu\text{mol/l}$ in Experiment 2; $P < 0.01$). Changes in the plasma concentration of AA could arise from changes in the amounts of AA absorbed from the gut or from changes in the demands for these AA; hence plasma concentrations are not reliable indicators of the limiting AA. The stage of lactation or the magnitude of milk yield could influence the demand for individual AA. Hence, in the present experiments, progressive decreases in the yields of milk protein, and hence the demand for the first-limiting AA, with the feather meal diet might explain the lack of effect of treatment on the plasma concentrations of His by the end of period 2.

In both experiments, although yields of milk fat were not affected by the treatment, the concentrations of milk fat in cows given the feather meal diet were markedly increased and, again, the differences were not evident when both groups received the control diet in period 3. It has been suggested that an imbalance of AA supply, arising both from a surplus or a deficit of AA, might be responsible for the increases in milk fat secre-

Table 6. The concentrations ($\mu\text{mol/l}$) of plasma free AA in Experiment 2.

AA ³	Period 1			Period 2			Period 3		
	Control ¹	Treatment	SED ²	Control	Treatment	SED	Control	Treatment	SED
His	24	21	6.9	18	14	5.0	19	26	4.0
Thr	121	107	19.7	112	113	17.8	118	116	12.1
Arg	80	88	10.8	86	74	6.8	79	81	7.3
Trp	36	35	3.1	44	38	5.9	34	37	2.6
Met	24	24	1.7	22	15	3.4*	28	25	2.4
Val	194	208	32.7	162	260	24.4**	164	185	14.1
Phe	36	41	2.6	37	48	2.7**	37	39	2.7
Ile	111	123	18.5	94	102	11.4	97	107	6.6
Leu	86	96	11.1	84	102	9.0	81	87	7.1
Lys	75	84	11.5	71	45	7.5**	63	71	6.6
Asp	0	0	0	3.1	3.4	0.3	3.8	3.7	1.0
Glu	51	57	5.1	42	43	2.7	46	49	2.2
Ser	95	103	10.5	83	118	11.9*	79	73	6.5
Gly	290	281	22.2	307	382	35.4	243	234	22.1
Ala	247	305	29.5	135	130	17.0	160	162	22.6
Tyr	58	63	3.9	65	86	8.8*	44	42	2.4
Asn	49	53	4.8	41	37	5.0	43	43	3.3
Tau	27	23	2.8	40	39	2.7	31	28	2.8
Glu	267	260	29.1	216	202	13.6	235	218	15.5
Orn	37	39	7.4	36	38	3.8	31	34	3.4
Pro	89	56	13.4*	52	95	16.5*	41	51	6.3
EAA	786	825	80.3	730	811	69.5	718	775	47.4
NEAA	1209	1240	61.5	1034	1159	85.9	969	925	57.2
TAA	1995	2065	118.1	1769	1965	147.7	1688	1699	91.9

¹The control group received fish meal throughout the experiments; the treatment group received fish meal in period 1 and then they were changed to feather meal in period 2 before returning to fish meal in period 3.

²SED, standard error of differences.

³EAA = essential AA, NEAA = nonessential AA, TAA = total AA.

* $P \leq 0.05$.

** $P \leq 0.01$.

tion (Chamberlain et al., 1992). Consistent with this, intravascular infusion of AA mixtures deficient in His markedly increased the yield and concentration of milk fat (Kim et al., 1999; 2001a; Cant et al., 2001) which returned to the starting level when His was reintroduced.

Kim et al. (2001a,b) calculated an efficiency of transfer of His from blood to milk of around 41% and commented that the figure was much lower than assumed

in protein rationing schemes (AFRC, 1992). The present experiments do not allow such a precise calculation as that of Kim et al. (2001a,b) but, with some assumptions, we can obtain some figures for comparison. When the cows returned to the fish meal diet, milk protein yield from the whole udder increased by 176 and 162 g/d in Experiment 1 and 2, respectively. The difference in His provided by the RDP of fish meal and feather meal was around 11 g/d (Chamberlain et al., 1992) which

Table 7. Mammary enzyme activities, total DNA and udder volume in Experiment 2.

	Period 1			Period 2			Period 3		
	Control ¹	Treatment	SED ²	Control	Treatment	SED	Control	Treatment	SED
Enzyme activities, $\mu\text{mol/min}$ per mg DNA									
Acetyl CoA carboxylase	0.25	0.21	0.037	0.25	0.22	0.041	0.25	0.22	0.072
Fatty acid synthetase	0.88	0.84	0.145	0.72	0.53	0.089	0.85	0.70	0.126
Galactosyltransferase	0.62	0.48	0.096	0.50	0.40	0.037*	0.48	0.38	0.088
Total DNA, g	20.3	19.9	3.22	16.4	19.6	3.55	18.4	17.7	3.96
Udder volume, l	12.9	12.2	1.08	12.5	12.6	1.03	11.3	12.4	1.28

¹The control group received fish meal throughout the experiments; the treatment group received fish meal in period 1 and then they were changed to feather meal in period 2 before returning to fish meal in period 3.

²SED, standard error of differences.

* $P \leq 0.05$.

amounts to around 9 g/d of absorbable His (AFRC, 1992); about 2 g/d of absorbable His could also come from an increased yield of microbial protein from the higher silage intakes on the fish meal treatment. The overall figure of about 11 g/d of absorbable His is roughly equivalent to the highest dose of His given intravenously by Kim et al. (2001a; 2001b). Assuming that milk protein contains 26 g His/kg (Kaufmann, 1980), these increases in protein yield correspond to crude efficiencies of transfer of His of 42% and 38% respectively, values that agree well with the estimate (41%) obtained in short-term experiments in which His was given intravenously. If corrections are made for absorbability in the small intestine, those results are also reasonably close to the transfer rate of 28% (about 33% when corrected for absorbability) calculated for abomasal infusion of His (Korhonen et al., 2000).

The results from the present experiments show that indicators of mammary function were largely unaffected in response to changes in dietary AA balance even though milk production was markedly affected. Although positive relationships between udder volume and milk yield have been reported in goats (Linzell, 1966) and cows (Knight, 2000), the present results lend these no support. It should be noted, however, that the measurement of udder volume might overestimate secretory tissue in the large, pendulous udders of older cows and underestimate it in heifers (Knight et al., 1995). Statistically significant effects on FAS and PCNA that occurred at isolated time points are difficult to interpret because there were differences between the two groups of cows for these variables when both consumed the control diet in Experiment 1. The fact that the two treatments supported levels of milk production that differed by around 21%, and yet no clear differences were detected in the measurements of mammary function, suggests either that the measurements were not sensitive indicators of mammary function or, alternatively, metabolic capacity of the mammary gland was in substantial excess of that required on the feather meal treatment. There is some support for the latter suggestion in that cows eating the feather meal diet respond to intravenous infusion of AA by markedly increasing milk secretion within 24 h (Kim et al., 1999; 2000), and such a rapid response would seem incompatible with a change in mammary function. In Experiment 2, there was a significant difference in GT between the feather meal and the fishmeal treatments at the end of period 2. However, the numerical difference of GT between the two groups was already around 20% in period 1 and this difference seemed to change little throughout the experiment. The lack of difference in the yield of milk fat was reflected in similar activities of the associated enzymes (ACC and FAS). However,

decreases in lactose yields in cows given the feather meal diet were not associated with corresponding changes in GT.

Little information is available on the interaction between changes in milk production and mammary function in the dairy cow. In a recent study, in cows under extreme dietary conditions, enzyme activities in the mammary gland changed in response to nutrient supply (Piperova et al., 2000). When the cows were given a diet containing a high ratio of concentrate to forage, a 45% reduction in the yield and concentration of milk fat was seen, and activities of FAS and ACC in the mammary gland were markedly decreased. On the other hand, a 17% increase in milk fat yield in response to bST treatment did not lead to changes in the level of ACC and FAS in the mammary gland (Beswick and Kennelly, 1998). Again, a large difference (28%) in milk yield between high and low genetic merit cows did not affect GT activities in the mammary gland (Sorensen et al., 1998).

CONCLUSIONS

The present results show that deficiencies in the dietary supply of specific AA for 6 wk, in both early/mid and mid/late lactation, markedly reduced milk yield. However, there was no adverse effect on the cow's ability to respond to a return to AA-adequate diet at either stage of lactation. Furthermore, despite the markedly lower milk yield in cows given the AA-deficient diet, there was no clear evidence of corresponding changes in measurements of mammary function.

ACKNOWLEDGMENTS

We thank Mrs. I. Stewart and Mr. J. Davidson for skilled technical assistance and Mr. S. Robertson and his staff for care of the animals during the experiment.

REFERENCES

- AFRC. 1992. Nutrient requirements of ruminant animals: protein. *Nutr. Abstr. Rev.* B62:787-835.
- Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux: Slough, UK.
- Beswick, N. S., and J. J. Kennelly. 1998. The influence of bovine growth hormone and growth hormone releasing factor on acetyl-CoA carboxylase and fatty acid synthase in primiparous Holstein cows. *Comp. Biochem. Physiol. Part C.* 120:241-249.
- Cant, J. P., D. R. Trout, F. Qiao, and B. W. McBride. 2001. Milk composition responses to unilateral arterial infusion of complete and histidine-lacking amino acid mixtures to the mammary glands of cows. *J. Dairy Sci.* 84:1192-1200.
- Capuco, A. V., D. L. Wood, R. Baldwin, K. Mcleod, and M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of bST. *J. Dairy Sci.* 84:2177-2187.

- Chamberlain, D. G., J. -J. Choung, and S. Robertson. 1992. Protein nutrition of dairy cows receiving grass silage diets: effects of feeding a protein supplement of unbalanced amino acid composition. *J. Sci. Food Agric.* 60:425–430.
- Choung, J.-J., and D. G. Chamberlain. 1995. The effects of intravenous supplements of amino acids on the milk production of dairy cows consuming grass silage and a supplement containing feather meal. *J. Sci. Food Agric.* 68:265–270.
- Kaufmann, W. 1980. Protein degradation and synthesis within the reticulo-rumen in relation to milk protein synthesis. Pages 152–158 in *Factors Affecting the Yields and Contents of Milk constituents of Commercial Importance* (Document 125). International Dairy Federation, Brussels.
- Kim, C.-H., J.-J. Choung, and D. G. Chamberlain. 1999. Determination of the first-limiting amino acid for milk production in dairy cows consuming a diet of grass silage and a cereal-based supplement containing feather meal. *J. Sci. Food Agric.* 79:1703–1708.
- Kim, C.-H., J.-J. Choung, and D. G. Chamberlain. 2000. Variability in the ranking of the three most-limiting amino acids for milk protein production in dairy cows consuming grass silage and a cereal-based supplement containing feather meal. *J. Sci. Food Agric.* 80:1386–1392.
- Kim, C.-H., J.-J. Choung, and D. G. Chamberlain. 2001a. Estimates of the efficiency of transfer of L-histidine from blood to milk when it is the first-limiting amino acid for secretion of milk protein in the dairy cow. *J. Sci. Food Agric.* 81:1150–1155.
- Kim, C.-H., T.-G. Kim, J.-J. Choung, and D. G. Chamberlain. 2001b. Effects of intravenous infusion of amino acids and glucose on the yield and concentration of milk protein in dairy cows. *J. Dairy Res.* 68:27–34.
- Knight, C. H. 2000. The importance of cell division in udder development and lactation. *Livestock Prod. Sci.* 66:169–176.
- Knight, C. H., J. R. Brown, and R. J. Dewhurst. 1995. Udder characteristics and relationship to efficiency of milk production. *Brit. Soc. Anim. Prod. Occasional Publication*, 19:79–85.
- Knight, C. H., and R. J. Dewhurst. 1994. Once daily milking of dairy cows: relationship between yield loss and cisternal milk storage. *J. Dairy Res.* 61:441–449.
- Knight, C. H., J. E. Hillerton, R. M. Teverson, and A. Winter. 1992. Biopsy of the bovine mammary gland. *Br. Vet. J.* 148:129–132.
- Knight, C. H., and M. Peaker. 1984. Mammary development and regression during lactation in goats in relation to milk secretion. *Q. J. Exp. Physiol.* 69:331–338.
- Korhonen, M., A. Vanhatalo, T. Varvikko, and P. Huhtanen. 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. *J. Dairy Sci.* 83:2596–2608.
- Labarca, C., and K. Paigen. 1980. A simple, rapid and sensitive DNA assay procedure. *Anal. Biochem.* 102:344–352.
- Linzell, J. L. 1966. Measurement of udder volume in live goats as an index of mammary growth and function. *J. Dairy Sci.* 49:307–311.
- Morrison, I. M. 1972. A semi-micro method for the determination of lignin and its use in predicting the digestibility of forage crops. *J. Sci. Food Agric.* 23:455–463.
- Oldham, J. D., W. H. Broster, D. J. Napper, and J. W. Siviter. 1979. The effect of a low-protein ration on milk yield and plasma metabolites in Friesian heifers during early lactation. *Br. J. Nutr.* 42:149–162.
- Piperova, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. Fritsche, K. Ku, and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. *J. Nutr.* 130:2568–2574.
- Sorensen, A., M. Alamer, and C. H. Knight. 1998. Physiological characteristics of high genetic merit and low genetic merit dairy cows: a comparison. Page 4 in *Proc. Brit. Soc. Anim. Sci.*
- Wilde, C. J., A. J. Henderson, and C. H. Knight. 1986. Metabolic adaptations in goat mammary tissue during pregnancy and lactation. *J. Reprod. Fertil.* 76:289–298.