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Effect of Dietary Rumen Undegraded Protein (RUP) Level on Nutrient Intake and Digestion of Lactating Dairy Cows

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ABSTRACT

The objective of this study was to examine the effect of different levels of rumen undegraded protein (RUP) in diets of lactating dairy cows on feed intake and nutrient digestion. The experiment was run according to completely randomized design with three treatments of complete feed rations containing different levels of rumen undegraded protein (RUP) and four replications for each treatment. In a digestibility trial, twelve Friesian Holstein cows were divided into three groups at each group consist of four cows and given three dietary treatments containing three levels of RUP, namely 7.43% of total crude protein /CP (P0), 8.49% of total CP (P1), and 9.45% of total CP (P2). Diets consisted of forages (King grass, *Pennisetum* hybrid) and concentrates at ratio of 50:50 in dry matter basis. The diets contained 18.25% CP and 61.75% total digestible nutrient (TDN). The cows fed diet (P0) were considered the control group. Drinking water was given *ad libitum*. This experiment was conducted for 21 days of adaptation period and seven days of collection period. In the collection period, samples of feed, refusal feed, and faecal were collected to get the chemical content includes dry matter (DM), organic matter (OM), ether extract (EE), crude fiber (CF), crude protein (CP), nitrogen-free extract (NFE) and total digestible nutrients (TDN). The data obtained were processed with one-way ANOVA (analysis of variance) and the difference existed between the means ($P<0.05$) would be analyzed with Duncan's new multiple range test. The results showed that cows fed diet P2 (9.45% RUP in CP) in had significantly higher feed and nutrient intake (DM, OM, CP, CF, EE, TDN) and higher DM and OM digestibility compared to the other two treatments.

Keywords: Dairy cows, Lactation, Nutrient digestion, Rumen undegraded protein

Introduction

The lactating dairy cattle, protein, which plays a vital role in body functions, is a major nutrient that can be limiting in the diet (Nisa *et al.*, 2008). Although the efficiency of crude protein (CP) intake in dairy cattle is higher than that in other ruminants, nitrogen (N) excretion in their milk and feces is approximately two to three times greater (Bahrami-Yekdangi *et al.*, 2014; Broderick, 2003). Nutrient requirement of dairy cows increases with milk production, but high producing cows in peak lactation are usually not able to consume sufficient dry matter (DM) to support an optimal milk production.

High production dairy cows require high energy and protein diets during lactation, because energy and protein are mobilized from body stores to support high milk production (NRC, 2001). The contribution of amino acid (AA) to gluconeogenesis has been considered important during lactation in the dairy cows, there is supportive evidence from observations either *in vivo* or *in vitro*. The other important demand for AA is to support milk protein synthesis and this requirement increases greatly at the onset of lactation.

Absorbed AA is essential for protein synthesis to the maintenance, reproduction, growth, and milk production of dairy cattle. The absorbed amino acids in the small intestine could be provided from two types of protein. The first is rumen degradable protein (RDP) in the rumen, which offers peptides, AA, and ammonia for microbial growth to generate microbial protein (Bach *et al.*, 2005). The former synthesized microbial protein provides approximately 70 to 80% of the required AA passing to the small intestine. The second is rumen undegraded protein (RUP), which is digested in the small intestine by the animals to produce AA (Chumpawadee *et al.*, 2006; Laudadio and Tufarelli, 2010).

Crude protein can be protected by several means, including with the identification of naturally protected protein, chemical treatment or modification, inhibition of proteolytic activity, and heat treatment (Nisa *et al.*, 2008). Energy losses associated with protein losses and fermentation incurred in transformation of dietary protein to microbial protein can be eliminated by feeding nutrients post-ruminally (Kamalak *et al.*, 2005). Therefore, the despite an increased supply of metabolism protein through increased dry matter intake (DMI) and rations formulated for lactation, however these conditions create a negative protein balance for cows in early lactation (Doepel *et al.*, 2009; Larsen and Kristensen, 2009).

Therefore, with proper balancing of RDP and RUP, some mobilization and repletion of body protein seems to help transition of cows to lactation. Protein synthesis of ruminal microbial alone is insufficient to meet the protein needs of high producing cows therefore, it is important to include feed in diets that have low protein degradabilities. Feeds such as corn gluten feed, corn gluten meal and fish meal, are low in ruminal degradability (NRC, 2001). Schwab (1995) reported that to optimize the amount of absorbable amino acids (AA) for high producing dairy cows, one of the diet formulation objectives is to provide adequate amounts of RUP. The objective of this study was to examine the effect of increasing levels of RUP, through replacement of soybean meal with formaldehyde-treated soybean meal, on feed intake and nutrient digestion in lactating dairy cows.

Materials and Methods

Time and place of experiment

The experiment was conducted in National Livestock Dairy Breeding Center and Forage (BBPTU-HPT) Baturraden, Banyumas, Central Java. Analysis of samples was conducted in Laboratory of Dairy Science and Milk Industry and Laboratory of Feed Technology, Faculty of Animal Science, Universitas Gadjah Mada (UGM), Yogyakarta.

Experimental design, diets and cows management

The experiment was run according to completely randomized design with three treatments of complete feed rations containing with different levels of rumen undegraded protein (RUP) and four replications for each treatment. This study used 12 lactating Friesian Holstein cows with the 2nd or 3rd lactation periods, and live body weight (BW) of 559.1±50.0 kg. The cows were devided into three treatments and grouped according to BW and lactation at each group consist of four cows. The cows in housed individually, permanent enclosure models stanchion barn with concrete floors. Each group was given a different feed treatment (Table 1), with the proportion forage to concentrate of 50:50 (DM basis). Group P0 cows were fed control diet (with RUP, 7.43% of CP), the second group cows were fed diet P1 (with RUP, 8.49% of CP) and the third group cows were fed diet P2 (with RUP, 9.45% of CP). The amounts of RUP in each treatment were provided by partial replacement of soybean meal with formaldehyde-treated soybean meal. The formaldehyde-treated was carried out on soybean meal following the method of Rochijan (2014), that is the formaldehyde concentration used to treated/protect soybean meal is 0.8%/kg DM. The formaldehyde solution was evenly sprayed on the soybean meal and stirred. Furthermore, the treated soybean meal was fermented for one night and aerated the next day for two to three days. King grass was chopped to size 5 to 10 cm lengths and given 3

Table 1. Ingredients and chemical composition of experimental diets (%DM)

Parameters	Treatments		
	P0	P1	P2
Feedstuffs:			
Forage	50	50	50
Copra meal	9.5	8.8	8.6
Soybean meal	6.0	5.6	5.4
Soybean meal-HCHO	0.0	3.6	4.9
Pollard	12.5	11.6	11.3
Corn gluten feed	8.5	7.9	7.7
Corn gluten meal	1.0	0.9	0.9
Casava waste	10	9.3	9.0
Mineral mix	2.5	2.3	2.3
Total	100	100	100
Nutrient (%):			
Dry matter	51.67	51.87	51.88
Organic matter	87.98	86.74	86.78
Crude protein	18.25	19.04	19.41
Crude fiber	23.56	23.07	22.97
Ether extract	3.14	3.04	3.02
Nitrogen-free extract	43.01	41.54	41.33
Total digestible nutrients[a]	62.03	61.51	61.72
Rumen undegraded protein[b] (% of CP)	7.43	8.49	9.45

[a] : The results of the formula of Hartadi *et al.* (2005).
[b] : The results of the analysis in sacco degradation from laboratory.
P0 : The first group cows were fed control diet (with RUP, 7.43% of CP).
P1 : The second group cows were fed diet P1 (with RUP, 8.49% of CP).
P2 : The third group cows were fed diet P2 (with RUP, 9.45% of CP).

hours after concentrate distribution. King grass (*Pennisetum* hybrid) and concentrate were offered to the cows three times a day at 07:00 am, 12:00 and 03:00 pm. The concentrate ingredients formulation and nutrient compositions of experimental diets for each group were described in Table 1. Drinking water were given by *ad libitum*.

Feed and fecal sample collection and preparation

The diets ingredients or sample (100 g) were composited for each collection period (twenty one days of adaptation period and seven days of the collection period), dried in a 55°C forced-air oven and analyzed to determine the content dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF) and ether extract (EE). Faeces were sampled daily (10% proportions) during each collection period. Faeces sample were composited for each collection period and analyzed for DM, OM, CP, CF and EE. The dried feed and faeces samples were used for chemical or nutrient analysis.

Statistical analysis

The data obtained were processed with one-way ANOVA (analysis of variance) and the difference existed between the means (P<0.05) would be analyzed with Duncan's new multiple range test (Steel and Torrie, 2003).

Results and Discussion

Nutrient composition

The results of chemical composition analysis of experimental diets showed that the levels of CP and TDN among the dietary treatments were not different, the data is presented in Table 1 (P<0.05). However, the RUP content of the diets varied according to the treatment groups. In treatment PI and P2, the amounts of formaldehyde-treated soybean meal replacing the non-treated soybean meal, so as to increase the RUP value. Treatment with formaldehyde appreciably increased the rumen undegraded protein content. It is well known that protein deficiency in lactating dairy cows can cause in reduction in milk production. Dietary supplementation containing optimal energy and protein will optimize the proliferation of rumen microbes and milk synthesis (Widyobroto *et al.*, 2016). Addition of rumen undegraded protein in a balanced energy and protein diet will protect the protein from rumen microbe's degradation, hence the amino acids will be absorbed directly in the small intestines of the cows to meet the requirement for milk synthesis.

Nutrient intake

Feed and nutrient intake of the animals among the dietary treatments is presented in Table 2. The results showed that the dietary treatments significantly affect the intake of dry matter, organic matter, crude protein, crude fiber, ether extract and total digestible nutrient (P<0.05). The results of DMI value (2.74±0.22 %BW) higher than the DMI of NRC (2.66±0.23 %). As expected, the organic matter intake is in line with DM intake. Based on NRC (2001), the requirement of DM for producing 10 kg of milk is 12.4 kg (2.23 %). Decruyenaere *et al.* (2009) explained that DM intake is affected by physiological status of the animals, thus Hadgu (2016) stated that nutrient intake in cattle was influenced by several factors such as the physical and physiological conditions of the animals, feed quality (physic and chemistry) and the environment.

The DMI is very important as it influence the nutrient intake to meet nutrient requirement of lactating dairy cows in maintaining their health and productivity (production and reproduction). As shown in Table 2, cows fed diets containing rumen undegraded protein level (9.45 CP%) had higher nutrient intakes (DM, OM, CP, CF, EE and TDN) compared to animals fed with low RUP level. There is a positive correlation between DMI with BW and milk production in this study. In this study, the RUP supplementation (P2) had a significant effect on DM, OM, CP, CF, EE and TDN consumption. The highest TDN consumption was observed in cows in treatment P2, followed by P1 and P0. A higher level of TDN in diet leads to the increase in the TDN consumption, and TDN consumption was influenced by the dietary intake. The higher level of DM intake will lead to the higher level of TDN intake. TDN was related to the supply of energy that is needed by the animals. A higher consumption of TDN provides more energy

Table 2. Body weight and nutrient intake in dairy cows receiving different levels of dietary RUP

Parameters	Group		
	P0	P1	P2
Body weight (kg/head)	559.0±70.9	564.0±57.5	554.3±36.6
Nutrient (kg DM/head/d):			
Dry matter	12.81±0,86[a]	13.13±1.07[a]	15.16±1.32[b]
Organic matter	10.58±0.71[a]	10.69±0.88[a]	12.40±1.09[b]
Crude protein	2.41±0.17[a]	2.50±0.20[a]	2.88±0.23[b]
Crude fiber	2.68±0.20[a]	2.56±0.28[a]	3.10±0.40[b]
Ether extract	0.42±0.03[a]	0.41±0.03[a]	0.47±0.04[b]
Total digestible nutrients	8.26±0.58[a]	8.50±0.67[a]	9.74±0.78[b]
Dry matter intake (%Body weight)	2.32±0.30	2.34±0.21	2.74±0.22

[a,b] = Means with different superscript within the same row are significantly different (P<0.05).
P0 : The first group cows were fed control diet (with RUP, 7.43% of CP).
P1 : The second group cows were fed diet P1 (with RUP, 8.49% of CP).
P2 : The third group cows were fed diet P2 (with RUP, 9.45% of CP).

Table 3. Nutrient digestibility in dairy cows receiving different levels of dietary RUP

Parameters (%)	Group		
	P0	P1	P2
Dry matter	56.27±3.16[a]	57.58±1.16[a]	61.62±2.38[b]
Organic matter	58.91±2.60[a]	59.98±1.35[a]	63.20±1.75[b]
Crude protein	76.36±2.14	76.02±1.16	77.17±2.80
Crude fiber	41.12±2.04	41.27±1.65	44.46±5.02
Ether extract	80.87±4.04	80.52±4.50	81.55±5.53

[a,b] = Means with different superscript within the same row are significantly different (P<0.05).
P0 : The first group cows were fed control diet (with RUP, 7.43% of CP).
P1 : The second group cows were fed diet P1 (with RUP, 8.49% of CP).
P2 : The third group cows were fed diet P2 (with RUP, 9.45% of CP).

supply that will be used for the metabolism (Astuti *et al.*, 2012; Hanifa, 2008). Suwandyastuti (2013) reported that the consumption of DM was related to the digestibility of DM, OM, CF and CP. However, McCormick *et al.* (2001) reported that DM consumption was not affected by CP content in the diet.

Nutrient digestibility

The average nutrient digestibility in of dairy cows is presented in Table 3. The mean nutrients digestibility were significant (P<0.05) different among the treatment group P0 with P2, especially the DM and OM digestibility. It has been shown that feed protection that has high biological value with formaldehyde treatment tended to have a greater effect on digestibility of DM and OM than that of non-formaldehyde treatment (Huhtanen *et al.*, 1985; Morgan *et al.*, 1989). Nevertheless, a reduction in the concentration of volatile fatty acids (VFA) associated with microbial amino acid metabolism suggested that protein in the formaldehyde-treated feeds was more resistant to microbial degradation. With increase resistance to microbial digestion, the amount of non-protein nitrogen (NPN) reaching the duodenum will be increased with formaldehyde-treated protein sources.

Higher amount of NPN at the duodenum in these diets will increase amounts of individual amino acids concentrations at the duodenum as compared to control diet. However, with the exception of glutamine, formaldehyde treatment did not alter the amino acid compositions of duodenal digesta. A decrease in the susceptibility of dietary protein to microbial attack usually reduces the quantity of microbial N reaching the small intestine and depresses the efficiency of microbial protein synthesis (McCarthy *et al.*, 1989; Hussein *et al.*, 1991). Bunnakit and Khampa (2011) reported that DMI and OM digestibility in Thai Native x Brahman cattle increased linearly while the level of RUP increased. In another study, the use of protein source with lower rumen degradability promotes an increase in the flow of nutrients and changes in the digestive parameters of the omasum, but compromise the production or microbial efficiency in dairy goats (Felisberto *et al.*, 2011).

Conclusions

Dietary rumen undegraded protein level at 9.45% of total crude protein in lactating dairy cows was effective in increasing nutrient intake and digestibility of dry matter and organic matter.

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