EFFECTS OF THE SUPPLEMENTAL LEVEL OF PROTECTED LYSINE ON PERFORMANCES OF HOLSTEIN DAIRY COWS

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Summary

The objective of this study was to investigate the optimum level of the rumen protected lysine (RPLys) for early lactating Holstein dairy cow. This experiment was carried out with 16 Holstein dairy cows for 106 days, and consisted of 4 treatments : T_1 (RPLys 0%), T_2 (RPLys 0.1%), T_3 (RPLys 0.2%) and T_4 (RPLys 0.3%). The results obtained are summarized as follows :

1. The daily intakes of feed were similar among treatments, but the digestibility of crude protein tended to increase 0.5-5.0% with increased level of RPLys, and also the crude fiber digestibility increased ($p < 0.05$).
2. The daily weight gain for cows in T_1 was 253 g, which was lower than any other treatments ($p < 0.05$). The highest was 521 g in T_3. Also, the body condition score was changed from 3.22 at initial to 3.45 at final. The lowest increase in body condition score as 0.09 was obtained in control and the highest as 0.60 in T_3 ($p < 0.01$).
3. The total milk production of groups T_2, T_3 and T_4 were higher than T_1, as well as total protein, total fat and total solid yield. Especially in T_4 treatment group milk yield was higher than other treatments. The content of fat was higher in T_2 and T_4 compared to other treatments. Other components of milk were not significantly different ($p > 0.05$). The persistencies of lactation were increased in all RPLys treatments, especially, rate of reduction in milk yield was lowest in T_4 ($p < 0.05$).
4. The total amino acid content in the plasma, as well as plasma lysine content showed no consistent trend with treatments.

(**Key Words** : Rumen Protected Lysine, Milk Yield, Milk Composition, Plasma Amino Acid)

Introduction

Milk yield has increased in response to postruminal administration of proteins and amino acids (AA) (Fisher, 1972; Clark, 1975; Spires et al., 1975; Schwab et al., 1976; Rogers et al., 1984) and ingestion of ruminally protected protein sources (Chalupa, 1975; Crooker et al., 1983; Kung and Huber, 1983; Sahlu et al., 1984; Netemeyer et al., 1982). Deficiencies of certain AA may limit milk production in lactating cows. Milk yields were greater during infusion or supplementation with methionine (Met) and lysine (Lys) in combination, than when either AA was infused or supplemented individually (Schwab et al., 1976; Bozak and Schwab, 1988). Rogers et al. (1987) did not observe consistent increase in milk yield upon supplementation of rumen protected Met (RPMet) and Lys (RPLys), but Lys improved Met utilization.

Some researchers (Chalupa, 1975; Clark, 1975; Schwab et al., 1976; Clark et al., 1978; Ahrar and Schingoethe, 1979; Schingoethe and Ahrar, 1979; Crooker et al., 1983; Drackley and Schingoethe, 1986) suggested that Met might be the most limiting AA for milk protein synthesis, however, direct evidence is limited.

In contrast, intravenous infusion of Met (Clark, 1975) or the feeding of a RPMet source (Broderick et al., 1970; Spires et al., 1975; Papas et al., 1984a,b; Yang et al., 1986) did not increase the production of milk, milk fat or milk proteins. The inconsistency of responses to supplemental RPMet may indicate that Met is not always the first-limiting AA, or that other AA or factors may be

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co-limiting or affecting the physiological utilization of Met for milk production.

Chow et al. (1990) reported that Lys may be the more limiting AA than Met in both high fat and high concentrate diets, and emphasized that more studies are needed to determine under which feeding conditions supplementary Met and Lys increase milk production. Holter et al. (1992) also reported that Lys deficiency in diets caused decreased milk production as Lys was the first-limiting AA during whole lactation periods with the second-limiting AA being isoleucine (Ile) in early lactation, and Met in mid and late lactation periods. Research results are, however, not sufficient to make any conclusion on the optimum level of RPLys supplementation, necessary to achieve maximum production in ruminants.

The objective of this study was to evaluate effects on the performance of milk production when RPLys was supplemented at different levels to early lactating Holstein dairy cows receiving total mixed rations.

TABLE 1. ANIMAL DATA AT THE BEGINNING OF A FEEDING TRIAL

Item	Level (%) of protected lysine[1]			
	T_1	T_2	T_3	T_4
Lactation day (day)	92.0 ± 57.87[2]	92.8 ± 54.82	93.5 ± 44.93	92.5 ± 45.57
Milk yield (kg/day)	25.7 ± 2.99	25.1 ± 1.61	25.0 ± 0.93	25.3 ± 2.34
Body weight (kg)	502.0 ± 46.97	530.3 ± 55.91	505.2 ± 33.66	497.7 ± 17.27
BCS[3]	3.1 ± 0.39	3.4 ± 0.38	2.9 ± 0.21	3.5 ± 0.42

[1] T_1 ; RPLys 0%, T_2 ; RPLys 0.1%, T_3 ; RPLys 0.2%, T_4 ; RPLys 0.3%.
[2] Means ± SD.
[3] BCS : body condition score.

Materials and Methods

Animals and Experimental design

This experiment was conducted over a 106-d period. Sixteen Holstein dairy cows were used in a feeding experiment at the lactation stage of between 92 to 93d post-calving. Average body weight (BW), daily milk yield and body condition score (BCS) on a scale from 1 to 5 were 550-530 kg, 25.0-25.7 kg/d and 2.9-3.5, respectively (table 1).

Animals were used in a completely randomized design to evaluate the effects of RPLys supplementation on performance, and were housed in a free-stall barn.

The following four dietary treatments were randomly assigned to individual cows with four cows per treatment; 1) T_1 (TMR + no supplemental RPLys), 2) T_2 (TMR + 0.1% RPLys), 3) T_3 (TMR + 0.2% RPLys) and 4) T_4 (TMR + 0.3% RPLys) as shown in table 1.

Diets and Feeding

A total mixed ration (TMR) was prepared by mixing 52% roughage and 48% concentrates composed of high protein concentrates (contained alfalfa pellet, soybean meal, corn gluten, corn germ and wheat bran) and commercial concentrates formulated for dairy cattle in a DM ratio of 3:7. Roughages composed of corn silage, rye silage and orchard grass hay were fed in a DM ratio of 25:45:30. The chemical composition of diets are shown in table 2.

Cows were housed in a free-stall barn and individually fed the TMR twice daily to appetite using automatic

TABLE 2. CHEMICAL COMPOSITION OF DIETS FOR HOLSTEIN DAIRY COWS

Items	High protein concentrate	Conventional concentrate	Corn silage	Rye silage	Hay	Total
	% DM basis					
Dry matter	87.40	86.30	24.60	16.20	87.50	62.60
Crude protein	27.70	22.40	11.30	10.16	13.70	17.50
Ether extract	3.39	4.55	3.28	6.29	1.68	4.14
Crude fiber	14.00	6.60	23.50	39.50	33.40	21.68
Crude ash	7.40	9.90	5.80	8.50	9.30	8.55
Ca	0.85	1.26	0.33	0.53	0.48	0.78
P	0.63	1.00	0.38	0.56	0.40	0.67

feeding doors with amounts fed and refusals recorded daily.

Nutrient digestibility

Sulphuric acid lignin and acid-insoluble ash (AIA) were used as an indigestible internal marker and digestibilities were calculated according to the method of Schneider and Flatt (1975). Samples of feces were collected from each cow three times per day for four consecutive days.

Body weight and body condition score

Cows were weighed and scored for body condition as described by Edmonson et al. (1989) once monthly on a scale from 1 to 5.

Milk production and composition

Cows were milked twice daily with milk weights recorded at each milking. Two 24-h (p.m. plus a.m.) milk samples were collected from each cow at 4 weeks interval throughout the experimental period. Milk samples were analyzed for the content of protein, lactose, solids not fat (SNF) and total solid (TS) using a infrared milk analyzer (Milkoscan, Hillerod-Moded 300, Denmark).

Somatic cell count (SCC) in milk was also measured by the method of Wiltbank et al. (1962).

Amino acid composition in plasma

Blood samples were collected from the jugular vein in heparinized tubes and plasma was separated by centrifugation at 2,000 × g for 30 min. at 4℃. Proteins were precipitated in 5 ml of plasma by addition of 5-sulfosalicylic acid (5% w/v) and centrifuged at 15,000 × g for 20 min., and stored at −20℃ until analysis.

Chemical analysis

The dry matter content of feeds was determined by drying at 100℃. The feces samples were dried in a 55℃ drying oven for 48 h, ground through a 1 mm screen in a Wiley mill, and analyzed for DM, crude fiber and Kjeldahl N according to AOAC (1984). Minerals in basal diets were analyzed by atomic absorption spectrophotometry after nitric/perchloric acid digestion (AOAC, 1984). Phosphorus was determined by the meta-Vanadate method (AOAC, 1984). Plasma amino acids were determined by the general procedures outlined by Spackman et al. (1958) using an automatic AA analyzer (LKB, Model 4150-alpha) after deproteinization of sample with sulfosalicylic acid.

Statistical analyses

Data obtained were analyzed as a completely randomized block design by Duncan's multiple range test using GLM (general linear model) procedures of SAS (1985).

Results and Discussion

Feed intake and nutrient digestibility

The effects of feeding RPLys on nutrient intake and digestibility are shown in table 3.

The daily intake of feed components was not affected by RPLys supplementation, and were maintained at a constant level over the course of the experiment. These results agree with the results of Papas et al. (1948b), Schwab and Bozak (1988a,b), and Williams et al. (1970) who reported that feeding RPLys and RPMet did not alter dry matter intakes (DMI). A depressed DMI was observed in the studies by Broderick et al. (1970), Papas et al. (1974), Seymour et al. (1990) and Yang et al. (1986), while on the other hand increased DMI was also found in studies by Casper et al. (1987), Illg et al. (1987) and Papas et al. (1984a).

DMD was significantly ($p < 0.05$) lower for cows fed RPLys than for cows in group 1. Chow et al. (1990) also reported decreased DMD with RPLys, whereas others (Oke et al., 1986; Wright and Loerch, 1988) observed no differences in DMD, which was decreased in other trials when large concentrations of RPLys was supplemented to the diets (Papas et al., 1984b; Williams et al., 1970).

TABLE 3. DAILY NUTRIENTS INTAKE AND DIGESTIBILITY BY HOLSTEIN DAIRY COWS AS INFLUENCED BY RPLys

Items	Treatment[1]			
	T_1	T_2	T_3	T_4
Intake (kg/day)				
Dry matter	22.2	22.4	22.3	21.8
Organic matter	19.2	19.3	19.2	18.8
Ether extract	1.47	1.48	1.48	1.44
Crude fiber	7.69	7.76	7.73	7.55
Digestibility (%)				
Dry matter	64.83^b	62.37^a	61.67^a	61.67^a
Crude protein	34.93^a	35.40^{ab}	38.26^b	40.00^c
Ether extract	73.96^b	74.38^b	69.81^a	71.92^a
Crude fiber	22.62^a	27.88^b	27.20^b	27.04^b

[1] T_1 ; RPLys 0%, T_2 ; RPLys 0.1%, T_3 ; RPLys 0.2%, T_4 ; RPLys 0.3%.

[a,b,c] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

Crude protein digestibility (CPD) tended to increase by 0.5-5.0%, varying by the level of RPLys, and also the crude fiber digestibility (CFD) presented similar tendency ($p<0.05$). Some researchers (Mowat and Deelstra, 1972; Oke et al., 1986) also reported an increased CPD in animals fed RPLys and/or RPMet, whereas Wright and Loerch (1988) observed no differences in CPD. Oke et al. (1986) and Wright and Loerch (1988) reported that feeding RPLys and/or RPMet did not alter CFD.

Body weight and body condition score

The average daily gain (ADG) and body condition score (BCS) are shown in table 4.

Average daily gain for cows in group T_3 was higher ($p < 0.01$) than for those in group T_1. The tendency of increased daily gain is supported by the report of Deetz et al. (1985) and Oke et al. (1986).

As there was no difference in feed intake (table 3) between treatments, feed conversion efficiency followed the same trend as rate of ADG.

Supplementing 0.3% RPLys increased ADG by 41% compared to the T_1 treatment, without affecting feed intake. This is similar to the increased growth observed when 300 g/d of fish meal was fed as a supplement to grass silage (Veira et al., 1990).

Several other experiments (Kirby et al., 1983; Gill and England, 1984; Veira et al., 1985, 1988, 1990, 1991) have shown that RPAA supplementations improved growth of various animals without affecting intake. This indicates that protected lysine is not a limiting factor for intake, but it does affect growth rate.

TABLE 4. BODY WEIGHT AND BODY CONDITION SCORE OF HOLSTEIN DAIRY COWS AS INFLUENCED BY THE LEVEL OF RPLys[1)]

Items	Treatments[2)]			
	T_1	T_2	T_3	T_4
Body weight				
Initial (kg)	502 ± 47.0	530 ± 56.0	505 ± 33.7	498 ± 17.3
Final (kg)	529 ± 43.1	569 ± 52.2	560 ± 29.7	543 ± 15.3
Weight gain (kg)	27 ± 12.1[a]	39 ± 6.4[ab]	55 ± 9.9[b]	45 ± 14.4[ab]
Daily gain (kg)	0.25 ± 0.114[a]	0.36 ± 0.060[ab]	0.52 ± 0.094[b]	0.43 ± 0.136[ab]
Body condition score				
Initial (A)	3.1 ± 0.39	3.4 ± 0.38	2.9 ± 0.21	3.5 ± 0.42
Final (B)	3.2 ± 0.15	3.5 ± 0.1	3.5 ± 0.19	3.6 ± 0.15
Change (B-A)	0.1 ± 0.34[a]	0.2 ± 0.32[a]	0.6 ± 0.25[b]	0.1 ± 0.33[a]

[1)] Mean ± SD.
[2)] T_1 ; RPLys 0%, T_2 ; RPLys 0.1%, T_3 ; RPLys 0.2%, T_4 ; RPLys 0.3%.
[a,b] Mean values with different superscripts within the same row are significantly different ($p < 0.01$).

Production performance

Milk production

The effects of supplementing RPLys on production performance of milk, fat and protein yields during whole experimental periods are shown in table 5.

Milk yields were increased by 4.1, 5.5 and 8.5% for T_2, T_3 and T_4 treatments, respectively compared to control (T_1) treatment. The result agreed with the result of Papas et al. (1984a,b) who found that supplementation with specific RPAA led to an increase of about 6% milk production.

Feeding RPAA increased yields of milk fat and fat-corrected milk (Chalupa, 1975; Chandler et al., 1976; Bhargava et al., 1977; Lundquist et al., 1983; Huber et al., 1984), but generally did not change milk production (Chandler et al., 1976; Bhargava et al., 1977; Lundquist et al., 1983; Huber et al., 1984).

Supplementing RPLys tended to increase fat yields without statistical significance. Supplying limiting amino acids could improve the ability of the liver to synthesize lipoproteins necessary for lipid transport to the mammary gland and other tissues (Oldham and Smith, 1982; Sniffen and Krick, 1987), although Pullen et al., (1989) reported liver contribution of VLDL to be smaller in early lactation.

Total protein yields tended to be greater for diets containing RPLys, but statistical differences were not

TABLE 5. DAILY MILK YIELD, FAT AND PROTEIN PRODUCTION AND MILK COMPOSITIONS OF HOLSTEIN DAIRY COWS AS INFLUENCED BY THE LEVELS OF RPLys[1)]

Items	Treatments[2)]			
	T_1	T_2	T_3	T_4
Daily production (kg/d)				
Milk	22.0 ± 3.40	22.9 ± 2.66	23.2 ± 2.51	23.9 ± 1.99
Fat	0.83 ± 0.13	0.90 ± 0.11	0.86 ± 0.09	0.95 ± 0.08
Protein	0.67 ± 0.10	0.73 ± 0.09	0.71 ± 0.06	0.74 ± 0.04
Composition (%)				
Fat	3.76 ± 0.06[b]	3.94 ± 0.08[a]	3.72 ± 0.08[b]	3.96 ± 0.08[a]
Protein	3.02 ± 0.10	3.21 ± 0.09	3.08 ± 0.06	3.09 ± 0.04
Lactose	4.31 ± 0.18	4.25 ± 0.10	4.35 ± 0.14	4.18 ± 0.12
SNF[3)]	7.94 ± 0.30	7.93 ± 0.18	8.03 ± 0.21	7.87 ± 0.18
Total solid	11.55 ± 0.33	11.76 ± 0.27	11.60 ± 0.25	11.76 ± 0.23

[1)] Mean ± SD.
[2)] T_1 ; RPLys 0%, T_2 ; RPLys 0.1%, T_2 ; 0.1%, T_3 ;0.2%, T_4 ; RPLys 0.3%.
[3)] SNF; Solids not fat.
[a,b] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

significant. Percentage increases in milk protein yields upon supplementation of Lys observed in our study were smaller than those observed by Schwab and Bozak (1988a), but larger than those observed Rogers et al. (1987). Even though milk protein responses differed, both Schwab and Bozak (1988a) and Rogers et al. (1987) found that supplementation with Lys led to greater increases in milk protein production than did supplementation with Met. On the other hand, Schingoethe et al. (1988) reported that adding RPMet led to significantly increased α- and β-casein in milk production.

Milk composition

Supplementing RPLys had no effect on the concentrations of milk lactose, solids not fat and total solid, but fat percentage was significantly higher for cows receiving diets supplemented with RPLys than those without RPLys except T_3 treatment (table 5).

Chamberlain and Thomas (1982), Rogers et al. (1984) and Seymour et al. (1990) reported that RPAA supplementation has increased milk fat secretion during mid-lactation of cows fed Met-deficient, corn-soybean meal and corn silage-soybean meal diets, respectively.

McGilvery (1983) reported that Met in particular may facilitate the transfer of blood lipid to milk by furnishing methyl groups for the synthesis of choline and phosphatidylcholine (lecithin), which in turn play a key role in lipid digestion, absorption, transport and metabolism (McGilvery, 1983). Radiotracer experiments demonstrated that in goats up to 28 and 4% of the Met pool may be converted to choline and plasma phospholipids, respectively (Emmanuel and Kennelly, 1984). Rogers et al. (1987) reported that Lys improved Met utilization.

The milk protein percentage was not significantly affected by RPLys. Illg et al. (1987), using the same source of RPLys used by Yang et al. (1986), reported an increase in the protein content with no change in other milk components. Other studies utilizing RPLys reported no effect on milk composition (Papas et al., 1984a,b; Williams et al., 1970).

Lactation persistency

The effect of supplementing RPLys on lactation persistency is shown in table 6.

The persistency of lactation was increased in all RPLys treatments, especially rate of reduction was significantly ($p < 0.05$) lower in T_4 treatment.

The result of present study is difficult to explain. Perhaps lysine supplementation improved lactation persistancy as reported by Holter et al. (1992) who also observed that Lys deficiency in diets caused decrease in milk production as Lys is the first-limiting AA during whole lactation periods.

Somatic cell counts in milk

The effect of supplemental RPLys on the SCC in milk

TABLE 6. DAILY MILK YIELD (kg) FROM HOLSTEIN DAIRY COWS[1)]

Treatment[2]	Preliminary	Days on trial				
		0	30	60	90
T_1	25.7 ± 2.99	24.1 ± 3.37	22.6 ± 3.01[a]	21.5 ± 2.87[a]	20.4 ± 3.53[a]
T_2	25.1 ± 1.61	24.0 ± 2.28	22.9 ± 3.17[a]	22.4 ± 3.19[ab]	21.4 ± 3.46[ab]
T_3	25.0 ± 0.93	24.5 ± 2.10	23.9 ± 3.03[b]	22.8 ± 3.00[ab]	21.2 ± 2.97[ab]
T_4	25.3 ± 2.34	25.0 ± 2.42	24.7 ± 2.91[b]	23.3 ± 2.98[b]	21.8 ± 2.09[b]

[1)] Mean ± SD.
[2)] T_1 ; RPLys 0%, T_2 ; RPLys 0.1%, T_3 ; RPLys 0.2%, T_4 ; RPLys 0.3%.
[a,b] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

of Holstein dairy cows is presented in table 7.

The SCC in milk was not affected by RPLys supplementation. The average SCC during whole experimental periods was 23.0 × 10^4/ml milk.

TABLE 7. CHANGE OF SOMATIC CELL COUNTS (× 10^4/ml) IN MILK OF HOLSTEIN DAIRY COWS[1)]

Treatments[2]	Days on trial			
	0	30	60	90
T_1	26.9±3.96	26.3±5.30	23.8±2.74	24.3±1.95
T_2	22.2±4.78	22.8±1.06	23.0±1.15	24.5±3.64
T_3	16.5±3.94	22.1±0.86	22.2±1.47	20.4±2.30
T_4	17.2±1.76	27.6±4.70	24.3±2.86	23.8±1.13

[1)] Mean ± SD.
[2)] T_1 ; RPLys 0%, T_2 ; RPLys 0.1%, T_3 ; RPLys 0.2%, T_4 ; RPLys 0.3%.

Amino acid composition of blood plasma

At the end of experiment total amino acid (TAA) in group T_1 and T_4 decreased by 0.34 and 0.10 μg/ml, but that of T_2 and T_3 treatment was increased by 0.34 and 0.54 μg/ml, respectively from the beginning of experiment. However, the differences were not statistically significant.

Concentrations of individual plasma amino acids were not significantly affected by treatments.

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