

Optimizing the Utilization of Animal Fat and Ruminant Bypass Proteins in the Diets of Lactating Dairy Cows¹

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ABSTRACT

Fifty cows were used to evaluate the lactational response to diets containing additional fat as tallow and increased amounts of RUP (bypass proteins) with or without molasses. Cows were blocked by parity and calving date and randomly assigned to one of five diets from wk 4 to 16 postpartum. Treatments were 1) control (soybean meal), 2) bypass proteins (blood meal, meat and bone meal, corn gluten meal, soybean meal), 3) molasses and bypass proteins, 4) fat and bypass proteins, and 5) molasses, fat, and bypass proteins. Cows were fed for ad libitum intake a total mixed diet that contained 25% corn silage, 25% alfalfa hay, and 50% concentrate mix (dry matter basis). Production of milk was higher for cows fed diets containing fat and bypass proteins; molasses and bypass proteins; and molasses, fat, and bypass proteins than for cows fed the diet with bypass proteins alone, but production was similar for cows fed the control diet and diets containing bypass proteins alone. Production of milk was similar for cows fed the diet with molasses and bypass proteins and for cows fed the diet with fat and bypass proteins. Milk protein percentages were higher for cows fed the diet with molasses and bypass proteins than for those fed the diet containing fat and bypass proteins. The dry matter intake, body weight gains, and body condition scores were unaffected by treatment. For all diets, Met, Lys, and Phe were the first three limiting essential amino acids for milk protein synthesis. Production was increased by including either fat or molasses with bypass protein, but there was no clear advantage of including both fat and molasses in the diet.

(**Key words:** animal fat, ruminally undegradable protein, lactating dairy cows)

Abbreviation key: AV = arteriovenous; BP = bypass proteins; EAA = essential AA; FBP = fat and BP;

MBF = mammary blood flow; MBP = molasses and BP; MFBP = molasses, fat, and BP; NEAA = nonessential AA.

INTRODUCTION

Diets containing supplemental fat often stimulate increased milk production (5, 14, 17) because of increased energy intake, improved efficiency of utilization of energy, or both. Milk production was also increased when the diet with supplemental fat contained increased amounts of ruminally degradable carbohydrates from dried whey (5, 17) or molasses (17), probably because dried whey (5) and molasses (30) provided more readily fermentable carbohydrates to stimulate microbial protein synthesis in the rumen. Supplementing the diets of ruminants with protein sources that are resistant to ruminal degradation improved N and AA flow to the small intestine (6). Some of the studies reviewed by Schingoethe (26) indicated increased production when high producing cows were fed diets containing higher amounts of RUP, but others showed little or no response. For instance, milk production was increased when cows were fed high quality protein, such as a blend of fish meal, blood meal, and soybean meal, in place of lower quality protein, such as corn gluten meal (8), even though both diets contained equal amounts of RUP. Blends of protein supplements often supported more milk production than did individual protein supplements fed alone (26). A blend of proteins containing animal proteins (e.g., blood meal and meat and bone meal) and plant proteins (e.g., corn gluten meal and soybean meal) appears to be a source of high quality protein with good ruminal bypass potential.

The objective of this study was to evaluate diets that were aimed at increasing milk and milk protein production while using animal fat and proteins in the diets. The intent was to provide sufficient ruminally fermentable carbohydrates as molasses to ensure optimal microbial protein synthesis, even when the diet contained supplemental fat, and to supplement the ruminal microbial proteins with high quality RUP.

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MATERIALS AND METHODS

Experimental Plan

Fifty Holstein cows (30 multiparous and 20 primiparous) were blocked by parity and calving date and randomly assigned within block to one of five treatment diets from wk 4 to 16 postpartum. Cows were fed a standard diet (corn plus soybean meal in concentrate mix) from wk 1 to 3 postpartum and produced at least 23 and 27 kg/d of milk during wk 3 for first and later lactations, respectively. Treatments were 1) control, 2) bypass proteins (BP), 3) molasses and BP (MBP), 4) fat and BP (FBP), 5) molasses, fat, and BP (MFBP). Incorporation of BP into the diets was based on calculations of the milk protein scores (26) of individual protein supplements making up the BP mixture. The blend of protein supplements was formulated such that soybean meal supplied one-half of the supplementary protein, and 16.7% of supplementary protein was supplied by each of blood meal, meat and bone meal, and corn gluten meal. Total mixed diets were fed for ad libitum intake. The total mixed diet consisted of 25% corn silage, 25% alfalfa hay, and 50% concentrate mixes (DM basis) (Table 1). Cows were housed in a free-stall barn and individually fed a total mixed diet once daily using Calan feeding doors (American Calan, Inc., Northwood, NH).

Data Collection and Analytical Procedures

Amounts of feed offered andorts were recorded daily from wk 4 to 16 postpartum. Body weights were

recorded for 3 consecutive d at the start and end of the experimental period and once weekly throughout the trial. Body condition scores were recorded once weekly using the scoring system described by Wildman et al. (29).

Cows were milked twice daily, and milk weights were recorded at each milking throughout the experimental period. Two 24-h (p.m. plus a.m.) milk samples (~450 ml) were collected from each cow during wk 3 postpartum (pretreatment), and one 24-h sample was taken weekly throughout the trial. Milk samples (40 ml) were analyzed for fat, protein, lactose, and SNF contents by the mid infrared spectroscopic method (Multispec; Foss Food Technology Corp., Eden Prairie, MN) (1). The SCC were determined using Fossomatic 90 (Foss Food Technology Corp.) as described by the AOAC (1).

Samples of concentrate mixtures, corn silage, and alfalfa hay were obtained weekly throughout the experiment and frozen at -20°C. Samples were combined into monthly composites, oven-dried at 55°C for 48 h, and ground through an ultracentrifuge mill (1-mm screen; Brinkmann Instrument Co., Westbury, NY). Samples were analyzed for DM, CP, ether extract, and ash (1). Permanganate lignin and ADF were determined by the procedures of Robertson and Van Soest (24). The NDF was determined as described by Van Soest et al. (28). Contents of Ca and Mg were determined by atomic absorption spectrophotometry (1), and P was determined by the AOAC official method 931.01 (1).

TABLE 1. Ingredient composition of total mixed diets for cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBP); fat and BP (FBP); or molasses, fat, and BP (MFBP).

Ingredient	Diet				
	Control	BP	MBP	FBP	MFBP
	(% of DM)				
Corn silage	25.00	25.00	25.00	25.00	25.00
Alfalfa hay	25.00	25.00	25.00	25.00	25.00
Corn, shelled rolled	31.10	34.00	25.75	30.95	22.70
Soybean meal, 44% CP	16.25	7.80	7.95	8.10	8.25
Blood meal	...	1.50	1.50	1.55	1.55
Meat and bone meal	...	2.40	2.45	2.50	2.55
Corn gluten meal	...	1.90	1.95	2.00	2.05
Molasses, dry	8.00	...	8.00
Tallow	2.50	2.50
Dicalcium phosphate	0.50	0.25	0.25	0.25	0.25
Limestone	0.50	0.50	0.50	0.50	0.50
Sodium bicarbonate	0.75	0.75	0.75	0.75	0.75
Magnesium oxide	0.25	0.25	0.25	0.25	0.25
Trace mineral salt	0.50	0.50	0.50	0.50	0.50
Vitamin premix ¹	0.15	0.15	0.15	0.15	0.15

¹Provided 4409 IU of supplemental vitamin A, 880 IU of vitamin D, and 22 IU of vitamin E/kg of DM.

Ruminal fluid was sampled from each cow once per month, and three samples per cow were collected during the trial at about 2 to 4 h after feeding. Samples were obtained by applying vacuum to an esophageal tube fitted with a suction strainer. To minimize saliva contamination of samples, the first 250 ml of ruminal fluid were discarded. Samples were collected into a 250-ml bottle and placed on ice. Samples were analyzed for VFA (3) and ammonia concentration (7).

Blood samples from the tail artery (coccygeal vessels), mammary vein (subcutaneous abdominal vein), and jugular vein were collected into serum tubes at the time that ruminal fluid was sampled. Samples were centrifuged for 20 min at $850 \times g$, and the serum was decanted and frozen. The jugular serum samples were analyzed for urea N (7). Serum samples from the coccygeal vessels and mammary vein were obtained from the three highest producing cows per treatment (wk 6 to 8 postpartum) and were analyzed for AA concentration (12, 20). Soluble protein in serum was precipitated with sulfosalicylic acid and removed by filtration. The serum was then diluted with lithium citrate buffer. The AA were resolved by ion-exchange chromatography using an isothermal format for the elution of the three-buffer, lithium HPLC gradient. The AA were quantified by postcolumn derivatization using ninhydrin (Pickering Laboratories, Inc., Mountain View, CA). The AA uptake from blood by the mammary gland was estimated using arteriovenous (AV) differences of AA

and mammary blood flow (MBF), estimated as described by Cant et al. (2), which assumes that Phe plus Tyr uptake equals Phe plus Tyr output.

Statistical Analysis

Data were subjected to least squares ANOVA for a randomized complete block design using the general linear models procedure of SAS (25); weeks were the repeated measures (19). Treatment means for milk production and composition were adjusted by analysis of covariance using data from wk 3 as covariates. This procedure used two separate runs of the general linear models procedure to obtain the proper regression slope for covariance adjustment of treatment means.

The sources of variation were replication (df = 9), diet (df = 4), covariate (df = 1), error a (df = 35), week (df = 12), week \times replication (df = 108), week \times diet (df = 48), and error b (df = 432). Diet was tested by error a; week and week \times diet were tested by week \times replication and error b, respectively. Three repetitions (df = 2) rather than 13 wk were included for measurements of ruminal and blood samples.

Differences among treatment least squares means were compared using orthogonal single degree of freedom contrasts to compare the control with the BP, MBP, FBP, and MFBP diets; the BP diet with the MBP, FBP, and MFBP diets; the MBP diet with the FBP diet; and the MBP and FBP diets with the MFBP. Statistical significance was declared at $P < 0.05$ unless otherwise noted.

TABLE 2. Chemical composition of total mixed diets for cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBP); fat and BP (FBP); or molasses, fat, and BP (MFBP).

Item	Diet				
	Control	BP	MBP	FBP	MFBP
DM, %	67.3	67.3	67.6	67.5	67.8
	(% of DM)				
CP	18.9	19.0	19.0	19.2	18.8
RUP, % of CP ¹	37.3	45.1	43.2	43.8	44.8
NE _L , ² Mcal/kg	1.65	1.65	1.63	1.74	1.72
Ether extract	2.75	2.90	2.70	5.65	5.30
NDF	30.8	31.1	32.6	30.8	32.4
ADF	18.9	18.4	20.2	18.5	20.2
Lignin	5.0	4.9	5.5	4.9	5.1
Ash	7.0	7.3	8.2	7.5	8.0
CA	0.74	0.91	1.05	0.93	1.02
P	0.47	0.48	0.47	0.48	0.44
Mg	0.33	0.33	0.38	0.36	0.35
Soluble residue ³	40.5	39.7	37.4	36.9	35.5

¹Calculated based on measured ruminal escape N content of soybean meal, blood meal, meat and bone meal, and corn gluten meal (16) and other values estimated from the NRC (21).

²Estimated from the NRC (21).

³100 - (CP + EE + NDF + ash).

RESULTS AND DISCUSSION

Nutrient Content of Diets

Chemical composition of the total mixed diet is presented in Table 2. All of the diets contained similar amounts of CP. Diets containing BP (i.e., BP, MBP, FBP, and MFBP diets) had more RUP and Ca than did the control diet. Increased Ca concentration was due to the meat and bone meal in those diets. Addition of 2.5% tallow increased ether extract and NE_L contents of FBP and MFBP diets. Fiber contents of the total mixed diet reasonably met the NRC (21) recommendations for high producing dairy cows. All diets provided sufficient ruminally fermentable carbohydrates to assure optimal microbial protein synthesis as indicated by soluble residue.

Lactational Response

Production of milk and 3.5% FCM was similar for cows fed the control diet and cows fed the other diets (Table 3). Cows fed the BP diet had a lower mean

milk production than did cows fed the MBP, FBP, and MFBP diets. Production of milk and 3.5% FCM was similar for cows fed the MBP and FBP diets, which indicates that energy (in the form of soluble carbohydrate, or fat, or both) is the nutrient that drives milk production when diets are fed that are high in RUP. Cows fed the MFBP diet had lower milk and 3.5% FCM production than did cows fed the MBP or FBP diets. Three cows among those fed the MFBP diet had high SCC, lowering the mean milk production of this group during wk 8 to 11 postpartum, which may at least partially explain the lower production when cows were fed that diet. Milk production efficiency (FCM/DMI) was unaffected by treatment. Diets containing fat, molasses, or both increased production of milk and 3.5% FCM (17). Stern et al. (27) summarized several studies in which animal and plant protein sources that were high in RUP were fed to dairy cows. Those researchers concluded that a high RUP diet might increase total N, nonmicrobial NAN, and AA flow to the duodenum, but microbial N flow might be decreased. Because the BP supplements used in this study were high in RUP, the greater quantity of

TABLE 3. Milk production and composition, DMI, production efficiency (3.5% FCM/DMI), BW, BW gain, and body condition scores (BCS) for cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBF); fat and BP (FBP); or molasses, fat, and BP (MFBP).

Item	Diet					SE	Contrast ¹			
	Control	BP	MBP	FBP	MFBP		1	2	3	4
	<i>P</i>									
Cows per treatment, no.	10	10	10	10	10					
Milk, ² kg/d	33.3	32.8	35.9	36.4	33.6	1.00	0.25	0.03	0.73	0.04
3.5% FCM, ² kg/d	32.4	32.6	35.2	35.0	32.8	0.84	0.13	0.09	0.88	0.03
Fat ²										
%	3.42	3.55	3.46	3.39	3.44	0.11	0.75	0.38	0.65	0.88
kg/d	1.13	1.16	1.23	1.21	1.15	0.03	0.20	0.37	0.70	0.11
Protein ²										
%	3.09	2.98	2.97	2.85	2.82	0.04	<0.01	0.02	0.02	0.04
kg/d	1.02	0.97	1.06	1.03	0.94	0.03	0.64	0.21	0.45	<0.01
Lactose ²										
%	4.82	4.81	4.87	4.78	4.80	0.04	0.99	0.76	0.17	0.50
kg/d	1.59	1.58	1.75	1.74	1.62	0.05	0.12	0.03	0.94	0.05
SNF ²										
%	8.62	8.49	8.54	8.37	8.26	0.06	<0.01	0.14	0.04	0.01
kg/d	2.83	2.78	3.06	3.03	2.79	0.08	0.36	0.06	0.81	0.01
SCC, ² ×10 ⁵ /ml	4.87	3.11	1.88	2.52	2.89	1.42	0.17	0.68	0.75	0.70
DMI, kg/d	22.0	21.3	22.1	21.7	21.8	0.81	0.77	0.55	0.73	0.89
FCM/DMI	1.47	1.53	1.59	1.61	1.50	0.05	0.21	0.65	0.78	0.25
BW, kg	598	607	599	614	554	17.52	0.84	0.39	0.54	0.02
BW Gain, kg/d	0.23	-0.04	0.05	0.26	0.02	0.78	0.21	0.27	0.20	0.30
BCS ³	2.70	2.77	2.70	2.86	2.76	0.10	0.54	0.96	0.26	0.86

¹Contrast 1 = Control versus BP, MBP, FBP, and MFBP; contrast 2 = BP versus MBP, FBP, and MFBP; contrast 3 = MBP versus FBP; and contrast 4 = MBP and FBP versus MFBP.

²Adjusted for covariance.

³Scored on a five-point scale where 1 = severe undercondition and 5 = severe overcondition (29).

TABLE 4. Ruminal VFA, ammonia, and urea N concentrations in serum of cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBP); fat and BP (FBP); or molasses, fat, and BP (MFBP).

Item	Diet					SE	Contrast ¹			
	Control	BP	MBP	FBP	MFBP		1	2	3	4
VFA, mol/100 mol										
Acetate (A)	62.9	63.2	64.7	63.7	64.4	0.58	0.17	0.19	0.26	0.80
Propionate (P)	20.5	18.4	19.0	19.3	19.1	0.57	0.02	0.26	0.75	0.89
Isobutyrate	1.4	1.3	1.2	1.4	1.2	0.05	0.21	0.19	0.05	0.19
Butyrate	11.8	11.8	11.9	12.3	12.2	0.31	0.40	0.44	0.31	0.70
Isovalerate	1.9	1.7	1.6	1.8	1.6	0.06	0.01	0.29	0.05	0.02
Valerate	1.6	1.5	1.5	1.4	1.5	0.05	0.07	0.58	0.29	0.58
Total VFA, μ mol/ml	109.9	103.6	103.2	99.7	99.8	2.83	0.01	0.41	0.39	0.65
A:P	3.14	3.43	3.43	3.35	3.40	0.11	0.02	0.13	0.62	0.98
Ammonia, mg/dl	16.8	12.0	9.8	9.7	9.0	1.25	0.01	0.09	0.95	0.59
Serum urea N, mg/dl	21.2	20.9	20.9	19.5	20.3	0.95	0.43	0.55	0.29	0.89

¹Contrast 1 = Control versus BP, MBP, FBP, and MFBP; contrast 2 = BP versus MBP, FBP, and MFBP; contrast 3 = MBP versus FBP; and contrast 4 = MBP and FBP versus MFBP.

RUP was possibly counterbalanced by less microbial protein synthesis. Addition of RUP to diets based on alfalfa silage had no effect on milk production (14). Repeated measures data showed a significant ($P < 0.01$) treatment \times week interaction. Milk production peaked 2 wk later (wk 10 postpartum) for cows fed the MBP, FBP, and MFBP diets than for cows fed the BP diet (data not shown). Cows fed MFBP diet had lower milk production during wk 8 to 11 postpartum.

Milk fat percentages were unaffected by diet. Supplementation of dairy cow diets with fat sometimes (5, 11), but not always (14, 17, 22), depressed milk fat percentages.

Milk protein percentages were affected by diet. A greater proportion of RUP in the diet did not increase milk protein percentages over the control, indicating a possible reduction in microbial protein synthesis. Milk protein percentages were higher for cows fed the MBP diet than for cows fed the FBP diet. However, milk protein production was similar for cows fed these two diets. Supplemental dietary fat often decreased milk protein percentages (2, 4, 5). Milk protein production was higher for cows fed the MBP and FBP diets than for cows fed the MFBP diet because of the higher milk production of cows fed these diets.

Milk lactose concentrations were unaffected by diet. Milk SNF percentages were higher for cows fed the control diet than for cows fed the other diets. Milk SNF percentages were higher for cows fed the MBP diet than for cows fed the FBP diet. Mean production of lactose was lower, and mean production of SNF tended ($P < 0.06$) to be lower, for cows fed the BP diet than for cows fed the MBP, FBP, and MFBP diets because of the increased milk production for cows fed these diets. In general, milk and milk component

production was lower for cows fed the MFBP diet than for cows fed diets containing fat or molasses. Milk SCC were unaffected by diet.

The DMI, BW gains, and body condition scores were similar for cows fed all diets. The lower BW for cows fed the MFBP diet were due to lower initial BW of some cows receiving this diet; BW gain and body condition score were unaffected for those cows.

Ruminal and Blood Urea N

Concentrations of VFA and ammonia from ruminal samples and concentrations of urea N in serum are presented in Table 4. Molar percentages of propionate, isovalerate, valerate, and ruminal ammonia were higher for cows fed the control diet than for cows fed diets containing BP, which was likely due to the higher RUP content of these diets that might have limited microbial activity. Cunningham et al. (10) reported reduced ammonia N as RUP in the diet increased. Concentrations of urea N in serum were unaffected by diet.

Blood AA

Concentrations of AA in the serum of the tail artery (coccygeal vessels) are presented in Table 5. Given the small number of observations per treatment ($n = 3$), $P < 0.10$ was used to determine differences (5). Mean concentrations of Leu and Phe in the arterial serum of cows fed the control diet were lower ($P < 0.08$) than those for cows fed the other diets; however, the converse was true for Thr. Mean concentrations of Leu, Val, and total essential AA (EAA) were lower ($P < 0.07$) for cows fed the BP diet than

those for cows fed the MBP, FBP, and MFBP diets. Concentrations of His and Thr were higher ($P < 0.03$), and, in general, concentrations of EAA were numerically higher, for cows fed the MBP diet than for cows fed the FBP diet. Reduced plasma or serum AA concentrations with supplemental fat have been reported (5). Mean concentrations of Thr were lower for cows fed the MFBP diet than those for cows fed the MBP and FBP diets. Calculations of AA concentrations of diets based on published data (16, 23) showed that all diets containing BP had similar EAA content. Therefore, lower concentrations of Thr in the arterial serum of cows fed the supplemental fat could not be attributed to AA concentrations of the diet containing fat. Ratios of total EAA to nonessential AA (NEAA) < 0.6 indicate protein deficiency (22). Ratios in this study ranged from 0.82 to 1.04, indicating an adequate protein supply from all diets. Higher total EAA concentrations in the arterial blood of cows fed the MBP, FBP, and MFBP diets explained the higher milk production by cows fed these diets than that by cows fed the BP diet. Mean concentrations of Cit were

lower ($P < 0.10$) for cows fed the BP diet than those for cows fed the MBP, FBP, and MFBP diets. Concentrations of Ala and Ser for cows fed the MFBP diet differed ($P < 0.09$) from concentrations for cows fed the MBP and FBP diets. Arterial concentrations of total NEAA were unaffected.

Mean venous (subcutaneous abdominal vein) concentrations of Arg, Leu, Phe, and Thr differed ($P < 0.09$) for cows fed the control diet from concentrations for cows fed the other diets (Table 6). Cows fed the MBP diet had higher ($P < 0.10$) venous concentrations of His and Thr than did cows fed the FBP diet. Concentrations of Met and Ser in venous serum were lower ($P < 0.09$) for cows fed the MFBP diet than for cows fed the MBP and FBP diets.

Mean arteriovenous difference of Arg was lower ($P < 0.01$), but those of Trp and Ala were higher ($P < 0.07$), for cows fed the control diet than for cows fed the other diets (Table 7). The mean AV differences of His and two branched-chain AA (Ile and Leu) were lower ($P < 0.10$) for cows fed the BP diet than for cows fed the MBP, FBP, and MFBP diets. The AV

TABLE 5. Concentration of AA in serum of coccygeal vessels of cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBP); fat and BP (FBP); or molasses, fat, and BP (MFBP).

AA	Diet					SE	Contrast ¹			
	Control	BP	MBP	FBP	MFBP		1	2	3	4
	($\mu\text{mol/dl}$)						<i>P</i>			
Arg	23.3	20.0	20.1	21.1	21.0	2.40	0.33	0.80	0.78	0.90
His	5.2	5.6	6.9	5.4	6.0	0.39	0.10	0.30	0.02	0.75
Ile	11.5	9.3	12.3	10.0	13.9	1.63	0.94	0.18	0.34	0.19
Leu	15.1	18.2	25.3	21.3	25.6	2.41	0.02	0.06	0.26	0.45
Lys	8.1	7.5	9.5	8.0	8.2	0.67	0.78	0.21	0.14	0.48
Met	1.6	1.9	2.0	1.7	1.5	0.19	0.47	0.47	0.39	0.13
Phe	4.4	5.1	5.8	5.3	5.2	0.43	0.07	0.47	0.38	0.51
Thr	11.0	8.2	10.8	8.0	7.0	0.72	0.01	0.63	0.02	0.02
Trp	3.4	3.1	3.1	2.8	2.7	0.30	0.16	0.51	0.47	0.49
Val	27.5	25.2	36.1	31.6	36.8	3.76	0.27	0.05	0.41	0.54
Total EAA ²	111.1	104.1	131.9	115.1	127.7	8.61	0.39	0.06	0.20	0.70
Ala	23.3	21.2	28.5	22.6	19.4	2.54	0.91	0.44	0.13	0.08
Asp	1.3	1.4	1.3	1.4	1.5	0.25	0.70	0.95	0.72	0.74
Asn	3.0	3.0	3.8	2.7	2.3	0.48	0.84	0.92	0.13	0.17
Glu	7.1	6.8	7.6	6.9	6.6	0.99	0.93	0.82	0.65	0.62
Gln	22.2	22.8	19.7	21.6	20.9	2.50	0.74	0.49	0.60	0.94
Gly	30.7	32.5	28.8	31.4	39.3	2.30	0.99	0.40	0.44	0.93
Pro	6.4	6.4	10.6	7.4	6.5	1.25	0.36	0.24	0.11	0.13
Ser	9.5	10.3	10.0	9.3	7.2	0.82	0.75	0.14	0.60	0.03
Tyr	5.0	4.4	6.4	4.7	4.1	0.68	0.85	0.42	0.11	0.11
Cit	11.9	9.1	9.8	13.2	14.0	1.50	0.81	0.09	0.13	0.20
Orn	4.5	3.8	5.3	4.1	4.2	0.56	0.79	0.25	0.17	0.43
Tau	5.4	5.6	6.7	6.0	5.8	1.05	0.59	0.63	0.64	0.70
Total NEAA ³	130.4	127.2	138.3	131.5	122.9	8.41	0.97	0.71	0.59	0.28
EAA:NEAA	0.85	0.82	0.95	0.87	1.04					

¹Contrast 1 = Control versus BP, MBP, FBP, and MFBP; contrast 2 = BP versus MBP, FBP, and MFBP; contrast 3 = MBP versus FBP; and contrast 4 = MBP and FBP versus MFBP.

²Total dietary essential AA.

³Total nonessential AA.

differences of EAA for cows fed the MBP and FBP diets were similar, but the AV differences of Pro and Orn were higher ($P < 0.03$) for cows fed the MBP diet than for cows fed the FBP diet. The AV differences of Glu and Pro were lower ($P < 0.09$) for cows fed the MFBP diet than for cows fed the MBP and FBP diets. Halfpenny et al. (13) suggested that Glu and Pro were potentially limiting to milk protein synthesis.

Uptake to output ratios for AA are presented in Table 8. Uptake of AA by the mammary gland is dependent on arterial concentrations of AA, the rate of MBF, and the extraction process by which carrier systems affect the transfer of blood AA across basal membranes of the secretory cells (18). The MBF (2) per kilogram of milk output (365, 480, 384, 415, and 433 L/d, respectively, for cows fed the control, BP, MBP, FBP, and MFBP diets) was unaffected by treatment, but cows fed the control diet had numerically lower blood flow, partially explaining the lower uptake to output ratio for Arg and the lower mean values for EAA for these cows than for cows fed the other diets. Low ($P > 0.10$) AV differences of His for cows fed the BP diet explained the lower ($P < 0.05$)

ratio for this AA for cows fed the BP diet. Uptake (AV difference \times MBF) of EAA by the mammary gland relative to EAA output in milk protein were ≥ 1 , except for Met. Uptake to output ratios for Met were < 1 for the control, MBP, FBP, and MFBP diets. Lower ratios for Met were reported (11), which could have been due to a lower estimate of Met by the AA analysis procedure, a lower estimate of MBF, or both. Estimates of MBF by the equation of Kronfeld et al. (15) were higher than those derived from the procedure of Cant et al. (2). However, the equation of Kronfeld et al. (15) was derived with data from low producing cows and was, therefore, not used in this study. Clark (9) indicated that the quantity of Arg extracted by the lactating bovine mammary gland was two- to fourfold greater than that quantity secreted in milk protein. For all diets containing BP, uptake to output ratios for Arg were > 4 . Excess uptake of EAA would be metabolized in the mammary gland. Many of the EAA are precursors of the NEAA (9). Phenylalanine is thought not to be catabolized by the mammary tissue (9). Output of most NEAA in

TABLE 6. Concentration of AA in serum of mammary vein of cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBP); fat and BP (FBP); or molasses, fat, and BP (MFBP).

AA	Diet					SE	Contrast ¹			
	Control	BP	MBP	FBP	MFBP		1	2	3	4
	($\mu\text{mol/dl}$)						<i>P</i>			
Arg	20.8	13.8	12.8	14.8	13.8	2.21	0.02	0.98	0.53	0.99
His	3.5	4.3	5.0	3.5	4.0	0.40	0.13	0.78	0.03	0.65
Ile	6.3	6.3	7.0	6.0	9.8	1.53	0.58	0.48	0.65	0.11
Leu	7.7	12.4	16.7	14.2	18.8	2.58	0.02	0.19	0.50	0.32
Lys	3.1	3.0	3.6	3.6	3.4	0.66	0.65	0.50	0.94	0.79
Met	0.5	0.8	0.8	0.7	0.5	0.11	0.15	0.29	0.57	0.07
Phe	1.9	3.1	3.6	3.1	3.1	0.51	0.04	0.72	0.49	0.67
Thr	7.1	5.5	7.0	5.1	4.5	0.73	0.08	0.94	0.09	0.11
Trp	2.3	2.6	2.7	2.2	2.5	0.33	0.64	0.77	0.36	0.85
Val	20.5	20.3	28.1	25.6	31.2	4.10	0.34	0.12	0.67	0.41
Total EAA ²	80.7	77.0	95.3	84.9	97.2	8.57	0.43	0.15	0.41	0.52
Ala	16.5	16.7	23.0	19.2	16.6	2.39	0.39	0.32	0.29	0.16
Asp	1.1	1.2	0.9	1.2	1.3	0.21	0.96	0.75	0.36	0.33
Asn	1.9	2.0	2.4	1.9	1.6	0.39	0.79	0.96	0.43	0.24
Glu	3.7	3.3	4.0	4.0	5.0	0.68	0.65	0.21	0.96	0.27
Gln	16.8	16.5	13.7	14.0	13.7	2.55	0.44	0.38	0.93	0.95
Gly	26.7	31.0	27.0	28.7	28.7	2.24	0.44	0.31	0.61	0.78
Pro	3.7	4.4	6.0	6.1	5.4	0.94	0.12	0.21	0.92	0.57
Ser	7.4	7.4	7.3	7.4	5.0	0.99	0.59	0.52	0.91	0.08
Tyr	2.6	2.7	4.1	2.8	2.4	0.61	0.56	0.59	0.19	0.18
Cit	11.1	8.8	8.6	12.6	13.1	1.37	0.84	0.13	0.06	0.16
Orn	2.3	2.0	2.5	2.4	2.0	0.60	0.94	0.63	0.94	0.58
Tau	4.9	4.4	6.0	5.0	5.2	0.95	0.80	0.38	0.51	0.79
Total NEAA ³	98.72	100.4	105.4	105.6	100.1	9.41	0.70	0.77	0.99	0.64

¹Contrast 1 = Control versus BP, MBP, FBP, and MFBP; contrast 2 = BP versus MBP, FBP, and MFBP; contrast 3 = MBP versus FBP; and contrast 4 = MBP and FBP versus MFBP.

²Total dietary essential AA.

³Total nonessential AA.

TABLE 7. Arteriovenous difference in concentration of AA for cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBP); fat and BP (FBP); or molasses, fat, and BP (MFBP).

AA	Diet					SE	Contrast ¹			
	Control	BP	MBP	FBP	MFBP		1	2	3	4
	($\mu\text{mol/dl}$)						<i>P</i>			
Arg	2.5	6.3	7.3	6.3	7.1	0.72	<0.01	0.45	0.33	0.71
His	1.7	1.3	1.9	1.9	1.9	0.27	0.92	0.08	0.96	0.83
Ile	5.2	3.0	5.2	4.0	4.1	0.66	0.16	0.09	0.20	0.58
Leu	7.4	5.8	8.6	7.1	6.8	0.78	0.74	0.09	0.21	0.32
Lys	5.0	4.5	5.9	4.4	4.8	0.65	0.87	0.51	0.12	0.63
Met	1.1	1.1	1.2	1.0	1.0	0.17	0.85	0.93	0.55	0.61
Phe	2.5	2.0	2.2	2.1	2.1	0.27	0.22	0.62	0.92	0.82
Thr	3.6	2.7	3.8	2.8	2.5	0.62	0.22	0.64	0.30	0.29
Trp	1.1	0.5	0.4	0.5	0.1	0.28	0.06	0.71	0.76	0.36
Val	6.9	4.9	8.0	6.0	50.6	0.93	0.44	0.16	0.15	0.25
Total EAA ²	30.4	27.2	36.6	30.1	30.5	3.48	0.85	0.22	0.22	0.52
Ala	6.8	4.5	5.5	3.4	2.7	0.96	0.03	0.61	0.15	0.18
Asp	0.2	0.2	0.4	0.2	0.2	0.10	0.39	0.59	0.29	0.23
Asn	1.2	1.0	1.4	0.7	0.8	0.27	0.48	0.91	0.12	0.44
Glu	3.4	3.5	3.6	2.9	1.7	0.56	0.50	0.26	0.46	0.04
Gln	5.4	6.3	6.0	7.6	7.2	1.30	0.38	0.67	0.41	0.80
Gly	4.0	1.5	1.8	2.7	1.7	1.12	0.13	0.66	0.59	0.69
Pro	2.7	2.0	4.6	1.3	1.1	0.78	0.63	0.71	0.01	0.08
Ser	2.1	3.0	2.7	1.9	2.2	0.61	0.64	0.32	0.39	0.89
Tyr	2.4	1.7	2.3	1.8	1.7	0.32	0.15	0.46	0.33	0.37
Cit	0.8	0.3	1.2	0.6	0.9	0.37	0.82	0.17	0.30	0.90
Orn	2.2	1.8	2.8	1.7	2.1	0.28	0.71	0.20	0.02	0.70
Tau	0.5	1.1	0.7	0.9	0.6	0.36	0.39	0.37	0.69	0.69
Total NEAA ³	31.6	26.8	32.9	25.9	22.9	4.78	0.41	0.93	0.31	0.28

¹Contrast 1 = Control versus BP, MBP, FBP, and MFBP; contrast 2 = BP versus MBP, FBP, and MFBP; contrast 3 = MBP versus FBP; and contrast 4 = MBP and FBP versus MFBP.

²Total dietary essential AA.

³Total nonessential AA.

milk exceed uptake, which is in agreement with results found by Drackley and Schingoethe (11), indicating the synthesis of these NEAA by the mammary gland (9, 13).

The efficiency of extraction of Leu and Phe from blood serum by the mammary gland was higher ($P < 0.05$) for cows fed the control diet than for cows fed the other diets (Table 9), indicating that these AA

TABLE 8. Ratios of AA uptake:output¹ by the mammary gland of cows fed the control diet and diets containing bypass proteins (BP), molasses and BP (MBP), fat and BP (FBP), or molasses, fat, and BP (MFBP).

AA	Diet					SE	Contrast ²			
	Control	BP	MBP	FBP	MFBP		1	2	3	4
Arg	1.4	5.2	4.7	4.3	5.3	0.64	<0.01	0.54	0.70	0.30
His	1.2	1.2	1.4	1.5	1.7	0.14	0.11	0.04	0.44	0.16
Ile	1.4	1.1	1.5	1.2	1.4	0.17	0.78	0.25	0.35	0.69
Leu	1.8	1.3	1.5	1.3	1.4	0.15	0.19	0.60	0.49	0.93
Lys	1.1	1.4	1.4	1.1	1.3	0.14	0.18	0.48	0.22	0.58
Met	0.7	1.1	0.8	0.8	0.9	0.13	0.26	0.21	0.83	0.76
Phe	1.0	1.0	1.0	1.0	1.1	0.07	0.54	0.76	0.60	0.39
Thr	1.8	1.1	1.2	1.0	1.0	0.13	0.58	0.70	0.29	0.37
Trp	1.8	1.0	0.8	1.0	1.3	0.53	0.12	0.63	0.80	0.41
Val	1.5	1.4	1.8	1.5	1.5	0.16	0.60	0.32	0.17	0.59
Total EAA ³	1.2	1.5	1.6	1.4	1.6	0.12	0.04	0.93	0.35	0.65
Tyr	1.0	0.9	1.0	0.9	0.9	0.07	0.45	0.93	0.32	0.57

¹Arteriovenous difference of AA (grams per day) \times mammary blood flow (liters per day)/AA output in milk (grams per day).

²Contrast 1 = Control versus BP, MBP, FBP, and MFBP; contrast 2 = BP versus MBP, FBP, and MFBP; contrast 3 = MBP versus FBP; and contrast 4 = MBP and FBP versus MFBP.

³Total dietary essential AA.

were low in arterial serum and were more limiting for cows fed the control diet. However, the converse was true for Arg. The extraction efficiencies of all AA were similar ($P > 0.10$) between cows fed the BP diet and cows fed the MBP, FBP, and MFBP diets. Extraction efficiencies were similar between cows fed the MBP diet and those fed the FBP diet and between cows fed the MBP and FBP diets and those fed the MFBP diet. Fat did not reduce the extraction of Ile and Val, in contrast to results by Casper and Schingoethe (4). For all diets, Met and Lys had the highest extraction percentages, which is in agreement with previous studies (4, 9). Amino acids that had high percentages of extraction are most likely to be limiting for milk synthesis: Met or Lys ranked first or second, and Phe was third-limiting for all diets. Often Phe, Met, Lys, His, and Thr are listed as the five most limiting AA to the mammary gland (9).

Transfer efficiency data (Table 9) definitely ranked Met, Lys, and Phe as first-, second-, and third-

limiting EAA, respectively. Similar ranking has been reported previously (4, 11). For each diet, Arg, Tyr, and Val appeared to be the least limiting AA. Transfer efficiency of arterial AA into milk protein indicated that His, Leu, Met, Phe, and Val were more limiting ($P < 0.06$) for cows fed the control diet than for those fed the BP, MBP, FBP, and MFBP diets.

CONCLUSIONS

Diets containing FBP, MBP, or MFBP increased milk production compared with a diet containing BP. Milk protein percentages, but not milk protein yields, were reduced by fat in the diet. Low concentrations of Leu, Val, and EAA in coccygeal serum and low AV differences of His, Ile, and Leu for cows fed the BP diet coupled with lower energy content of the BP diet, might explain the lower milk production for cows fed this diet than for cows fed the MBP, FBP, and MFBP diets. The cows fed a diet containing 2.5% tallow and

TABLE 9. Amino acid extraction and transfer efficiencies from serum by the mammary gland of cows fed the control diet and diets containing bypass proteins (BP); molasses and BP (MBP); fat and BP (FBP); or molasses, fat, and BP (MFBP).

AA	Diet					SE	Contrast ¹			
	Control	BP	MBP	FBP	MFBP		1	2	3	4
	Extraction ² (%)						P			
Arg	10.2 (10) ³	31.9 (7)	37.2 (5)	29.9 (8)	34.2 (5)	3.66	<0.01	0.67	0.19	0.89
His	33.2 (7)	22.6 (8)	27.1 (8)	34.8 (6)	33.0 (6)	4.41	0.45	0.11	0.24	0.71
Ile	45.4 (5)	35.0 (4)	37.4 (4)	39.8 (4)	32.7 (7)	5.89	0.24	0.66	0.82	0.29
Leu	48.9 (4)	33.0 (6)	33.7 (7)	33.5 (7)	29.5 (8)	5.31	0.02	0.91	0.98	0.55
Lys	62.8 (2)	60.0 (1)	61.6 (1)	55.8 (2)	58.8 (2)	6.50	0.61	0.87	0.54	0.99
Met	69.1 (1)	57.5 (2)	58.4 (2)	60.0 (1)	68.0 (1)	6.18	0.26	0.53	0.86	0.27
Phe	56.7 (3)	39.1 (3)	41.7 (3)	42.1 (3)	41.6 (3)	6.27	0.04	0.87	0.61	0.82
Thr	35.2 (6)	34.0 (5)	34.0 (6)	36.4 (5)	35.6 (4)	6.07	0.98	0.85	0.78	0.96
Trp	30.4 (8)	15.7 (10)	13.6 (10)	19.1 (10)	5.6 (10)	8.5	0.10	0.77	0.66	0.33
Val	25.7 (9)	19.8 (9)	22.0 (9)	19.7 (9)	17.5 (9)	4.41	0.25	0.99	0.71	0.54
Tyr	49.9 [4] ⁴	37.6 [4]	35.9 [6]	40.5 [4]	43.5 [3]	6.8	0.19	0.77	0.64	0.54
	Transfer ⁵ (%)									
Arg	7.9 (10)	6.4 (10)	8.2 (10)	7.1 (10)	6.4 (10)	1.12	0.49	0.52	0.48	0.38
His	28.9 (7)	19.5 (7)	19.7 (7)	23.3 (7)	19.4 (7)	3.09	0.03	0.72	0.43	0.59
Ile	33.1 (5)	31.0 (4)	29.1 (4)	32.0 (5)	22.9 (5)	4.38	0.39	0.56	0.65	0.18
Leu	41.5 (4)	25.6 (6)	22.5 (6)	24.7 (6)	20.6 (6)	3.74	0.01	0.51	0.69	0.52
Lys	59.1 (2)	45.9 (2)	44.8 (2)	50.9 (2)	45.1 (2)	7.32	0.16	0.90	0.57	0.77
Met	97.7 (1)	57.4 (1)	69.5 (1)	73.4 (1)	78.6 (1)	9.88	0.03	0.18	0.79	0.57
Phe	58.3 (3)	37.0 (3)	39.3 (3)	41.5 (3)	38.7 (3)	6.45	0.02	0.71	0.82	0.84
Thr	29.8 (6)	30.9 (5)	27.5 (5)	34.9 (4)	36.9 (4)	5.05	0.64	0.71	0.33	0.38
Trp	17.0 (9)	13.9 (9)	17.0 (8)	17.4 (8)	18.5 (8)	2.69	0.92	0.26	0.90	0.69
Val	17.5 (8)	14.0 (8)	12.1 (9)	12.8 (9)	10.9 (9)	2.04	0.05	0.40	0.79	0.56
Tyr	48.7 [4]	39.9 [3]	35.8 [4]	44.2 [3]	47.1 [2]	6.72	0.38	0.76	0.40	0.41

¹Contrast 1 = Control versus BP, MBP, FBP, and MFBP; contrast 2 = BP versus MBP, FBP, and MFBP; contrast 3 = MBP versus FBP; and contrast 4 = MBP and FBP versus MFBP.

²Arteriovenous difference of AA (micromoles per deciliter) \times 100/AA concentration (micromoles per deciliter) in serum of coccygeal artery.

³Numbers in parentheses indicate the ranking of limiting essential AA.

⁴Ranking of Tyr if it were considered a dietary essential AA.

⁵AA Output (grams per day) in milk \times 100/[AA concentration in serum of coccygeal artery (grams per liter) \times mammary blood flow (liters per day)].

BP had similar milk production but reduced milk protein percentages compared with cows fed the diet containing 8% molasses and BP. Adequate amounts of energy in the form of soluble carbohydrate may be required to promote higher milk production and to prevent milk protein percentage depression with diets containing high concentrations of good quality RUP. There was no clear advantage of feeding both molasses and fat with BP.

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