

The effect of protected soybean meal as a protein supplement on blood metabolites of lactating dairy cows

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ABSTRACT: The objective of this study was to determine the effect of undegradable dietary protein (UDP) using protected soybean meal supplementation on blood metabolites of lactating dairy cows. Eighteen early lactating Friesian Holstein cows of 1 to 3 years old were used in this study and observed twelve weeks. All cows received forage and concentrate in a ratio of 62:38 (DM basis). Adaptation to the diet was conducted for 4 weeks. The cows were divided into 3 groups with different dietary treatments: control diet without UDP and mineral mix (T0), control diet + UDP 40 g/L milk + mineral mix (T1), and control diet + UDP 60 g/L milk + mineral mix (T2). Fresh water was also offered ad libitum in the pen. In the days 14 and 7 pre-treatment, and in the days 1, 7, 14 and 21 post-treatment, cows were weighted and their blood were collected for chemical analysis. Means between treatments were analyzed using one-way analysis of variance (ANOVA). The results obtained from this study revealed that blood metabolites pattern of lactating cows before and after UDP supplementation subjects to considerable variations. The UDP supplementation had a significant effect on the concentration of Ca. For comprehensive assessment of the effect of UDP supplementation on blood metabolites at different stages of parturient period, further detailed studies should be performed

Keywords: blood metabolites; dairy cow; soybean meal; transition period; undegradable dietary protein (UDP).

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INTRODUCTION

The transition period (i.e., the period between 3 weeks before and 3 weeks after parturition) is marked by extensive dietary, metabolic, endocrine, and immunological changes in dairy cows (Bell, Burhans, and Overton, 2000). It is one of the most critical physiological stages since most of the metabolic and infectious diseases occur during this period, including mastitis, retained fetal membranes, displaced abomasum, dystocia and ketosis, which can reduce a cow's productive life (Curtis *et al.*, 1985). Therefore, a successful transition is important for minimizing metabolic disorders and optimizing productivity and profitability for the upcoming lactation.

In general, besides improving nutritional status and feed management in high yielding dairy cows during transition, the use of supplements are also important to improve the productive and reproductive performance of dairy cows. The use of rumen degradable protein (RDP) and undegradable dietary protein (UDP) has been evaluated in lactating animals (Curtis *et al.*, 1985; Robinson, McQueen, and Burgess, 1991; Hassan and Saeed, 2012; Setiadi, Widyobroto, and Rustamaji, 2003). There is evidence that increasing proportions of UDP have been shown to increase nutrient intake (Setiadi, Widyobroto, and Rustamaji, 2003). UDP is a protein that is not degraded in the rumen and reaches the small intestine. Protein is the first limiting nutrient for growing cattle. Providing the rumen with protein supplementation that degrades slowly and maintains an optimal ruminal concentration of ammonia is recommended to improve animal productivity. However, understanding the effect of UDP supplementation on productive and reproductive performance is still complex. To date, there are limited studies assessing the effect of UDP on nutritional status and productive performance of lactating

animals (Curtis *et al.*, 1985; Robinson, McQueen, and Burgess, 1991; Hassan and Saeed, 2012; Setiadi, Widyobroto, and Rustamaji, 2003), especially in cows raised under tropical conditions such as in Indonesia (Setiadi, Widyobroto, and Rustamaji, 2003).

Blood plays an important role in carrying end products of metabolism processes out of body tissues and supplying these tissues with different nutrients that may need. These nutrients differ in concentrations according to differences in diets and ingredients or diet components or treatments may apply on diets via their interaction in metabolic pathways naturally occurring in different body tissues (Hassan and Saeed, 2012). The concentration of blood metabolites has been used to evaluate the nutritive background of many studies on the causes of reproductive disorder and periparturient disorders (Whitaker, 1997; Kida, 2003; Jóźwik *et al.*, 2012). Blood metabolites are mainly used to evaluate the body's internal condition and metabolic processes in the body (Scamell, 2006). Blood parameters such as glucose, NEFA, BHB, total protein, and total cholesterol that may reflect nutritional status of the cows are of great interest to be assessed in the transition period (Stengärde *et al.*, 2008). Therefore, it is important to select proper biochemical indicators determining the work of different organs or systems.

The objective of this study was to determine the effect of undegradable dietary protein (UDP) using protected soybean meal supplementation on blood metabolites in dairy cows.

MATERIALS AND METHODS

Animals and diets

Eighteen lactating Friesian Holstein cows (1 to 3 years old), with 7 months of gestation, average body weight of 550 kg, BCS \pm 3.5 and 20 liters of milk production were used in this study. They were grouped

into 3 different treatments (each consisted of 6 cows): control diet without UDP and mineral mix (T0), control diet + UDP 40 g/L milk + mineral mix (T1), and control

diet + UDP 60 g/L milk + mineral mix (T2), as shown in Table 1.

Table 1. Average amount of feed offered on each treatment (% DM)

Feed ingredient	Percentage (%)		
	T0	T1 and T2	Proportion
Forage			50
Concentrate:			
Coconut meal	20	19	9.5
Soybean meal	12	12	6
Pollard	25	25	12.5
Corn gluten feed (CGF)	18	17	8.5
Corn gluten meal (CGM)	2	2	1
Cassava waste pulp	21	20	10
Mineral mix	2	5	2.5
Total	100	100	100

Undegraded protein

The undegraded protein used in this study was soybean meal protected by formaldehyde. Soybean meal was analyzed for its protein content and its degradation value, then it was protected by formaldehyde. The use of formalin (formaldehyde 37%) in making protected soybean meal was performed according to Suhartanto *et al.* (2014). Each 100 gr of soybean meal DM was mixed with 0.8 mL of formalin (37% formaldehyde). Distilled water was used to dilute formalin. As much as 150 kg of soybean meal were weighted and placed on a plastic sheeting. Soybean meal was flattened and sprayed with formaldehyde according to the treatment, and mixed. It was boiled for 1 night and aerated on the next day for 2-3 days.

Feeding management

The feeding treatment was carried out for 3 weeks before parturition until 100 days of lactation period, with an adaptation period of 2 weeks before the experimental treatment. T0 feed was given 2 times per day, while T1 and T2 feeds were given 4 times per day. The increased feeding frequency was conducted with the aim at increasing its DMI consumption. Feeding

was provided as TMR (Total Mixed Ration).

Blood analysis

Blood samples (3 ml for each sample) were collected from caudalis artery of each cow before and after parturition and kept in vacutainers containing EDTA,. All of the blood samples were centrifuged at 3000 rpm for 15 minutes to separate blood cells and plasma. The plasma was taken using a micropipette and then transferred to 1 mL eppendorf tubes. After that, it was stored in a freezer at -20°C for blood metabolites analysis including mineral Ca, P, glucose, urea-N, total protein, cortisol and NEFA concentration.

Statistical analysis

Statistical analysis was performed using SPSS version 24.0. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) analysis where there were significant differences among treatments. Differences at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Table 2. Average blood metabolites of lactating dairy cows on each treatment

Blood metabolite	Treatment						Sig.
	T0		T1		T2		
	Prepartum	Postpartum	Prepartum	Postpartum	Prepartum	Postpartum	
Mineral Ca (mg/dL)	27.27±7.90 ^a	19.08±3.67 ^a	39.95±12.86 ^b	23.35±7.08 ^b	47.19±11.36 ^b	27.92±4.97 ^b	*
Mineral P (mg/dL)	1.60±0.30	1.38±0.31	1.56±0.18	1.46±0.26	1.74±1.16	1.47±0.31	ns
Glucose (mg/dL)	49.36±12.51	39.44±14.37	54.89±8.83	37.12±7.66	55.90±4.31	40.19±12.53	ns
Urea-N (mg/dL)	33.89±9.66	33.57±8.60	37.88±7.15	36.75±8.08	37.00±5.10	36.02±6.62	ns
Total protein (g/dL)	6.59±0.78	6.35±0.92	6.78±1.27	6.85±1.42	6.92±1.13	6.91±1.37	ns
Cortisol (ng/mL)	12.46±11.97	16.74±16.84	11.36±7.89	15.38±11.04	11.49±8.33	13.64±11.16	ns
NEFA (mg/mL)	40.05±14.74	44.97±13.72	35.14±4.91	47.62±18.92	32.24±16.11	50.04±21.12	ns

Note: *Means in same row with different superscript showed significantly different ($P < 0.05$); ns = non significant

Blood metabolites concentrations as affected by each treatment are shown in Table 2. UDP supplementation had a significant effect on the concentration of Ca ($P < 0.05$). The highest concentration of Ca (47.19±11.36 mg/dL) was found in T2 prepartum, while the lowest Ca concentration was observed in T0 postpartum (19.08±3.67). A higher trend for the level of glucose and urea-N was observed in cows fed T1 and T2 compared to those fed T0 during prepartum period. In this study, the concentration of P, glucose, urea-N, total protein, cortisol and NEFA were not influenced by UDP supplementation ($P > 0.05$).

The aim of this study was to assess the effect of UDP supplementation on blood metabolites around parturition of lactating dairy cattle. In the dairy industry, alterations of blood metabolic profiles in and around parturition of high yielding dairy cows are a great challenge (Yousuf *et al.* 2016). Therefore, the UDP supplementation is aimed to reduce various problems in and around peri-parturient period.

The results of this study indicated that there was an increasing trend for the

concentration of Ca after UDP supplementation, both in pre- and postpartum. The findings of Piccione *et al.*, 2012 and Yousuf *et al.* (2016) showed that Ca serum levels decrease during the post partum period and increase constantly during all the lactation periods, while the P serum level decrease during the end of lactation. The Ca concentrations of 8.90 mg/dL in prepartum and 8.30 mg/dL in postpartum were reported in the lactating dairy cows, which were lower than the findings of the current study.

In this study, the concentration of glucose was not influenced by UDP supplementation. It may be attributed to the homeostatic mechanism in glucose regulation. The control of blood glucose is an excellent example of homeostatic control via negative feedback. This is where the corrective response is triggered by a deviation from normal levels is turned off by a return to normal levels. For example, low blood glucose results in the production of glucagon and this raises blood glucose. Consequently, as glucose levels rise, the stimulation to produce glucagon is turned off. Regulation of blood glucose is a complex process like most of

other physiological processes (Ramsay and Woods, 2014). There are many hormones besides insulin and glucagon that play important functions in regulation of blood glucose, such as somatostatin (Guthrie and Guthrie, 2002). The results of this study were in agreement with the findings reported by Robinson, McQueen, and Burgess (1991) and Setiadi, Widyobroto, and Rustamaji (2003) who reported that UDP supplementation did not significantly affect the blood glucose concentration. However, the finding of the current study are comparable with the earlier study who reported that concentrations of blood glucose are 65.8 ± 13.5 mg/dL and 49.8 ± 8.6 mg/dL in prepartum and postpartum, respectively (Yousuf *et al.* 2016). Furthermore, Amirifard *et al.* (2016) reported 67.40 mg/dL (prepartum) and 62.61 mg/dL (postpartum) for the blood glucose concentrations of lactating dairy cows.

Blood urea, total protein and albumin concentrations are generally used as indicators of protein metabolism. Urea concentration in blood and milk is one of the indicators of the nutritional status. In our study, no statistically significant differences ($P > 0.05$) of blood urea, total protein and cholesterol were found in the cows. These findings coincide with the published study who reported that the concentration of blood urea of lactating dairy cows is 36.75 mg/dL (Ervayuz *et al.*, 2008). A similar observation was made by Alvarez *et al.* (2006) and Wadhwa *et al.* (2012) who reported that plasma urea nitrogen showed no significant difference when lactating cows were supplemented with high moisture corn-based concentrate and Ca salts of rice bran oil, respectively. Tyagi, Thakur, and Shelke (2009) reported a decrease in the plasma urea nitrogen level when cows fed bypass fat supplementation. In this study, we observed the increasing trend for the level of cholesterol at postpartum in all groups. The increased

cholesterol levels in lactating cows compared with the period before calving may be in relation to the increased requirements for cholesterol of the glands producing steroid hormones, which is in line with studies reported by Schweigert (1990) and Van Den Top *et al.* (1996).

Negative energy balance in dairy cows induces lipolysis and lipid mobilization, and the concentration of non-esterified fatty acids (NEFA) is a useful indicator of lipid mobilization and fatty acid oxidation (Wathes *et al.*, 2009). The results of the present study indicated that UDP supplementation had no significant effect on the concentration of NEFA, either in prepartum or in postpartum period ($P > 0.05$). However, the NEFA concentrations at postpartum of cows in T2 and T3 tended to be higher compared to T0. Grummer, Mashek, and Hayirli (2004) reported that the increased level of NEFA in plasma leads to the increase in ketogenesis by hepatocytes. Furthermore, as defined by Bell (1995), the metabolic adaptations that support the onset of lactation include increased mobilization of fatty acids from adipose tissue and increased hepatic gluconeogenesis.

CONCLUSION

The results obtained from this study revealed that blood metabolites pattern of lactating cows before and after UDP supplementation subjects to considerable variations. The UDP supplementation had a significant effect on the concentration of Ca. For comprehensive assessment of the effect of UDP supplementation on blood metabolites at different stages of parturient period, further detailed studies should be performed.

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