Effects of Rumen Undegradable Protein and Minerals Proteinate on Early Lactation Performance and Ovarian Functions of Dairy Cows in the Tropics**

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ABSTRACT : A 90 d study was designed to investigate the effects of rumen undegradable protein (RUP) and a mixture of Cu, Zn and Mn proteinate (CZMP) on milk yield and composition and ovarian functions during rainy months. Twenty four Holstein× indigenous cows in their 2nd and 3rd lactation were randomly allocated to total mixed rations (TMR) containing soy bean meal (SBM) as a source of rumen degradable protein (RDP), SBM plus CZMP, and formalin treated SBM (FSBM) as a source of RUP. Maximum and minimum temperature humidity index during the experimental period were 83.6-84.7 and 75.4-76.1. There were no differences (p>0.05) in intakes of dry matter, crude protein and net energy and in contents of butterfat, lactose and minerals. Cows on TMR containing FSBM not only lost less weight (-278, -467 and -433 g/d) with more intake of RUP (0.92, 0.58 and 0.59 kg/d) but also produced more milk (19.27, 18.23 and 18.13 kg/d) and 4% fat corrected milk (18.57, 17.57 and 17.51 kg/d) with more protein (3.06, 2.81 and 2.80%), solids-not-fat (8.69, 8.38 and 8.38%) and less milk urea N (9.3, 15.4 and 15.0 mg/dl) compared with those on TMR containing SBM and SBM+CZMP, respectively (p<0.01). However, cows on TMR containing SBM and SBM+CZMP did not differ in these respects (p>0.05). Whereas incidence of cystic ovaries at 20 and 90 d pospartum was less (p<0.01) in cows on TMR containing SBM+CZMP (37.3 and 12.5%) than those on TMR containing SBM (62.5 and 25%), it was nil for cows on TMR containing FSBM. Cows in all three group differed (p<0.01) from each other for the recurrence of first observed estrus with those on TMR containing FSBM having least days (22, 36 and 47 d) compared with their counterpart on TMR containing SBM+CZMP and SBM, repectively. The results suggest that RUP is one of the limiting factors affecting milk yield and its composition and ovarian functions during early lactation of dairy cows in the tropics. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 6 : 806-811)***

Key Words : Rumen Undegradable Protein, Minerals Proteinate, Early Lactation, Ovarian Functions, Tropics

INTRODUCTION

The biological value of protein for dairy cows is directly related to the energy status of the dairy cows and the balance of absorbed amino acids relative to their requirement (Van Straalen et al., 1994). Reduced energy consumption and increased energy maintenance requirement during heat stress often results in considerably more protein being metabolized to meet energy requirements of dairy cows (Beede and Collier, 1986). Dairy cows under heat stress often have negative nitrogen balance (Kamal and Johnson, 1970; Hassan and Roussel, 1975) and thus less protein is available for productive functions. An adequate supply of dietary protein to dairy cows in the tropics is necessary in order to optimize milk yield and fertility.

It has been observed that under thermal stress, dairy cows produce more milk in high protein diets (Hassan and Roussel, 1975) and in diets medium (Higginbotham et al., 1989) and low (Taylor et al., 1991) in rumen degradable protein (RDP). Intakes of dietary protein that maximize milk yield can cause changes of reproduction and fertility unless release of ammonia within the rumen is controlled (Visek, 1984). However, RDP or rumen undegradable protein (RUP) in excess of requirement can contribute to reduced fertility (Bulter, 1998) resulting economic losses approximately 50 million US$ a year in Thailand. Ferguson and Chalupa (1989) suggest that amount and degradability of dietary protein can explain protein-fertility relationships.

The ability to provide adequate protein to dairy cows depends on moderate the balance between the availability of nitrogen for microbial growth in the rumen and for productive functions (Ferguson and Chalupa, 1989). Too little RDP reduce microbial growth in the rumen while increasing availability of protein for digestion and absorption at the small intestine, whereas too much RDP increase ruminal ammonia (NH_3) and blood urea resulting in less protein available for productive functions (Ferguson et al., 1988). However, information is limited on the influence of amount and degradability of protein as well as trace minerals proteinate on yield and composition of milk and reproduction for dairy cows in the tropics. An objective of this study was to evaluate diets containing two protein sources of different degradabilities and a mixture of trace

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mineral proteinate on early lactation performance and ovarian functions of dairy cows in the tropics.

MATERIALS AND METHODS

Before the study was started, all cows were examined with particular reference to pathologic features of reproductive tracts and only normal and healthy cows were used. Twenty four Holstein×indigenous (93.75×6.25%) cows in their 2nd and 3rd lactation were randomly allocated to dietary treatments according to completely randomized design. All the cows were in early lactation.

Dietary treatments consisted of soybean meal (SBM) as a source of RDP, SBM plus a mixture of Cu, Zn and Mn proteinate (CZMP; Applied Force Ltd., Thailand) and formalin treated SBM (FSBM; Cows Friend Ltd., Thailand) as a source of RUP. Paragrass hay was made from perennial paragrass. The paragrass was cut, chopped into 2-3 cm length and sun dried for total mixed rations (TMR). Diets contained CP approximately 10% above the NRC (1988) requirement and were isonitrogenous and isocaloric as shown in table 1.

Experimental diets were offered *ad libitum* at 06:00, 10:00 and 18:00 h and were sampled weekly and bulked for later analysis of chemical composition. Feed intake and milk yield were daily measured. Milk samples of 50 ml were weekly collected at consecutive a.m. and p.m. milking in bottles containing 2-bromo-2-nitro-1, 3-propadiol and stored at 5ºC for composition analysis. Cows were weighed once each week immediately following the a.m. milking prior to accessing feed and water. The trial was conducted during rainy months from the end of May to the beginning of September (90 d). Ambient temperature and relative humidity were recorded before morning feeding with thermograph and hygrograph (Classella, London). The temperature humidity index (THI) was calculated following the equation: THI=td-(0.55-0.55RH) (td-58), where td is the dry bulb temperature (ºF) and RH is the relative humidity expressed as a decimal (NOAA, 1976).

Experimental cows were hold at the holding area for 30 min. at before and after a.m. (4:30 h) and p.m. (15:30 h) milking for observation for signs of estrus. The observation was done again at 11:00 and 22:00 h. Signs of estrus were determined by observing “mounting” behavior or “standing-for-mounting” nervousness and increased physical activity, swelling of the vulva, and clear stringy mucous discharge from the vulva and then rectal palpation were done. Cystic ovaries were determined at 20 d and 90 d postpartum by rectal palpation.

Crude protein (CP), ether extract (EE), ash and DM contents of the experimental diets were determined according to the AOAC (1980). Neutral detergent fiber (NDF) and neutral detergent insoluble nitrogen (NDIN) were measured following the method of Van Soest et al. (1991). Total non-fiber carbohydrates (TNFC) is calculated following the equation; TNFC=100-CP-EE-(NDF-NDIN) -ash. Milk compositions were measured with MilkoScan (Foss Electric, Denmark). Milk (0.2 ml) was deproteinized with 1.8 ml of TCA (3%), allowed to stand for 5 min, and centrifuged at 3,500×g for 30 min. at 2ºC. The clear supernatant was pipetted carefully from the solidified milk fat and analyzed at 535 nm for milk urea nitrogen (MUN) using a colorimetric diacetyl monoxine procedure (number 535; Sigma Diagnostic, St. Louis, MO). Statistical analysis was carried out by SAS (1989).

Table 1. Ingredients of total mixed rations (TMR)

Ingredients	TMR containing		
	SBM	SBM+ CZMP	FSBM
Whole cotton seed	11	11	11
Soy bean meal (SBM)	18.6	18.6	-
Formalin treated soybean meal (FSBM)	-	-	18.6
Cassava chips	36.8	36.5	36.8
Molasses	2.8	2.8	2.8
Urea	0.9	0.9	0.9
Monocalcium phosphate	0.7	0.7	0.7
Dicalcium phosphate	0.7	0.7	0.7
$CaCO_3$	0.3	0.3	0.3
$NaHCO_3$	0.6	0.6	0.6
NaCl	0.4	0.4	0.4
Premix	0.2	0.2	0.2
Cu, Zn and Mn proteinate (CZMP)	-	0.3	-
Paragrass hay	27	27	27
Total	100	100	100

RESULTS

Chemical composition of the dietary treatments was similar. Values of nutrient composition on a DM basis for total mixed rations (TMR) were as follows: CP, 15.87%; NDF, 28.11%; EE, 3.60%; TNFC, 41.27%; and calculated net energy for lactation (NE_l), 1.57 (Mcal/kgDM). Calculated RUP for TMR containing SBM, SBM plus CZMP and FSBM was 24.1, 24.1 and 38.5% of CP, respectively.

Means for environmental conditions during the experimental period are presented in table 2. Daytime temperature was much higher than the nighttime while daytime relative humidity was much lower than the nighttime. The combination of moderately high temperature and extremely high humidity led to high temperature humidity index (THI) during rainy months.

Dietary treatment effects on nutrient intakes and live

Table 2. Means for environmental conditions during the experimental period

Item	Value				
	May	Jun.	Jul.	Aug.	Sep.
Maximum temperature, ºC	33.5±2.4	33.2±2.4	33.6±2.4	33.1±2.3	33.4±2.1
Minimum temperature, ºC	24.7±1.0	24.4±0.9	24.7±1.2	24.3±0.4	24.1±0.2
Maximum relative Humidity, %	96.0±4.0	95.0±3.0	96.0±4.0	98.0±2.0	97.0±2.0
Minimum relative humidity, %	53.0±8.0	57.0±9.0	59.0±10.0	60.0±8.0	60.0±10.0
Maximum THI*	83.6±1.3	83.8±2.0	84.7±1.2	84.2±1.6	84.6±0.9
Minimum THI*	76.1±1.3	75.4±1.1	76.1±1.6	75.5±0.6	75.9±0.2

*THI-Temperature humidity index.

weight are presented in table 3. Daily intakes of DM, CP and NE_l for all cows were not significantly different ($p>0.05$). However, daily intakes of RUP for dairy cows fed TMR containing SBM and SBM plus CZMP were significantly lower than those fed TMR containing FSBM ($p<0.01$). Dairy cows fed TMR containing SBM and SBM plus CZMP significantly lost more weight than those fed TMR containing FSBM ($p<0.01$).

Dietary treatment effects on milk composition, yield and 4% fat corrected milk are shown in table 4. With an exception of fat, lactose and mineral contents, protein, solids-not-fat and total solids were significantly lower for dairy cows fed TMR containing SBM and SBM plus CZMP than those fed TMR containing FSBM ($p<0.01$). Milk yield and 4% fat corrected milk were also significantly lower for dairy cows fed TMR containing SBM and SBM plus CZMP than those fed TMR containing FSBM ($p<0.01$). Milk urea nitrogen in dairy cows fed TMR containing SBM and SBM plus CZMP were significantly higher than those fed TMR containing FSBM ($p<0.01$).

Dietary treatment effects on ovarian functions are presented in table 5. Incidence of cystic ovaries and days to first observed estrus were significantly higher ($p<0.01$) for dairy cows fed TMR containing SBM than those fed TMR containing FSBM. However, incidence of cystic ovaries and days to first observed estrus were significantly improved for dairy cows fed TMR containing SBM with supplementation of a mixture of Cu, Zn and Mn proteinate, compared to

Table 3. Live weight changes and voluntary intake of total mixed rations (TMR)

Item	TMR containing			SE
	SBM*	SBM + CZMP*	FSBM*	
Nutrient intake				
Dry matter, kg/d	15.06	15.52	14.96	2.35
Dry matter, %BM	3.32	3.36	3.19	0.20
Crude protein, kg/d	2.39	2.46	2.37	0.05
Undegraded intake protein, kg/d	0.58 [a]	0.59 [a]	0.92 [b]	0.03
Net energy**, Mcal/d	23.64	24.37	23.49	5.76
Live weight change				
Initial weight, kg	474	481	482	5.69
Final weight, kg	432	442	457	5.97
Average weight, kg	453	462	469	8.68
Daily gain, g/d	-467 [a]	-433 [a]	-278 [b]	20.1

* SBM-soy bean meal; CZMP-Cu, Zn and Mn proteinate mixture; FSBM-formalin treated SBM.

** Net energy for lactation (Mcal/kgDM)=(0.0245×TDN)-0.12.

[a,b] Means within a row without a common superscript letter differ ($p<0.01$).

Table 4. Composition and yield of milk in dairy cows fed different total mixed ration (TMR)

Item	TMR containing			SE
	SBM*	SBM + CZMP*	FSBM*	
Butter fat				
%	3.76	3.78	3.75	0.06
kg/d	0.69	0.68	0.72	<0.01
Milk protein				
%	2.81 [a]	2.80 [a]	3.06 [b]	0.02
kg/d	0.52 [a]	0.51 [a]	0.59 [b]	<0.01
Lactose				
%	4.87	4.88	4.93	0.08
kg/d	0.89	0.88	0.95	0.01
Minerals				
%	0.70	0.70	0.70	<0.01
kg/d	0.13	0.13	0.14	<0.01
Solids-not-fat				
%	8.38 [a]	8.38 [a]	8.69 [b]	0.02
kg/d	1.53 [a]	1.52 [a]	1.67 [b]	0.02
Total solids				
%	12.14 [a]	12.16 [a]	12.44 [b]	0.10
kg/d	2.21 [a]	2.20 [a]	2.40 [b]	0.05
Milk yield, kg/d	18.23 [a]	18.13 [a]	19.27 [b]	2.10
Fat corrected milk (4%), kg/d	17.57 [a]	17.51 [a]	18.57 [b]	2.75
Milk urea N, mg/dl	15.4 [a]	15.0 [a]	9.3 [b]	0.3

* SBM-soy bean meal; CZMP-Cu, Zn and Mn proteinate mixture; FSBM-formalin treated SBM.

[a,b] Means within a row without a common superscript letter differ ($p<0.01$).

Table 5. Ovarian activities in dairy cows fed different total mixed rations (TMR)

Item	TMR containing			SE
	SBM*	SBM + CZMP*	FSBM*	
Incidence of cystic ovaries, %				
at 20 d postpartum	62.5 [a]	37.3 [b]	nil [c]	5.4
at 90 d postpartum	25 [a]	12.5 [b]	nil [c]	1.9
Days to first observed estrus**	47 [a]	36 [b]	22 [c]	4.09

* SBM-soy bean meal; CZMP-Cu, Zn and Mn proteinate mixture; FSBM-formalin treated SBM.

** Excluding cystic ovary cows.

[a,b,c] Means within a row without a common superscript letter differ ($p<0.01$).

those fed TMR containing SBM ($p<0.01$).

DISCUSSION

Protein nutrition can affect milk yield through the availability of NH_3, peptides and amino acids for microbial growth in the rumen and of amino acids for milk protein synthesis (Bequette et al., 1998). It can also affect reproduction through toxic effects of ammonia and its metabolites on gametes and early embryos (Ferguson and Chalupa, 1989) and by exacerbation of negative balance of energy (Oldham, 1984). In this study, CP content of dietary treatments was approximately 10% above the NRC (1988) recommendation and undegraded intake protein (UIP) calculated from data of NRC (1988) were 24.1, 24.1 and 38.5% of CP for TMR containing SBM, SBM plus CZMP and FSBM, respectively. In this study, UIP for TMR containing FSBM slightly exceeded the upper level of 37% of CP recommended by NRC (1988), whereas it was well below the NRC (1988) recommendation of 34% for TMR containing SBM and SBM plus CZMP. It is likely that TMR containing FSBM, in this study, will adequate provide RDP for microbial growth and RUP for productive functions of dairy cows.

During rainy months in the tropics, ambient temperature, typically, is 2-4ºC lower than summer months while relative humidity is 8-16% higher. The combinations of moderately high ambient temperature and extremely high relative humidity during rainy months contributes to extremely high THI (Johnson, 1987) which is close to the THI during summer months (Kanjanapruthipong et al., 2001). The maximum THI of 84.7 reported, in this study, exceeded the lower range of danger zone of 78 for survival of Holstein cows (Johnson, 1987) and the minimum THI of 75.4 exceeded the upper critical point of 72 for optimal productivity (Johnson, 1987). These environmental conditions suggest the extremely stressful conditions to dairy cows under rainy months in the tropics.

Hassan and Roussel (1975) observed increased DMI in dairy cows fed 21% CP, compared to those fed 14% CP. Similarly, Higginbotham et al. (1989) compared diets with two CP contents (18.4 and 16.1%) and degradabilities (65 and 58%) and observed increased DMI in dairy cows fed diets high in CP than those fed diets medium in CP, irrespective of degradability. Whereas, Taylor et al. (1991) reported increased DMI in dairy cows fed diets high in CP (17.8%) and low in degradability (47%) than those fed diets high in CP (18.1%) and medium in degradability (61%). In this study, DMI did not differ in dairy cows fed diets medium in CP (15.9%) and two degradabilities (75.9 and 61.5%). These results suggest that under heat stress diets high in CP stimulate DMI of dairy cows and effect of degradability in diets on DMI is not consistent.

Circulating NH_3 can originate from both RDP and RUP (Ferguson and Chalupa, 1989). The quantity of NH_3 that is produced and the amount that escapes from the rumen directly reflects both dietary RDP and the availability of the fermentable carbohydrates to support microbial growth and protein synthesis (Firkins, 1996). The available amino acids that are not taken up for milk protein synthesis or deposited elsewhere are deaminated by the liver to yield energy substrates and NH_3 (Oldham, 1984). As urea that is synthesized from the NH_3 by the liver circulates through the blood, it equilibrates all tissue (Chalmers et al., 1971). Plasma urea nitrogen (PUN) and MUN are useful indicators of protein metabolism and status in dairy cows (Roseler et al., 1993). Higher MUN in dairy cows fed TMR containing SBM and SBM plus CZMN in this study may be due to excess RDP, while a good value of MUN in dairy cows fed TMR containing FSBM is likely to reflect the balance between the amount and degradability of CP in the diets.

During an early stage of lactation, dairy cows are in a negative energy balance. A negative energy balance can be exacerbated by feeding excess RDP and RUP as it requires energy to metabolize excess protein (Oldham, 1984). Diets high in RDP increase ruminal NH_3 (Visek, 1984; Stokes et al., 1991) and blood urea-N (Higginbotham et al., 1989) and thus increase energy cost of synthesizing and excreting the RDP as urea (Oldham, 1984). In the present study, dairy cows fed TMR containing SBM and SBM plus CZMP lost more weight than those fed TMR containing FSBM, though net energy intake were similar. Therefore, dairy cows in particular in early lactation on high RDP diets in the tropics can be in a severe negative energy balance.

An increased supply of RUP for digestion and absorption at the small intestine in ruminants will increase glucose availability in circulation due to increased gluconeogenesis, sparing of glucose from oxidation and increased glucose from glycerol released from adipose tissue breakdown (Oldham, 1984). It is evident that dairy cows under heat stress produce more milk on a diet high in

RUP, when the dietary protein fed to dairy cows in hot environments should not exceed the NRC (1988) requirement by more than 10-15% CP (Huber et al., 1994). Taylor et al. (1991) reported that on diets averaged 18.3% CP, dairy cows fed 51% RUP yielded more milk (3.4 kg/d) than those fed 37.8% RUP. On diets averaged 15.9% CP in this study, dairy cows fed 38.5% RUP produced more milk (1.1 kg/d) than those fed 24.1% RUP. These results suggest that increased RUP in the diets will increase glucose availability for milk secretion.

McGuire et al. (1989) reported that net flux of $\propto$ amino N was reduced 35% by thermal stress compared with thermal comfort and hence less protein is available for productive functions. Increased intake of RUP from 580 g/d to 920 g/d as presented in table 3 will increase protein for digestion and absorption at the small intestine. Increased milk protein as a result of increased intake of RUP reported in this study is likely due to increase amino acids available for mammary protein secretion.

Cystic ovaries can be a serious cause of reproductive failure in dairy cows because it prolongs the postpartum interval to first estrus and conception in probably 10 to 30% of the dairy cows (Kesler and Garverick, 1982). The frequency of detecting cystic ovaries is higher in early postpartum cows. Whitmore et al. (1974) reported that 71% of cystic ovaries developed within 45 d postpartum. Delays in onset of normal ovarian activities thus limiting the number of estrus cycles before breeding, may account for the observed decrease in fertility (Butler and Smith, 1989). In this study, higher incidence of cystic ovaries and longer days to first estrus were observed in dairy cows fed TMR containing SBM and SBM plus CZMP than those fed TMR containing FSBM. It is likely that for dairy cows in the tropics, excess RDP can reduce fertility possibly via the exacerbation of negative energy balance (Butler and Smith, 1989), the detrimental effects of NH_3 and its metabolites on ovarian functions (Swanson, 1989) and the deficiency of amino acids relative to requirement (Ferguson and Chalupa, 1989).

In addition, supplementation with complexed trace minerals appeared to improve ovarian functions. O’Donoghue et al. (1995) reported that days to first estrus reduced from 25 to 20 days postpartum in dairy cows supplemented with a mixture of CU, Zn and Se proteinate. Similar results were observed in this study in dairy cows fed TMR containing SBM supplemented with a mixture of Cu, Zn and Mn proteinate.

Milk yield and its compositions and ovarian functions during early lactation can be significantly improved with diets high in RUP for dairy cows in the tropics. Additionally supplementation of a mixture of Cu, Zn and Mn proteinate to diets high in RDP appeared to improve ovarian functions during early lactation in dairy cows in the tropics. Results of this study suggest that RUP is one of the limiting factors affecting productivity of dairy cows in the tropics.

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