Responses to post-ruminal infusions of casein and arginine, and to dietary protein supplements in lactating goats

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1. In Expt 1 a study was made in goats of responses in terms of milk production, nitrogen utilization and plasma amino acids to abomasal infusions of casein (45 g/d) in goats given 2·5 kg/d of a ration containing crude protein (N × 6·25) at 109 (L_1) or 146 (H_1) g/kg.

2. In Expt 2 a study was made in goats of responses in terms of milk production, nitrogen utilization, plasma amino acids and growth hormone levels to abomasal infusions of casein (45 g/d) or arginine (25 g/d) in goats given 2·3 kg/d of a ration containing crude protein (N × 6·25) at 104 g/kg (L_2). These observations were made also in goats given a ration containing crude protein at 136 g/kg (H_2).

3. Milk production in Expt 1 was 2·75, 2·45 and 2·76 kg/d on L_1 + casein, H_1 and H_1 + casein treatments respectively, the response to casein infusion being significant ($P < 0·05$). Milk production in Expt 2 was 1·90, 2·04, 1·96 and 1·96 kg/d on L_2, L_2 + casein, L_2 + arginine and H_2 treatments respectively, and the differences were not significant.

4. Total N intake in Expt 1 was 49, 58 and 64 g/d on L_1 + casein, H_1 and H_1 + casein treatments respectively. Faecal N was similar on the three treatments (14 g/d), urinary N was 15, 23 and 30 g/d and milk N was 14, 12 and 14 g/d on the respective treatments.

Total N intake in Expt 2 was 33, 40, 43 and 44 g/d on L_2, L_2 + casein, L_2 + arginine and H_2 treatments respectively. Faecal N was similar on the four treatments (12 g/d), urinary N was 7, 10, 13 and 14 g/d and milk N was 9, 9, 8 and 8 g/d on the respective treatments.

5. The concentration of indispensable amino acids in plasma was increased by casein infusion in both experiments. It was 1279, 825 and 1133 μM/l on L_1 + casein, H_1 and H_1 + casein treatments respectively in Expt 1, and 1081, 1582, 1055 and 1163 μM/l on L_2, L_2 + casein, L_2 + arginine and H_2 treatments respectively in Expt 2.

6. The concentration of arginine in plasma was doubled 1 h after the onset of arginine infusion in Expt 2, Growth hormone levels in plasma were not increased when arginine levels rose following arginine infusion. and did not differ between treatments.

7. The results of the two experiments showed that the stimulatory effect on milk production of intra-abomasal infusion of casein was not reproduced by increasing the dietary intake of protein or by infusing arginine. The results of the second experiment showed that abomasal infusion of arginine did not stimulate production of growth hormone and that growth hormone apparently was not implicated in the effects of casein infusion on milk production.

Results of a number of experiments have shown that cows and goats fed on diets containing levels of crude protein (nitrogen × 6·25) which were adequate by accepted feeding standards give increased yields of milk when infused post-ruminally with casein. In ten experiments with cows, the results of which were summarized by Clark (1975), the mean increase in milk production, weighted according to the number of cows in each experiment, was 8·4%. In similar studies with goats (Ranawana & Kellaway, 1977*a*, *b*) the weighted mean increase in milk production was 19·9%.

In seeking explanations for the previously mentioned phenomenon, Clark (1975) suggested that the infused casein may increase milk yield directly by supplying limiting amino acids, or alternatively indirectly by supplying carbon for gluconeogenesis or effecting changes in hormone balance. Results of subsequent studies with cows (Clark *et al.* 1977) and goats

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(Ranawana & Kellaway, 1977*b*) showed that when glucose or casein, in equal amounts, were infused abomasally, there were similar increases in glucose entry rates. In spite of this, milk yields were not increased by infusion of glucose. These observations suggest that the stimulation of milk production effected by abomasal infusion of casein is not attributable to gluconeogenesis.

Attempts to mimic the effects of post-ruminal casein infusion on milk production by intravenous (Fisher, 1969, 1972; Fisher & Erfle, 1974) or intra-abomasal infusion of amino acids (Schwab *et al.* 1976) have not succeeded. An alternative and practical means of increasing the supply of amino acids for intestinal absorption, in order to mimic casein infusion, is to feed protected protein. An earlier attempt to do this with formaldehyde-treated casein was not wholly successful (Kellaway *et al.* 1974). In the experiments reported here we compared casein infusions with different dietary levels of soya-bean meal and meat meal.

The possibility that post-ruminal supplementation with casein elicits release of galactopoietic hormones is supported by some circumstantial evidence. Thus, it has been demonstrated in humans that intravenous infusion of relatively large amounts (15–30 g) of individual amino acids, in particular arginine, substantially increases plasma levels of growth hormone (Knopf *et al.* 1965). Similar responses have been obtained following intravenous injection of arginine in ruminants (Machlin *et al.* 1968; Davis, 1972; Hertelendy *et al.* 1969; Reynaert *et al.* 1972). Furthermore, injections of pituitary extracts (Meites, 1961) or purified growth hormone (Machlin, 1973) significantly increased milk production in cows. In one of the present studies we attempted to determine whether increased milk production obtained after post-ruminal casein infusion might be attributable to an increase in endogenous growth hormone secretion. Milk production and composition and plasma growth hormone levels were followed in goats fed on a basal diet supplemented by intra-abomasal infusion of casein or arginine.

EXPERIMENTAL

Design and treatments

Expt 1. The experiment was conducted in a 3×3 Latin Square design so that each animal was subjected to each treatment. Goats were given 2·5 kg/d of a ration containing either 100 g (L_1) or 146 g (H_1) crude protein/kg (see Table 1) alone or in combination with an infusion into the abomasum of 45 g casein (C)/d. The treatments were as follows: L_1+C, H_1 and H_1+C.

Expt 2. The experiment was conducted in a 4×4 Latin Square design, so that each animal was subjected to each treatment. Goats were given 2·3 kg/d of a ration containing either 104 g (L_2) or 136 g (H_2) crude protein/kg (see Table 1) alone or in combination with an infusion into the abomasum of either control infusate (I), 45 g (C)/d or 25 g arginine (A)/d. The treatments were as follows: L_2+I, L_2+C, L_2+A and H_2.

Animals and management

Multiparous Saanen goats in early lactation were used in both experiments. The goats had similar live weights (approximately 40 kg) and milk yields before commencing the experiments and all were free of abnormalities of the mammary glands. The surgical preparation and management of the animals was similar to that described previously (Ranawana & Kellaway, 1977*a*).

Table 1. *Composition of basal diet fed to goats (g/kg)*

	Expt 1		Expt 2	
Diet ...	L_1	H_1	L_2	H_2
Ingredient				
Rolled barley	620	520	620	560
Lucerne chaff	200	200	200	200
Oat straw	100	100	100	100
Molasses	70	70	70	70
Soya-bean meal	—	100	—	—
Meat meal	—	—	—	70
Bone meal	10	10	10	—
Analysis				
Dry matter	879	875	905	913
Acid detergent fibre	183	154	—	—
Lignin	59	37	—	—
Nitrogen	17·1	23·3	16·6	21·8
Crude protein (N × 6·25)	109	146	104	136

Diets

The composition of the diets is given in Table 1. They were fed in pelleted form at levels which were approximately 90% of *ad lib.* intake. All four diets provided minimum intakes of 150 g digestible crude protein (N × 6·25) and 20 MJ metabolizable energy daily, which by extrapolation from sheep requirements for protein (Agricultural Research Council, 1965) and energy (Ministry of Agriculture, Fisheries & Food, 1975), were sufficient for the production of 2·0 and 3·3 kg milk/respectively. This level of feeding was selected to avoid the confounding of treatment effects with varying levels of food intake.

Infusates

Expt 1. The casein infusate was prepared and infused using a Perpex Pump (LKB Produkter, Sweden) as described by Ranawana & Kellaway (1977*a*).

Expt 2. The infusion solution contained sodium hydroxide (8 g/l) in distilled water. In this was dissolved either casein (50 g/l) or L-arginine (400 g/l). The solutions were kept at 4° and pumped directly into the abomasum using a Perpex Pump (LKB Produkter, Sweden). Casein was administered as a constant infusion at the rate of 45 g/d (900 g infusate/d), the control infusate was given as a constant infusion (900 g/d), whereas the arginine infusate was given at the rate of 25 g/d (62·5 g infusate) during a period of 1 h commencing at 09.00 hours each day and was followed with control infusate for the remaining 23 h.

Experimental procedures

Expt 1. Each experimental period of 16 d was comprised of 10 d for adjustment, 5 d for milk and N balance measurements and 1 d for blood measurements. Collection and analytical procedures for milk, N balance and blood were as described previously (Ranawana & Kellaway, 1977*a*).

Expt 2. Each experimental period of 12 d was comprised of 7 d for adjustment and 5 d for milk, N balance and blood measurements. Collection and analytical procedures for milk and N balance were as described previously (Ranawana & Kellaway, 1977*a*). Jugular venous blood was obtained from indwelling polyvinyl-chloride cannulas on the 12th and 1st days of each treatment period. Pooled samples of 35 ml were collected over half hour intervals commencing 08.30, 09.30, 10.00 and 15.00 hours on the 12th day and at 08.30

Table 2. *Expt* 1. *Production of milk and milk constituents for goats given a diet containing high* (H_1) *or low* (L_1) *levels of protein alone or in combination with an intra-abomasal infusion of casein* (C)

(Values presented are means of three measurements and standard errors of treatment means are indicated)

	Treatment			
	H_1	L_1+C	H_1+C	SEM
Milk yield (g/d)	2447^{b}	2747^{a}	2763^{a}	48·9*
Total solids				
g/d	309^{b}	339^{a}	331^{ab}	7·2*
g/l	126·5	123·6	119·7	3·0
Fat				
g/d	93·6	97·6	94·5	8·2
g/l	38·3	35·7	34·2	3·0
Solids-not-fat				
g/d	216^{b}	242^{a}	236^{a}	2·6**
g/l	88·1	88·0	85·5	1·1
Total protein				
g/d	$78·9^{b}$	$88·9^{a}$	$87·2^{ab}$	2·6*
g/l	32·4	32·3	31·5	0·9
Casein				
g/d	$61·6^{b}$	$69·1^{a}$	$66·1^{ab}$	2·0
g/l	25·2	25·1	23·9	0·6
Casein/total protein	0·78	0·78	0·76	0·01
Lactose				
g/d	115·5	131·0	130·5	4·7
g/l	47·1	47·4	47·2	1·3

a, *b*. Values with unlike superscripts differed significantly.
* $P < 0·05$, ** $P < 0·01$.

hours on the 1st day of each period (i.e. −0·5, +0·5, +1, +6 and +23·5 h relative to the commencement of arginine infusion).

During the collection, blood samples were kept chilled, heparin was used as anticoagulant and, immediately after collection of the 35 ml portion, plasma was prepared using a refrigerated centrifuge. A portion of 10 ml of each plasma sample was deproteinized with 500 mg sulphosalicylic acid (Perry & Hansen, 1969) after addition of 1·25 μM carnosine and 2·5 μM norleucine, in a volume of 50 μl, which served as internal standards. The remaining plasma, together with the deproteinized plasma, was stored at −16° until analysed.

Amino acid analyses were carried out on the first of each batch of pooled samples. These samples were collected just before the change-over to another treatment. Analytical procedures were as described by Ranawana & Kellaway (1977*a*).

Growth hormone in plasma was determined by the talc radioimmunoassay described by Wallace & Bassett (1970). Concentrations of growth hormone were expressed in terms of a purified preparation of ovine growth hormone.

The data were analysed using conventional Latin Square analyses and are presented as treatment means.

Table 3. *Expt* 1. *Dry matter* (DM) *and nitrogen balance values for goats given a diet containing high* (H_1) *or low* (L_1) *levels of protein alone or in combination with an intra-abomasal infusion of casein* (C)

(Values presented are means of three measurements and standard errors of treatment means are indicated)

	Treatment			
	H_1	L_1+C	H_1+C	SEM
DM intake from diet (g/d)	2160	2198	2188	16·0
DM digestibility	0·69	0·68	0·72	0·014
N intake				
Basal diet (g/d)	$57·73^b$	$42·69^a$	$57·46^b$	0·37**
Total (g/d)	$57·73^b$	$49·39^a$	$64·16^c$	0·37**
N output				
Faeces (g/d)	14·28	14·78	14·04	0·58
Urine (g/d)	$23·21^b$	$15·26^a$	$29·51^c$	0·94**
Productive N				
Total (g/d)	20·25	19·36	20·61	0·69
Milk (g/d)	$12·37^b$	$13·93^a$	$13·66^{ab}$	0·42*
N absorbed (g/d)	$43·45^b$	$34·61^a$	$50·12^c$	0·54*
N retained (g/d)	7·88	5·43	6·95	0·65
N digestibility	$0·75^b$	$0·70^a$	$0·78^b$	0·01*
Milk N:absorbed N	$0·29^b$	$0·40^a$	$0·27^b$	0·012**
Total productive N:absorbed N	$0·47^b$	$0·56^a$	$0·41^b$	0·024*
Retained N:absorbed N	0·18	0·16	0·14	0·02

a, *b*, *c*. Values with unlike superscripts differed significantly.
* $P < 0·05$, ** $P < 0·01$.

RESULTS

Expt 1

Milk production

Results for the production of milk and milk constituents are presented in Table 2. Milk production was significantly increased ($P < 0·05$) during the periods when casein was infused.

Production of total solids (*TS*) was only significantly increased, relative to production on treatment H_1, during the L_1+C treatment ($P < 0·05$).

No significant differences were recorded between treatments for fat content of milk and fat production. The production of solids-non-fat was significantly greater ($P < 0·01$) during casein infusions. Levels of total protein and casein in milk were unaffected by treatment but total productions of casein and protein were significantly greater ($P < 0·05$) on treatment L_1+C than for treatment H_1.

N balance

Dry matter (DM) and N balance values are shown in Table 3. Basal intakes and digestibilities of DM were not significantly different ($P > 0·05$).

The basal intake of N was significantly higher for treatments H_1 and H_1+C than for treatment L_1+C. The outputs of N in faeces were not significantly different but the quantities of N absorbed ($P < 0·01$) and N digested ($P < 0·05$) were significantly higher for the H_1 and H_1+C treatments than for treatment L_1+C. The quantity of urinary N during treatment H_1 was significantly greater ($P < 0·01$) than on L_1+C, and on H_1+C was greater

Table 4. *Expt* 1. *Levels in arterial plasma of urea N and glucose (mg/l) for goats given a diet containing high* (H_1) *or low* (L_1) *levels of protein alone or in combination with an intra-abomasal infusion of casein* (C)

(Values presented are means of three measurements and standard errors of treatment means are indicated)

	Treatment			
	H_1	L_1+C	H_1+C	SEM
Urea N	211^{ab}	132^{a}	243^{b}	27·4
Glucose	681	551	600	41·5

a, b. Values with unlike superscripts differed significantly ($P < 0.05$).

Table 5. *Expt* 1. *Levels of free amino acids in arterial plasma (μM/l) for goats given a diet containing high* (H_1) *or low* (L_1) *levels of protein alone or in combination with an intra-abomasal infusion of casein* (C)

(Values presented are means of three measurements and standard errors of treatment means are indicated)

	Treatment			
	H_1	L_1+C	H_1+C	SEM
Indispensable amino acids				
Threonine	76^{b}	130^{a}	95^{ab}	12·1*
Valine	166^{b}	283^{a}	224^{ab}	18·1**
Methionine	17^{b}	33^{a}	23^{ab}	2·0*
Isoleucine	99^{b}	154^{a}	127^{ab}	8·4*
Leucine	101^{b}	191^{a}	163^{a}	6·1**
Phenylalanine	39	55	52	4·0
Lysine	97	178	154	27·7
Histidine	137	172	172	21·5
Arginine	93	83	126	21·0
Total	825^{b}	1279^{a}	1133^{a}	70·0*
Dispensable amino acids				
Aspartic acid	29	40	32	4·0
Serine	142	195	147	14·0
Glutamic acid + glutamine	388	380	373	30·2
Proline	154^{b}	347^{a}	233^{a}	12·8**
Glycine	672^{ab}	746^{a}	573^{b}	30·0*
Alanine	288^{b}	433^{a}	354^{ab}	18·3*
Cystine	28	36	34	2·4
Tyrosine	90^{b}	124^{a}	103^{ab}	6·8*
Ornithine	54	80	91	11·4
Total	1844^{b}	2379^{a}	1939^{ab}	54·0**
Total amino acids	2669^{b}	3658^{a}	3073^{ab}	122**
Indispensable:dispensable amino acids	0·46	0·54	0·59	0·024

a, b. Values with different superscripts differed significantly.
* $P < 0.05$, ** $P < 0.01$.

for the other two treatments ($P < 0.01$). No significant differences were recorded for total productive N. Milk N on treatment L_1+C was significantly greater than for treatment H_1 ($P < 0.05$). The goats were in positive N balance on all treatments.

The proportions of N absorbed (NA) that appeared as total productive N (TPN) and milk N (MN) were calculated to serve as indices of the efficiency of N utilization. Both TPN:NA ($P < 0.05$) and MN:NA ($P < 0.01$) were highest in the L_1+C treatment.

Table 6. *Expt 2. Production of milk and milk constituents for goats given either a high* (H_2) *or low protein diet* (L_2) *alone or in combination with an intra-abomasal infusion of casein* (C), *arginine* (A) *or control infusate* (I)

(Values presented are means of four measurements and standard errors of treatment means are indicated)

	Treatment				
	L_2+I	L_2+C	L_2+A	H_2	SEM
Milk yield (g/d)	1900	2041	1959	1955	95·3
Total solids					
g/l	118·2^a	115·4	118·3^a	117·9^a	0·67*
g/d	224·6	235·5	230·8	230·4	11·87
Fat					
g/l	36·8^a	34·0	38·1^a	37·0^a	0·72**
g/d	69·8	69·5	74·7	72·5	4·72
Solids-not-fat					
g/l	81·4^a	81·4^a	80·3^b	80·9^{ab}	0·31*
g/d	154·7	166·0	156·2	157·9	7·32
Total protein					
g/l	28·4^{ab}	28·7^b	26·6^a	27·6^{ab}	0·53*
g/d	54·4	58·6	52·0	53·9	0·52

a, b. Values with unlike superscripts differ significantly.
* $P < 0·05$, ** $P < 0·01$.

Plasma urea N and glucose

Effects of treatments on these parameters can be seen in Table 4. Concentrations of urea N in plasma were significantly higher ($P < 0·05$) for treatment H_1+C than for L_1+C. Although plasma glucose concentration was highest during treatment H_1, this difference was not statistically significant ($P > 0·05$).

Plasma amino acids

Arterial concentrations of free amino acids are shown in Table 5. Levels of total indispensable amino acids were significantly increased ($P < 0·05$) during casein infusions. Concentrations of threonine, valine, methionine and isoleucine were significantly higher ($P < 0·05$) for treatment L_1+C than treatment H_1. Concentrations of leucine and total branched-chain amino acids were significantly elevated ($P < 0·01$) by the casein infusions (L_1+C, H_1+C).

Concentrations of total dispensable amino acids were significantly higher ($P < 0·01$) during treatment L_1+C than for H_1. Levels of alanine and tyrosine were significantly greater on L_1+C than on H_1 ($P < 0·05$) and glycine concentration was significantly greater on L_1+C than on H_1 ($P < 0·05$). Proline levels in plasma were significantly increased by infusions of casein ($P < 0·01$).

Total amino acid concentration in arterial plasma was significantly higher ($P < 0·01$) during L_1+C than for treatment H_1. The ratio of indispensable to dispensable amino acids was significantly larger ($P < 0·05$) during treatment H_1+C than for treatment H_1.

Expt 2

Milk production

The production of milk and milk constituents can be seen in Table 6. On the H_2, L_2+A and L_2+C treatments milk production was increased by 2·8, 3·1 and 7·4% respectively, relative to L_2+I but these differences were not significant ($P > 0·05$).

Table 7. *Expt* 2. *Dry matter* (DM) *and nitrogen balance values for goats given either a high* (H_2) *or low* (L_2) *protein diet alone or in combination with an intra-abomasal infusion of casein* (C), *arginine* (A) *or control infusate* (I)

(Values presented are means of four measurements and standard errors of treatment means are indicated)

	Treatment				
	L_2+I	L_2+C	L_2+A	H_2	SEM
DM intake from diet (g/d)	1972	2072	2072	2025	42·15
DM digestibility	0·73	0·73	0·73	0·73	0·01
N intake (g/d)					
Basal diet	32·63	34·29	34·29	43·98	0·55
Total	32·63	$40·02^a$	$42·70^b$	$43·98^b$	0·51**
N output (g/d)					
Faeces	11·59	11·75	11·97	12·42	0·76
Urine	$7·43^a$	$10·19^{ab}$	$13·37^b$	$13·56^b$	1·70*
Productive N (g/d)					
Total	$13·62^a$	$18·08^b$	$17·36^b$	$18·00^b$	1·08*
Milk	8·53	9·19	8·15	8·45	0·52
N absorbed (g/d)	21·05	$28·27^a$	$30·73^{ab}$	$31·56^b$	0·80*
N retained (g/d)	$5·09^a$	$8·89^{ab}$	$9·21^{ab}$	$9·55^b$	1·23*
N digestibility	$0·64^a$	$0·71^{ab}$	$0·72^b$	$0·72^b$	0·02*
Milk N:absorbed N	$0·41^a$	$0·33^{ab}$	$0·27^b$	$0·27^b$	0·03*
Total productive N:absorbed N	0·65	0·64	0·57	0·58	0·05
Retained N:absorbed N	0·24	0·31	0·30	0·31	0·05

a, *b*. Values with unlike superscripts differed significantly.
* $P < 0·05$, ** $P < 0·01$.

Infusion of casein caused significant reductions in the contents in milk, but not total yield, of TS and fat ($P < 0·05$), in comparison with the period when the control diet (L_2) was fed. Infusion of arginine led to a significant reduction ($P < 0·05$) in the content of solids-not-fat relative to the content when the control diet was fed. Over all, changes in milk composition effected by alteration of diet were only small and total yields of milk constituents were not affected by dietary treatment.

Nitrogen balance

N balance results are given in Table 7. Intake and digestibility of DM were similar on all treatments. Total N intake was significantly lower ($P < 0·01$) than for all other treatments and total N intake for L_2+C was significantly lower ($P < 0·01$) than for L_2+A and H_2. Total productive N was significantly lower on L_2+I ($P < 0·05$) than on the other three treatments.

Plasma amino acids and growth hormone

The amino acid contents of plasma collected after goats had been on a particular treatment for 12 d are given in Table 8. Statistical comparison of treatments was not possible because analyses were carried out on one pooled sample for each treatment. However, the concentration of indispensable amino acids was 46% higher with casein infusion than on the L_2 diet alone, which was the same proportional increase seen in *Expt* 1 (Table 5). Amino acid concentrations on L_2+I, L_2+A and H_2 treatments were similar.

Arginine and growth hormone levels in plasma collected at the onset of treatment periods are given in Table 9. Arginine levels were doubled 1 h after commencing arginine infusion, but growth hormone levels remained unchanged. Further, growth hormone levels were similar for the four treatments.

Table 8. *Expt 2. Levels of free amino acids in venous plasma (μM/l) from goats given either a high* (H_2) *or low* (L_2) *protein diet alone or in combination with an intra-abomasal infusion of casein* (C), *arginine* (A) *or control infusate* (I)

(Mean values with their standard errors)

	Treatment							
	L_2+I		L_2+C		L_2+A		H_2	
Indispensable amino acids	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Threonine	59	1·1	77	6·3	61	5·5	71	1·4
Valine	273	60·4	470	4·2	288	27·1	350	6·6
Methionine	19	1·8	23	2·7	17	0·8	17	1·2
Isoleucine	135	2·5	134	3·1	110	2·9	130	1·6
Leucine	107	2·9	131	2·4	86	1·2	110	2·4
Phenylalanine	41	1·3	47	2·7	45	2·9	38	1·1
Lysine	143	2·8	258	6·9	149	11·9	130	9·1
Histidine	190	7·6	302	8·5	169	13·9	171	14·7
Arginine	114	7·6	140	0·5	130	17·4	146	5·5
Total	1081		1582		1055		1163	
Dispensable amino acids								
Aspartic acid	29	0·1	57	1·6	38	1·7	39	4·1
Serine	155	5·6	196	1·8	171	4·0	140	0·8
Glutamic acid	242	5·8	278	4·4	224	4·6	243	6·4
Glutamine	188	8·0	58	2·4	54	1·7	71	2·0
Proline	133	10·9	224	10·9	98	3·9	122	4·2
Glycine	1042	6·1	1031	64·9	894	55·4	965	4·9
Alanine	464	1·8	425	4·3	414	35·8	336	4·5
Cystine	8	0·1	9	0·9	6	0·1	15	0·2
Tyrosine	65	0·7	96	1·1	75	0·6	60	0·6
Ornithine	70	0·1	85	2·4	75	4·6	73	8·3
Total	2396		2459		2049		2064	
Total amino acids	3477		4051		3104		3227	
Indispensable: dispensable amino acids	0·31		0·39		0·34		0·36	

Table 9. *Expt 2. Levels of growth hormone (ng/ml) and arginine (μM/l) in venous plasma from goats given either a high* (H_2) *or low* (L_2) *protein diet alone or in combination with an intra-abomasal infusion of casein* (C), *arginine* (A) *or control infusate* (I)

(Values presented are means of four measurements and standard errors of treatment means are indicated. Blood samples were collected at intervals before and after the start of a treatment period)

	Period after infusion (h)	Treatment				
		L_2+I	L_2+C	L_2+A	H_2	SEM
Growth hormone	−0·5	9·2	6·3	3·2	3·7	2·65
	+0·5	9·2	4·9	3·7	3·0	2·56
	+1	8·7	3·7	3·8	4·2	2·82
	+6	5·7	6·3	4·0	4·3	1·85
	+23·5	7·0	4·2	3·1	4·5	1·87
Arginine	−0·5	—	—	139	—	—
	+1	—	—	251	—	—

DISCUSSION

Abomasal infusion of casein increased milk production by 12·6% ($P < 0·05$) in Expt 1 and 7·4% (not significant, $P > 0·05$) in Expt 2. The mean response from these two experiments and two earlier experiments (Ranawana & Kellaway, 1977*a*, *b*), weighted for the number of goats in each experiment, is 14·9%. Even greater increases in milk production were recorded after intra-abomasal infusions of casein in cows which were deliberately underfed (Ørskov & Grubb, 1977; Ørskov *et al.* 1977) and in goats which were fed on a low protein diet (Farhan & Thomas, 1977).

Of particular interest is the increase in milk yield in animals fed on diets supplying adequate amounts of energy and protein, as judged by accepted feeding standards, and in positive N balance. In spite of this, infusion of casein stimulated milk production. We did not measure flow of amino acids to the intestines in these experiments, but productive N was similar on all treatments in Expt 1 (Table 3) and on all treatments except L_2+I in Expt 2 (Table 7). These observations indicate that similar quantities of amino acids were absorbed on these treatments, and that the proportion directed to milk synthesis was increased only by casein infusion.

Ørskov *et al.* (1977) suggested that supplementation of the diets of cows during early lactation with a protein-rich concentrate, such as soya-bean meal or fish meal, may be efficacious in increasing peak milk yields and thus total lactation yield. The results of the present studies do not support this proposal. Goats used in the present studies were in early lactation and neither the soya-bean meal nor the meat meal diet was effective in mimicking the response obtained when casein was infused post-ruminally.

The contents of indispensable amino acids in soya-bean meal and meat meal, which were the protein supplements fed in Expts 1 and 2 respectively, do not differ markedly from those in casein, with the exception of methionine which is lower in the protein meals. Attempts to stimulate milk production in lactating cows by infusing methionine or methionine plus lysine into the abomasum (Schwab *et al.* 1976) or jugular vein (Fisher, 1969, 1972; Fisher & Erfle, 1974) were unsuccessful. Further, abomasal infusions of ten indispensable amino acids were found to be ineffective in stimulating milk production (Schwab *et al.* 1976). Moreover, the latter workers found that intra-abomasal infusion of sodium caseinate increased milk production in only one of four experiments with cows.

Other attempts to reproduce the stimulatory effect of intra-abomasal infusion of casein on milk yield by increasing the intake of protein which would be digested in the intestines have led to variable results. When grazing cows were given formaldehyde-treated casein, Wilson (1970) recorded an increase of 5% in milk production in one experiment and none in another. Similarly, Kellaway *et al.* (1974) recorded an increase of 7% in milk production in one experiment with lactating cows and no response in another. In contrast, Stobbs *et al.* (1977) recorded an increase of 20% in milk production of cows. It appears from the results of recent studies that formaldehyde treatment of protein may render such protein unsuitable for the lactating animal due to the formation of resistant cross-linkages which reduce the availability of tyrosine, tryptophan and histidine (Sidhu & Ashes, 1977).

Levels of indispensable amino acids in plasma were significantly increased when casein was infused in both experiments (Tables 5 and 8). If the amounts of amino acid absorbed were similar for all treatments in Expt 1 and all but treatment L_2+I in Expt 2, it is difficult to explain why plasma levels of amino acids were not similar, in view of the previous report that levels of plasma amino acids correlate closely with amounts of amino acids entering the abomasum in lactating goats (Ranawana & Kellaway, 1977*a*).

A mechanism by which milk production is stimulated by abomasal infusion of casein may be the release of galactopoietic hormone or hormones, in particular growth hormone

(Clark, 1975). The results of Expt 2 certainly do not support the proposal that casein administered post-ruminally stimulates milk production by virtue of the release of growth hormone. First, neither infusion of casein nor arginine raised the level of growth hormone in plasma (Table 9). Certainly, infusion of arginine resulted in a substantial increase in the level of arginine in peripheral blood and similarly, after infusion of casein the level of arginine (and indeed most indispensable and dispensable amino acids) in plasma was increased. In spite of the previously mentioned increases in levels of arginine, and other amino acids, there was no measurable effect on growth hormone in plasma. Secondly, in spite of the increase in milk production in Expt 2, non-significant though it was, there was a decrease in the efficiency of N utilization for milk production when casein was infused. Similar decreases were observed after infusion of arginine and feeding of meat meal. Examination of the results obtained by Ranawana & Kellaway (1977*a*, *b*) as well as Expt 1 (Table 2) shows that the efficiency of utilization of absorbed N for milk production usually was unchanged, relative to its utilization when the basal ration was fed, when casein was infused post-ruminally. This is inconsistent with the increase in milk yield being mediated by growth hormone. In this connexion, Machlin (1973) demonstrated that growth hormone administration gave rise to an increase in milk production with a concomitant increase in the efficiency of utilization of food for milk production.

Although the results presented here do not support the theory that milk production responses after post-ruminal supplementation with casein are mediated by growth hormone, recent studies with lactating goats by Oldham *et al.* (1977) provided evidence that abomasal infusion of casein led to a significant increase in levels of growth hormone in plasma. These contrasting observations raise the need to resolve whether growth hormone is implicated in increases in milk production obtained following post-ruminal supplementation with casein. The possibility that other galactopoeitic hormone(s) may be involved should be considered.

An alternative mechanism may be that casein provides an optimal arterial supply to the mammary gland of amino acids critical for biosynthesis of milk. This possibility is considered plausible in spite of failure of some workers to mimic the production response to casein after intra-abomasal infusion of mixtures of amino acids (Ranawana, 1976; Schwab *et al.* 1976). In connexion with the latter, it is considered that the introduction into the duodenum of abnormally large amounts of a small number of amino acids may result in impairment of absorption of other amino acids. Indeed, Williams (1969) demonstrated that individual amino acids are absorbed at different rates suggesting different affinities of receptors within the duodenum for particular amino acids, and the amino acids most commonly found to be first limiting for milk synthesis (methionine, phenylalanine, lysine) are among those absorbed fastest from the duodenum. Further, it has been demonstrated by Johns & Bergen (1973) that amino acids do compete with each other for transport from the intestinal lumen into blood.

Clearly, further studies will be necessary to determine the mechanism by which post-ruminal supplementation with casein gives rise to a stimulation in milk production, and the means by which this effect can be mimicked with dietary supplements.

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