The Effect of Supplementary Protein on In Vivo Metabolism of the Mammary Gland in Lactating Dairy Cows

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ABSTRACT

Four lactating cows equipped with rumen and duodenal cannulas were fed a diet of grass silage and concentrates containing either 12.4 or 17.2% CP (DM basis) in a change-over design. Additional protein was supplied as white fish meal. Fish meal did not affect molar proportions of VFA in the rumen, but duodenal NAN supply was increased .69 g/g of N in supplementary feed. In Experiment 2, three lactating dairy cows that had been prepared with catheters across the mammary gland were fed the same diets using a switchback design. Blood samples were taken to determine changes in metabolite flux to the mammary gland. In both experiments, milk production and protein yield were nonsignificantly increased by addition of fish meal. Milk urea output was increased from 3.18 to 4.74 g/d by fish meal supplementation, reflecting increased arterial concentrations of urea. Concentrations of glucose, VFA, and BHBA in blood showed no substantial changes because of dietary supplementation of fish meal. Supply of essential AA increased 26% with fish meal supplementation, mammary uptake increased 34%, but milk protein output only increased 5%. The low efficiency of conversion of supplementary protein to milk protein appears to be related to the inability of the gland to utilize the additional AA.

(**Key words**: protein, mammary gland, metabolism, lactation)

Abbreviation key: **EAA** = essential AA, **NEAA** = nonessential AA.

INTRODUCTION

Additional protein supplied to a lactating dairy cow usually increases milk protein output, but the extent of the increase is not easily predictable (22). Particular problems arise from the difficulty of estimating the subsequent partitioning of the additional AA, which are absorbed from the small intestine, toward milk protein synthesis. The differing results (19, 39) appear to be related to the energy and protein status of the cows used: cows given low energy diets tend to utilize most of the additional protein as an energy source. Studies (34) with abomasal infusion of mixtures of individual AA instead of casein or casein hydrolysate indicate that most of the effect of abomasal casein infusion is due to specific essential AA (**EAA**), but more recent work (33) has shown that these effects decrease with ongoing lactation. The use of protected AA supplements (12, 15) increased milk protein concentration in specific instances, but relatively little information exists on the mecha-

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nism of these changes. When supplied directly to the mammary gland (21, 24, 27), AA uptake by the gland increases, but increases in milk protein output were not established, and the fate of these additional AA is unclear. The length of infusion may have been insufficient to show such changes in output. Other work (10) has shown that intragastric supplementation with Pro can positively affect milk production and protein content; the response varies with stage of lactation. However, in another experiment (37), Arg, which can be utilized by the mammary gland to synthesize Pro, failed to produce a similar effect.

Rook and Balch (32) showed that intraruminal infusion of VFA can also affect milk protein concentration, supporting a possible relationship between energy and protein metabolism. The use of some energy to remove an increased hepatic load for conversion of NH_3 to urea has been shown in steers (40), but others (13) indicated that additional protein may stimulate gluconeogenesis. Increasing available energy by duodenal starch infusions had no effect on milk protein production, although milk protein production increased when starch was combined with duodenal casein infusion (4), suggesting that milk protein synthesis is strongly linked to energy metabolism and that those two aspects of metabolism should not be considered separately.

This experiment formed an initial study on the control of milk protein production, using a supplementary protein source typical for the United Kingdom. A low protein basal diet was used so that a response in milk protein output to the supplementary protein could be observed, and energy in the diet was held constant. The amount of fish oil in the fish meal was thought to affect rumen digestion, so the first part of this study (Experiment 1) served to quantify the effect of fish meal supplementation on rumen digestion and duodenal flow of nutrients. The second part of the study examined the effects of these dietary and digestive changes on mammary gland metabolism. A preliminary report of this work has been published (25).

MATERIALS AND METHODS

Experiment 1

Two diets based on grass silage were fed to four multiparous Holstein-Friesian cows (average BW, 660 kg) in midlactation that had been previously prepared with rumen and duodenal T-piece cannulas (6). Cows were offered twice daily either low protein (124 g of CP/kg of DM) basal concentrate or a high protein (172 g of CP/kg of DM) concentrate containing white fish meal (Provimi 66; Provimi Ltd., Hull, Humberside, England) as the additional protein source (Table 1; 9 kg/d of DM) and grass silage (Table 1; 8 kg of DM/d). This amount of feed should have provided the minimum dietary protein required for maintenance and lactation according to Agricultural Research Council (1) guidelines. Experimental design was a simple cross-over with 4-wk periods, including a 2-wk change-over design between diets and 2 wk for measurements, with a total of 8 wk for completion of the measurements. Both Cr-EDTA and Yb acetate were continuously infused into the rumen as digesta flow markers (6) at rates of 3.4 and .8 g/min, respectively. After 7 d of infusion, rumen and duodenal samples were taken hourly using automated samplers over at least 2 d of each experimental period (6). These samples were combined for analysis, resulting in one measurement per sample day. All cows were milked twice daily at 0730 and 1600 h, at which times they also were fed their daily ration in two equal portions. Milk samples were obtained from each cow on four consecutive milkings during the digesta sampling period for each dietary treatment. After preparation of two digesta phases (17), the samples of duodenal digesta were analyzed for OM, N, NH_3 N, Cr, and Yb (5). The daily amounts of DM, OM, total N, and NAN flow to the small intestine were estimated from the procedure described by Faichney (17). The VFA proportions in acidified rumen samples were determined by gas chromatography (5). Comprehensive analysis of the silage and concentrates used previously described methods (5, 36).

Experiment 2

Four additional Holstein-Friesian cows from the same herd, but in their second lactation, were prepared with catheters placed in the external pudic artery and milk vein (i.e., across the mammary gland), as previously described (27). The cows had calved 2 wk prior to surgical preparation, and 3 wk more were allowed for recovery before experimentation. The cows

were milked twice daily at 12-h intervals (0600 and 1800 h) and were fed the same concentrates and silage as in Experiment 1 but were fed four times daily at 0630, 1230, 1830, and 0030 h. The amount of feed offered was 18.25 kg/d of DM consisting of 10.25 kg of concentrate and 8 kg of silage. The basal diet provided the minimum requirement of dietary CP according to the Agricultural Research Council (1), as in Experiment 1. The experimental design consisted of a switchback: two cows were fed the dietary sequence low protein, high protein, and then low protein, and a second pair of cows were fed the high protein, low protein, and then high protein diets. Results for one cow on the latter sequence had to be deleted because of a mastitis infection caused by *Escherichia coli* during period 2, which seriously affected subsequent milk production and composition.

Each 5-wk experimental period included 1 wk for diet change-over, 2 wk for adaptation to diet, and 2 wk for measurements. During change-over, the cows were offered grass silage and a 1:1 (wt/wt) mixture of the two concentrate types and were milked twice daily. Milk samples were taken from each milking over the last 2 wk of each period, and paired blood samples were taken at 30-min intervals over 13 h, starting 30 min before the a.m. milking on the same day during the final week. All blood samples were placed immediately on ice in heparinized tubes (10 U/ml of blood) prior to preparation. Samples for oxygen analysis were taken separately into heparinized syringes and analyzed immediately. Blood flow across one-half of the mammary gland was determined by dye dilution using *p*-aminohippuric acid infusion at 90 mg/min, as previously described (27), throughout the 13 h. The cross-over of blood from one side of the venous circulation to the other was estimated to be minimal (<2%, results not shown) in the standing cow (26). Data relating to arterial supply of nutrients to and uptake by the mammary gland (Tables 4 and 5) were determined across one-half of the mammary gland, but no

TABLE 1. Concentrate formulations and the chemical composition of all feeds.

	Low protein	High protein	Silage
Component (kg/tonne of fresh weight)			
Barley	628	571	
Wheat	94	85	
Corn	125	114	
Molassed sugar beet pulp	20	96	
NIS[1]	105	18	
Molassine meal	26	24	
Fish meal (Provimi 66)[2]	0	91	
Chemical composition			
DM, g/kg of fresh	870	872	308
OM, g/kg of DM	926	947	915
N, g/kg of DM	19.82	27.58	28.25
CP, g/kg of DM	123.9	172.3	176.6
Gross energy, MJ/kg of DM	17.23	17.58	17.78
NDF, g/kg of DM	200	200	496
ADF, g/kg of DM	75	81	309
Starch, g/kg of DM	411	370	5.0
Water-soluble carbohydrates, g/kg of DM	68	65	103
Acetic acid, g/kg of DM			14.2
Propionic acid, g/kg of DM			1.1
Isobutyric acid, g/kg of DM			.7
n-Butyric acid, g/kg of DM			.5
Lactic acid, g/kg of DM			151.1
pH			3.88

[1]Nutritionally improved straw (alkali treated).

[2]Provimi Ltd., Hull, Humberside, England.

difference between halves was assumed in the discussion of mammary metabolism.

Sample Preparation and Analysis

Milk. Concentrations of milk fat, protein, and lactose were determined by infrared milk analysis as previously described (27). Urea concentration (Experiment 2 only) in milk stored at –20°C was determined enzymatically using a commercially available kit (Roche Diagnostic Products, Welwyn Garden City, Hertfordshire, England) by a method originally designed to measure urea concentrations in plasma but adapted for measurement of urea in milk on a discrete analyzer (Cobas Mira® Chemistry Analyzer; Roche Diagnostic Products).

Blood. Oxygen concentrations were determined on fresh blood using a blood gas analyzer (IL 1302 Instrumentation Laboratory, Warrington, Cheshire, England). Hemoglobin content of whole blood was determined using a colorimetric method (Boeringer Mannheim UK Ltd., Lewes, East Sussex, England). Blood samples (.5 ml) for AA analysis were laked with an equal volume of ice cold doubly distilled water containing .1 m*M* norleucine as an internal standard and 2.5% (vol/vol) 2-mercaptoethanol. Samples were stored at –40°C until required for analysis, at which time they were deproteinized by the addition of .25 ml of 31.5% (wt/vol) sulfosalicylic acid to produce a final concentration of 6.3% (wt/vol). The pH of the supernatant was adjusted to 2.2 by the addition of .055 ml of 4 *M* LiOH before analysis on a Beckman 6300 AA analyzer (Beckman Instruments, High Wycombe, Buckinghamshire, England). Inclusion of 2-mercaptoethanol in the laking solution was intended to protect Met from oxidation when glutathione was removed. Unfortunately, the 2-mercaptoethanol produced a large peak in the chromatogram, which disrupted the baseline in the initial acid region. Concentrations of Met and Val could not be determined routinely, even with the inclusion of 2-mercaptoethanol, because of problems with baseline and resolution of peaks. Furthermore, Arg concentrations are not presented because the laking procedure released reactive arginase from the red blood cells, causing a rapid decrease in Arg concentrations in the sample and a concomitant increase in concentrations of Cit and Orn. Concentrations of VFA were determined on whole blood as previously described (35). Packed cell volume was determined on the sampling day.

Plasma. All other analyses were carried out on plasma; determination of *p*-aminohippuric acid used previously described techniques (27), and glucose (hexokinase), urea, and BHBA were determined using commercially available kits (glucose and urea, Roche Diagnostic Products; BHBA, Sigma, Poole, Dorset, England). Lactate was determined enzymatically following the method of Bergmeyer (8). All analyses were adapted for the discrete analyzer.

Statistical Analysis

Data from experiment 1 were analyzed as a two-period cross-over design in which cows and periods were the blocking factors. Two degrees of freedom existed for the error term for significance testing of treatment effect using the *F* distribution. In Experiment 2, the design became unbalanced because of the loss of a cow. The data were analyzed using the REML method of Genstat (General Statistics Package; Lawes Agricultural Trust, Rothampstead, Hertfordshire, England), in which treatment was the fixed effect, and cows and period were the random effects. The treatments were estimated with .80 efficiency in the stratum for units. Recovery of information about treatment effects from cow and period strata gave identical results using REML or the combined means facility of the ANOVA directive in Genstat. Three degrees of freedom existed for the error term to perform *F* tests in ANOVA or Wald tests in REML.

RESULTS

Experiment 1

Feed intake was 16.4 and 16.5 kg/d of DM (SE of the difference = .14) on the basal and supplemented diets, respectively. The inclusion of fish meal in the concentrate had no effect on the molar percentages of the rumen VFA (Table 2) or on rumen digestion or duodenal flow. Total N intake was almost 70 g/d higher on the diet containing fish meal, which increased the flow of total N to the small intestine by 53 g/d, of which 46 g/d, equivalent to .69 g/d of extra N supplied, were present as NAN. The in-

TABLE 2. Effect of fish meal supplementation on rumen VFA molar proportions and OM and N digestion in dairy cows (Experiment 1).

	Basal	Fish meal	SED[1]
Rumen VFA, mol/100 mol			
Acetate	57.4	57.4	.59
Propionate	19.5	19.9	1.64
n-Butyrate	17.0	17.4	.80
OM Intake (OMI), kg/d	15.66	15.70	.086
Duodenal OM flow, kg/d	8.74	8.90	.539
OM Disappearance anterior to duodenum, g/kg of OMI	443	432	34.2
N Intake, g/d	374	441**	5.8
Total N flow at duodenum, g/d	426	479†	17.5
Duodenal NH_3 N flow, g/d	10	17	.9
Duodenal NAN flow, g/d	416	462	16.5

[1]Standard error of the difference.

†$P < .10$.

**$P < .01$.

crease in NAN flow, although nonsignificant ($P = .110$), agreed with predicted values based on previous studies (5) and Agricultural Research Council (1) guidelines. No significant effects on milk production or composition occurred (Table 3), although production of milk and milk protein tended to be higher with fish meal inclusion.

Experiment 2

Feed Intake. The DMI was 17.17 and 16.85 kg/d (basal and supplemented diets, respectively; SE of the difference = .20), and N intake was 399 and 469 g/d (basal and supplemented diets, respectively; SE of the difference = 25.2).

Milk Production. Recorded milk production in Experiment 2 (Table 3) was substantially lower than in Experiment 1, but the trend in response to fish meal supplementation showed a similar, nonsignificant increase over the basal diet. However, in this experiment, the concentrations of milk fat and protein were considerably higher than in Experiment 1. Mean fat content was 47.7 g/kg; values were slightly lower for cows on the fish meal diet than for those on the basal diet, and mean protein content was 37.7 g/kg; the response to fish meal (2.1%) was significant ($P < .10$). Consequently, overall fat production was unaffected by treatment, but protein production nonsignificantly increased, as in Experiment 1. Of greater interest, however, is a comparison of the relative production between the two experiments; despite the 20% lower milk production in Experiment 2 than in Experiment 1, the combined production of fat and protein was very similar. Fat production was marginally higher, and protein production was lower, in Experiment 2. As expected, lactose output in Experiment 2 agreed with the lower milk volume.

As a consequence of fish meal supplementation, urea concentration in milk was significantly ($P < .001$) increased, resulting in a significant ($P < .01$) increase in milk urea output, although, as a proportion of milk N, urea N was low on both treatments (<3 and 4% of total milk N for the basal and fish meal treatments, respectively).

Metabolite Flux. Blood flow to the whole mammary gland was unchanged by diet, averaging 11.4 L/min on the basal diet and 10.4 L/min on the protein-supplemented diet, which is equivalent to 750 and 663 L of blood flow/L of milk produced, respectively. Fluxes of metabolites were calculated after correction to whole blood using the packed cell volume. Arterial supplies were substantial for oxygen, glucose, urea, and acetate; arterial supplies of BHBA and lactate were smaller, and the supply of propionate was negligible (Table 4). Only the supply of urea was significantly ($P < .01$) increased by the inclusion of fish meal in

TABLE 3. Effect of fish meal supplementation on production and composition of milk and the output of milk components in Experiments 1 and 2.

Production and composition	Basal	Fish meal	SED[1]
	Experiment 1		
Milk production, kg/d	27.5	28.3	1.14
Composition, g/kg			
Fat	36.5	35.7	1.26
Protein	31.6	32.1	.45
Lactose	46.7	46.2	.40
Component output, kg/d			
Fat	1.010	1.006	.0763
Protein	.868	.907	.0461
Lactose	1.293	1.311	.0593
	Experiment 2		
Milk production, kg/d	21.9	22.6	.69
Composition, g/kg			
Fat	48.6	47.2	1.04
Protein	37.4	38.2†	.29
Lactose	48.0	47.6	.59
Urea	.148	.217***	.0012
Component output, kg/d			
Fat	1.062	1.060	.0571
Protein	.817	.860	.0374
Lactose	1.053	1.079	.0429
Urea, g/d	3.18	4.74**	.192

[1]Standard error of the difference.

†$P < .10$.

**$P < .01$.

***$P < .001$.

the diet. Similarly, the mammary uptake of these metabolites was largely unaffected by fish meal supplementation (Table 4). Mean oxygen uptake by the mammary gland amounted to approximately 29% of arterial supply, and uptakes of acetate, BHBA, and glucose were 67.4, 40.0, and 18.7% of supply, respectively. Despite a significant increase in output of urea in milk (Table 3), no uptake of urea could be detected, although the uptake required to account for the milk production, .13%, was within the range of analytical error. Interestingly, although arterial supply of lactate showed no change, venous output of lactate by the mammary gland, as indicated by a negative uptake, was significantly ($P < .05$) higher on the basal diet.

AA Flux. The effect of fish meal supplementation on the arterial supply of AA to the mammary gland and their subsequent uptake by the gland is shown in Table 5; EAA and nonessential AA (NEAA) are grouped according to their relative uptakes by the mammary gland (14). Under this scheme, Tyr is a mammary EAA. Supply of EAA was increased, although nonsignificantly, by 26% from 4.65 to 5.88 mmol/min; supply of Lys significantly increased ($P < .05$), and supplies of Leu and Ile were nonsignificantly increased. In response to these changes, uptake of EAA by the udder was significantly ($P < .05$) increased from 1.18 to 1.59 mmol/min, which represents a 34% increase, by the fish meal supplementation; relative extraction rates of arterial supply were 26 and 27% for the basal and supplemented diets, respectively. All measured EAA tended to have increased uptakes; responses in Tyr, Leu, and Lys were significant ($P < .05$).

Supply of NEAA was largely unaffected by protein supplementation, although large increases in the uptake of Ser and Ala were not significant. Total AA uptake was increased ($P < .10$) by 29% by protein supplementation.

DISCUSSION

The purpose of this study was to examine the nutrient metabolism of the mammary gland of lactating dairy cows, specifically in relation to the control of milk protein secretion. A dietary supplement (i.e., white fish meal) was used to increase AA availability to the cow, and Experiment 1 indicates that associated effects of the supplement within the rumen were minimal.

Although the response in duodenal NAN supply to the protein supplement was nonsignificant, Experiment 1 shows that the cows receiving the diet containing fish meal probably had an elevated supply of approximately 200 g/d of absorbed AA. In contrast, the response in milk protein output was only 39 g/d and was 19, 14, and 10% of the increased absorption, duodenal supply, or intake of protein (AA), respectively. Similarly, if the duodenal data on NAN supply (Experiment 1) are related to cows used in Experiment 2, efficiencies of 16, 12, and 8%, respectively, can be calculated. However, milk protein concentration did not change substantially in either experiment.

This lack of a highly positive production response hinders interpretation of the data

TABLE 4. Effect of fish meal supplementation on metabolite supply to and uptake by the lactating mammary gland.

	Arterial supply			Uptake		
	Basal	Fish meal	SED[1]	Basal	Fish meal	SED
	(L/min)					
Blood flow	11.4	10.4	1.35	. . .	. . .	. . .
	(mmol/min)					
Acetate	18.2	17.6	1.96	11.9	12.2	1.03
Propionate	.8	.6	.10	.3	.2	.07
BHBA	7.7	6.9	1.05	3.0	2.8	.35
Oxygen	67.0	61.8	8.17	19.3	17.7	2.25
Glucose	44.2	39.2	4.97	6.5	7.2	1.23
Lactate	4.0	3.6	1.20	−1.1	−.2*	.15
Urea	27.1	40.9**	5.50	−.7	−.2	.70

[1]Standard error of the difference.

*$P < .05$.

**$P < .01$.

relating to nutrient utilization by the mammary gland. Of the non-AA metabolites examined, only urea showed a significant increase in arterial supply in response to protein supplementation, but lactate output from the mammary gland was significantly reduced. Significant changes in AA metabolism were confined to the arterial supply of Lys and the mammary uptake of Tyr, Lys, Leu, and total EAA. Calculated AA compositions (16) of the diet indicated that the supplemented diet would have approximately a twofold increase in Lys con-

TABLE 5. Effect of fish meal supplementation on AA supply to and uptake by the lactating mammary gland.

	Arterial supply			Uptake		
	Basal	Fish meal	SED[1]	Basal	Fish meal	SED
	(μmol/min)					
EAA[2] in mammary gland						
Tyr	544	512	59.2	102	144*	7.6
Phe	522	490	58.4	126	144	7.2
His	602	784	82.8	120	128	17.8
Ile	1028	1172	102.4	226	300	28.8
Leu	1016	1320	116.2	304	436*	24.0
Lys	950	1578*	195.6	310	436*	30.2
NEAA in mammary gland						
Asp	6416	5962	789.4	−80	−60	46.6
Glu	2018	1784	353.6	484	392	42.4
Gln	3646	3138	218.2	494	486	132.2
Ser	1278	1234	183.8	30	142	53.6
Ala	2172	2348	394.2	14	196	62.6
Pro	798	828	228.4	114	96	116.8
Total AA	20,764	21,302	1947.6	2224	2864†	303.8
NEAA	16,328	15,294	1018.4	1056	1252	204.6
EAA	4646	5880	577.8	1184	1590*	91.6

[1]Standard error of the difference.

[2]EAA = Essential AA; NEAA = nonessential AA.

†$P < .10$.

*$P < .05$.

centration in the concentrate, and other EAA would increase from 130 for Phe to 191% for Met. In a similar experiment in growing steers (5), in which fish meal was added at 5% of DM to a grass silage diet, AA concentrations of duodenal digesta were determined. The increase in flow of the AA was small, 7.5%, but in agreement with individual concentrations of AA in fish meal. In the current experiment, fish meal inclusion was approximately 5.5% of DM. The changes here are similar to those for diets containing silage and fish meal fed to growing steers (5) in which AA flows into the duodenum increased by 23% for Met and 11 and 12% for Lys and Leu, respectively. Duodenal flows of Phe, Tyr, and Ser were not increased in those experiments (5) until a higher amount of fish meal was fed. The occurrence of similar effects in the present experiment could explain why arterial supply of these AA was not increased by fish meal supplementation.

Given the magnitude of the milk protein response in Experiment 2 and assuming that this response was accompanied by a quantitative increase in AA uptake by the mammary gland, the increased AA uptake can be estimated to be 234 μmol/min of total AA. In contrast, for the 12 AA in Table 5, which are estimated to account for approximately 75% of total casein AA, the measured increase in AA uptake was 640 μmol/min, which is a significant ($P < .1$) difference. Therefore, failure to establish a significant ($P < .05$) response in total AA uptake by the mammary gland was in part related to the limited response in milk protein output. The results of this study refer to only three cows, and data from additional cows would have been highly valuable. However, future studies probably will not include more than six cows because of the problems in long-term maintenance of catheter patency.

Despite the limitations discussed, the data presented in this study are biologically credible and, furthermore, provide interesting concepts for subsequent consideration and possible experimentation.

The data relating to supply and uptake of acetate suggest no effect of treatment, which agrees with the lack of response in milk fat output in either experiment. Furthermore, the mean extraction rate of acetate was high on both diets ($\bar{X} = 67\%$), which agrees with earlier findings (9, 28) and is equal to .79 mol of acetate/L of milk produced, in close agreement with a theoretical value of .80 mol/L (22) when 50% of milk fat is assumed to be synthesized de novo from acetate.

Glucose supply was unaffected by treatment, and, on both diets, mammary extraction rate amounted to between 17 and 20% of arterial supply, which concurs with other data (9, 24, 28). In consequence, glucose uptake averaged 6.8 mmol/min. Based on glucose requirements of the mammary gland for lactose synthesis and the provision of glycerol and NADPH for fat synthesis, 89% of glucose uptake can be accounted for on both treatments. These calculations assume that glucose oxidation in the mammary gland can account for $\leq 7\%$ of CO_2 production in lactating cows (3) but does not account for a possible contribution of glucose to NEAA synthesis within the mammary gland. Overall, glucose uptake by the mammary gland was equated with 79.9 g/kg of milk produced, which is in broad agreement with the proposed theoretical value of 70 g/L (22). Oxygen uptake by the mammary gland amounted to 18.5 mmol/min, indicating net utilization of 29% of arterial supply for both treatments. Based on previous measurements of total gaseous exchange for similar cows on similar but not identical diets (7), mammary oxygen consumption can be estimated to be approximately 25% of whole body oxygen consumption.

The decreased output of lactate by the mammary gland in response to increased protein supply in the diet is of biochemical interest. In vitro work (18) has indicated that lactate could be oxidized in the mammary gland, especially when acetate concentrations are <1.0 m*M*. If arterial acetate concentrations can be equated with the incubation medium used in vitro (18), the mean of 1.6 m*M* in the present study suggests that lactate oxidation in the mammary gland was of reduced significance, although its significance cannot be eliminated without appropriate tracer studies. As for the origin of the elevated output on the basal diet compared with virtually zero output on the supplemented diet, the provision of extra AA within the mammary gland by pro-

tein supplementation may have permitted a more complete oxidation of glucose through intermediates of the tricarboxylic acid cycle. In this context, a link between lactate and Ser metabolism has been suggested (N. E. Smith, 1992, personal communication), which may partially explain the apparent increase in Ser uptake on the supplemented diet.

The appearance of a small but significant increase in urea concentration in milk for cows receiving the supplemented diet agrees with earlier reports (30). However, given that the mammary gland does not possess all of the urea cycle enzymes, the urea output in milk probably resulted from the transfer of urea in blood through leaky junctions within the mammary gland. Overall, however, urea in milk production accounted for only .13% of the arterial supply of urea.

This study was designed to relate dietary supply of AA to AA utilization by the mammary gland. Changes in total AA supply to the gland were not significant; although EAA supply was substantially increased (27%) as a consequence of the imposed treatment, a commensurate reduction occurred in NEAA supply. The uptake of EAA by the mammary gland appeared to respond directly to the increased EAA supply; basal and supplemented treatments showed a net EAA extraction of 25.5 and 27.0% of supply, respectively. The increased EAA uptake on the fish meal diet (406 μmol/min) was accompanied by an increased uptake of NEAA (234 μmol/min), and overall net NEAA extraction was greater on the treated (8.3%) than on the control (6.5%) diet.

Data relating to the arterial supply of individual EAA do not provide evidence of a consistent effect of the imposed treatment. The results are consistent with expectations based on duodenal AA analysis in other work (5); major increases were confined to Leu, Ile, His, and Lys. Overall, these AA accounted for all (102%) of the increased supply and 84% of the increased uptake of EAA by the mammary gland, although extraction rates of individual AA were either marginally increased (Ile from 22 to 26% and Leu from 30 to 33%) or decreased (His from 20 to 16% and Lys from 33 to 28%) by treatment. These extraction rates were in broad agreement with previous data (11, 28).

The responses in mammary uptake of individual AA, driven largely by the variable responses in arterial supply, suggest that the role of the liver in deamination of AA (31) and, particularly, in the apparent resistance of branched-chain AA to hepatic catabolism (29) merits further investigation, but preferential use of specific AA by nonmammary tissues cannot be ignored.

Additionally, the dietary supplement may have preferentially increased the relative proportions of specific AA in the absorbed AA fraction, although experimentation with growing heifers fed grass silage with or without a fish meal supplement gave little indication of this effect (5).

Comparison of measured uptake of individual AA with calculated output in milk (Table 6) indicated that, for the 6 EAA examined, His was considerably (30%) in excess of output on both diets. Uptake of Lys increased from approximate balance with output (5%) on the control diet to a substantial excess on the fish meal diet (40%), whereas Leu and Ile gave similar values; uptake ranged from 75 to 107% of output.

In contrast, on both diets, uptakes of Phe and Tyr did not meet their respective outputs in milk. This apparent shortfall in the uptake of specific free EAA suggests the existence of an alternative source of AA, given that these AA cannot be synthesized by the mammary gland. Studies with growing animals (38) have suggested that low molecular weight peptides may be absorbed from the gastrointestinal tract and subsequently used by peripheral tissues. The metabolism of peptides by the mammary gland has not been conclusively established, but, based on the data from the present study, elimination of a possible involvement in the synthesis of milk proteins would be premature. However, before peptides are determined to play a substantial role in mammary AA metabolism, other diets should be examined, including those with a greater milk protein output response than in the present experiment.

Previous studies (14) have also demonstrated differences between AA in their relative extraction rates from arterial supply and their net uptakes in relation to output in milk; several groupings of AA have been proposed. The data of the present study agree with these

TABLE 6. Daily uptake of individual AA by the whole lactating mammary gland compared with calculated daily output in milk protein.[1]

	Uptake		Output		Uptake relative to output	
	Basal	Fish meal	Basal	Fish meal	Basal	Fish meal
	(mmol/d)				(%)	
EAA[2]						
Tyr	147	207	210	221	70	94
Phe	181	207	240	252	76	82
His	174	184	135	142	129	130
Ile	325	432	384	405	85	107
Leu	438	628	586	616	75	102
Lys	446	628	425	447	105	140
NEAA						
Glu	697	564				
Gln	711	700	1145[3]	1205	123	105
Ser	43	205	384	404	11	48
Ala	20	282	314	330	6	81
Pro	164	138	620	653	26	21

[1]The AA output in the milk proteins calculated as protein production less 5% as NPN using AA compositions from the literature (2).

[2]EAA = Essential AA; NEAA = nonessential AA.

[3]Combination of Glu and Gln residues after hydrolysis.

groupings in many respects. One group of AA represented by Leu and Lys showed increases in uptake in accordance with increased supply, and a second group, represented by Tyr and Phe, showed increased uptake despite no increase in arterial supply. In contrast, a third group, including His and Pro, showed no change in uptake, despite modest increases in supply. These data therefore suggest that arterial supply per se is not the sole factor controlling AA uptake by the mammary gland and that regulatory mechanisms within the mammary gland may play a more important role in the synthesis of milk proteins.

For NEAA, the discrepancies between uptake and output for Ser and Ala may be explained by the ability of these AA, with Asp, to be synthesized from glucose within the mammary gland (20), although the source of N for these AA is not known. Similarly, Pro can be synthesized in the mammary gland from Arg, Cit, or Orn (23), which may explain the low uptake to output ratio for Pro. Further investigation of the role of the interaction of Agr and Pro in the mammary gland should be conducted in the context of results obtained by duodenal infusion of Pro (10), in which production of milk protein and milk fat was stimulated in midlactating dairy cows.

CONCLUSIONS

Although the modest response in milk protein output to the dietary protein supplement has limited interpretation of this experiment, the data from an examination of arteriovenous differences in nutrient utilization in the mammary gland apparently are biologically credible.

Results relating to mammary utilization of acetate, BHBA, and glucose and to oxygen consumption agree with previous data and with the percentages of milk fat and lactose secretion on the two diets. In contrast, the data on AA metabolism by the mammary gland suggest interesting effects, including the possible involvement of AA that are bound by peptides. More specifically, however, milk protein output responses apparently are not driven by arterial AA supply alone, thus establishing the importance of intramammary mechanisms to control AA uptake and the subsequent secretion of milk proteins.

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