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Profiles of Blood Metabolites and Milk Production of Lactating Buffalo Fed Local Feed Resources in Sijunjung, West Sumatera

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Abstract | This research aimed to evaluate the effect of local feed resources on the blood metabolites (protein, blood glucose, prolactin hormone) and milk production of lactating buffalo in Pematang Panjang, Sijunjung. This research used four female lactating buffaloes, aged 4 - 6 y.o. The research method used was Latin Square Design. The treatments were A: supplementary forage, B: cassava leaves, C: *katuk* leaves, and D: gliricidia leaves. The addition of cassava leaves, *katuk* leaves, and gliricidia leaves each was given at 5 kg/ head/ day and the data collection period was repeated (4 replications). Each treatment was carried out for 10 days and the adaptation period was 1 (one) week for the next treatment. Basal feed was in the form of forage obtained during grazing and concentrates that are usually given by the graziers (farmers). The data analysis was performed using Analysis of variance (ANOVA) and differences between treatments [were tested] with Duncan's Multiple Range Test. The results show that the feeding of cassava leaves, *katuk* leaves, and gliricidia leaves as a food supplement can significantly increase blood protein, blood glucose, and buffalo milk production levels, and they do not affect the prolactin hormone. From the research of the research it can be concluded that cassava leaves, *katuk* leaves, and gliricidia leaves can be used as ruminant animal feed.

Keywords | Cassava leaves, *Katuk*, Blood metabolites, Milk production, Buffalo

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INTRODUCTION

Buffalo cattle family in Indonesia are *swamp* buffalo and *river* buffalo; *river* buffalo is only found in North Sumatra, while *swamp* buffalo spreads almost across all regions in Indonesia, especially in the provinces of North Sumatra, West Sumatra, West Java, Central Java, East Java, and Yogyakarta Special Region. *Swamp* buffalo is raised mainly as working and meat types, while in some regions is used as dairy type (Wirdahayati, 2008). In several in West Sumatra locations, such as Agam, Sijunjung, and Solok, buffalo milk is used to be processed into *dadib*, fermented milk in bamboo tubes.

Swamp buffalo milk production in West Sumatra is around

1.00 - 1.53 liters/day (Roza, 2013). The low milk production is due to genetic factors, raising conditions, and feed quality. The raising of buffalo cattle is generally carried out simply. It is highly dependent on the availability of natural grass, which is low in quality and inadequate in amounts, and application of technical aspects just reached 40% (Aritonang and Roza, 2010). Blood metabolites are strongly influenced by the number of nutrients consumed, age, stress, and health or external factors such as changes in environmental temperature, infectious germs, etc. (Guyton & Hall 1997 in Sudarman et al. 2019).

Supplementary feeding for cattles are generally intended to supply cattle with various nutrients needed because they cannot be sufficient from the available basal ration



(basal feed) (Tangdilintin, 2002) feed needs to be added to increase the availability of nutrients in the blood. The protein and blood glucose are the main raw materials for milk synthesis, as components of milk protein and fat at the same time, prolactin hormone is essential for the beginning and maintenance of milk secretion because this hormone maintains part of the enzyme for milk synthesis. Therefore, improving feed management by providing forage feed commonly found around the graziers but not yet utilized as animal feed, such as cassava leaves, *katuk* leaves, and *gliricidia* leaves.

Cassava leaves (*Manihot esculenta*, Crantz) and dried cassava leaves (hay) are a source of protein and can be used as a feed supplement for ruminants (Wanapat and Kang, 2015). A higher by-pass protein of cassava leaves in rumen is a causative factor that causes of increasing of milk fat and protein (Wanapat, 2009). *Katuk* leaves (*Sauropusandrogynus (L.) Merr*) has been known to increase breast milk production. *Katuk* leaves are expected to be a feed supplement to increase the quality and quantity of dairy buffalo. Giving *katuk* leaves to the level of 170 grams/head/day increases milk production to 45% and the quality of FH dairy cows, especially protein levels and milk fat levels (Sutomo et al. 2019).

Gamal leaves (*Gliricidiasepium*) are available abundantly as a hedge plant in rubber plantations in West Sumatra around the grazing area and not utilized yet as animal feed. Even though *gamal* leaves is a tree legume plant containing high protein element with a crude protein level of 25.7% in a dry material, crude fiber level of 23.9%, and in vitro digestibility of dry material is 48–77% (Herawati and Royani, 2017) they are very suitable to be combined with grass feed for ruminant animal feed. *Gamal* leaves are expected to be a feed supplement so that it can increase the productivity of buffalo. Supplementation of local feed resources conducted in this research aimed to improve the availability of nutrients in the blood to improve total protein, blood glucose, prolactin hormone, and milk production of lactating buffalo. It is expected that the results of this research can provide information on the benefits of fresh cassava leaves, *katuk* leaves and *gamal* leaves as feed supplements to improve cattle productivity.

MATERIALS AND METHODS

This research was carried out in Kanagarian Pamatang Panjang, Sijunjung, West Sumatra. It was carried out in female lactating swamp buffaloes, which are milked and traditionally raised the age of 4–6 years. The design used was Latin Square Design with four treatments, i.e., without additional feed (A) cassava leaves (B), *katuk* leaves (C), and *gamal* leaves (D) and four replications per treatments.

The linear model for Latin Square Design is:

$$Y_{ij}(t) = \mu + Bi + Kj + P(t) + \varepsilon_{ij}(t)$$

Table 1: The Feed Composition of Buffalo of this Study (kg/ head/ day)

No	Feed Composition (kg)	Treatment			
		A (kg)	B (kg)	C (kg)	D (kg)
1	Forage	Grass field	Grass field	Grass field	Grass field
2	Concentrate				
	- Tofu pulp	2.0	2.0	2.0	2.0
	- Bran	2.0	2.0	2.0	2.0
	- Corn	0.5	0.5	0.5	0.5
	- Palm kernel	0.5	0.5	0.5	0.5
3	Cassava leaves	-	5.0	-	-
	<i>Katuk</i> Leaves	-	-	5.0	-
	<i>Gliricidia</i> Leaves	-	-	-	5.0

Note: Roza et al (2015)

The data obtained were analyzed using Analysis of variance (ANOVA). If there is a difference between treatments, the comparison of treatment means was followed by Duncan's Multiple Range Test (DMRT) Steel and Torrie (2002).

The observed variables were:

Total Blood Protein: Determined by the biuret method (Weichselbaum, 1946)

Blood Glucose Levels: Determined by the biuret method

Prolactin hormone: Determined by enzyme-linked immunosorbent assay (ELISA) method (DRG, 2009)

Milk Production: Determined by the conversion method (7% FCM/ day) (Raafat and Saleh, 1962 in Gaafar et al., (2009))

BLOOD SAMPLING

Blood samples were taken before and after treatment through the jugular vein as much as 10 ml using a 10 ml syringe; the blood samples were then put in coagulant EDTA blood tube and stored in a cool box. The blood samples were further analyzed in the Biochemical Laboratory of the Faculty of Medicine of Universitas Andalas. Serum separation was carried out by centrifugation at 4000 rpm for 10 minutes.

Measurement of blood serum content was carried out on glucose, total protein, and levels using a Microlab 300 spectrophotometer in the Biochemical Laboratory of the Faculty of Medicine of Universitas Andalas. The collected examination data was in the form of glucose, total protein and prolactin hormone measured using the ELISA method.

RESEARCH PROCEDURES

This research used four buffaloes (Latin square design) in their second lactation period during the 3rd and 4th

month of lactation. Buffaloes were supplementary feeding in the fresh form of cassava leaves and *katuk* leaves, while *gamal* leaves were withered first with 5 kg/head fed out each morning, followed by 3 kg of concentrate. In the daytime, the buffalo foraged in the field. A period of adaptation was allowed so that the buffalo could adjust to the livestock feed regime, which lasted for one week. One treatment was used for ten days, the next one week for adaptation after receiving the first treatment, and then the next ten days, the animal received the subsequent treatment.

RESULTS AND DISCUSSION

TOTAL BLOOD PROTEIN

The average of the buffaloes total blood protein of this study could be seen in Table 2

Table 2: Average of Total Blood Protein, Blood Glucose and Prolactin Hormone of this study

Parameter	Treatment			
	A	B	C	D
Total Blood Protein (g/dl)	6.04± 0.40 ^c	7.12± 0.06 ^a	7.01± 0.39 ^a	6.34± 0.67 ^b
Blood Glucose (mg/dl)	55.32± 1.97 ^c	62.12± 1.48 ^a	60.95± 1.21 ^a	58.57± 1.76 ^b
Prolactin (ng/ml)	12.91± 0.12	13.20± 0.26	12.98± 0.18	13.06± 0.20

Note: ^{abc} Different superscripts within column is significant different (P < 0.01).

The statistical results show that the feeding of cassava leaves, *katuk* leaves, and gliricidia leaves influence the total buffalo blood protein. Treatment B was highly significant (P < 0.01) compared to other treatments, treatment B and treatment C was not different (P < 0.05) but significantly different (P < 0.01) from treatment D and treatment A. The high total of buffalo blood protein with the feeding of cassava leaves, *katuk* leaves, and gliricidia leaves the high protein content of its, it will be followed by increased intake of protein ration into the body which is needed for cattle basic life and milk production.

Non significant difference in total blood protein on treatment B and treatment C was the cause by both cassava leaves, and *katuk* leaves contain high protein and other contents in the form of beta carotene, vitamin A, tannins, and steroids. With tannins in cassava and *katuk* leaves, the protein in cassava and *katuk* leaves cannot be degraded by microbes in the rumen (by-pass protein). Furthermore, amino acids will be absorbed by the blood, thereby improving the total blood protein used to synthesize milk protein. As suggested by Patra and Saxena (2011), condensed tannins may offer an effective strategy to protect dietary protein from degradation by forming stable complexes in

the rumen environment. However, cassava leaves and *katuk* leaves are rich in mineral sources, especially calcium and micro minerals. Roza et al. (2013) stated that giving Cassava leaves flour as a feed supplement up to 10% can increase buffalo productivity. The micro minerals such as iron play an important role in forming of pigments and proteins in the blood that had a high affinity for oxygen to form hemoglobin, which is also a blood protein. Iron is also required for the production of red blood cells (a process known as hematopoiesis), but it's also part of hemoglobin (that is the pigment of the red blood cells) binding to the oxygen (Gupta, 2014).

Total blood protein of treatment C higher than treatment D, even though the protein content of treatment D is higher (20 - 30%) than treatment C (15 - 20%), it is because *katuk* leaves contain tannins which are able to protect the protein of feed ingredients so that they are not degraded in the rumen. As stated by Mustarichie et al. (2019) *katuk* plants contain tannins, saponins, flavonoids, alkaloids, proteins, calcium, phosphorus, vitamins A, B, and C. With this condition, the protein is bound by tannins, After leaving the Rumen this bond will be destroyed in the abomasum and duodenum so that the protein can be digested and absorbed in blood vessels which are useful for growth and formation of red blood cells (erythrocytes). Treatment A (control) has the lowest total blood protein of lactating buffalo, which was not given supplementary forage. It was because the buffalo only consumed the grass available in the field. During the grazing, the buffalo only consume field grass, which contains 5.82% of crude protein, and *Imperata cylindrical* containing 2.8% of crude protein. Total blood protein in this study was in the normal range i.e., 6.06 - 7.12 g/dl. Other studies also got in the range 5.63 - 8.10 g/dl (Khan et al. 2009), 7.46 g/dl (Sudarman et al., 2019). This indicates that the blood protein concentration is quite good in buffalo and shows the fulfillment of nutrients in the ration given, both in quality and quantity (Roza, et al. 2019).

BLOOD GLUCOSE LEVELS

The blood glucose levels in this study (Table 2) showed that the feeding of cassava leaves, *katuk* leaves, and gliricidia leaves influence the buffalo blood glucose levels. DMRT test showed that treatment B was highly significant (P < 0.01) higher than treatment D and treatment A, but not significantly different from treatment C. It meant that the feeding of cassava leaves, *katuk* leaves, and gliricidia leaves was highly significant (P < 0.01) improved blood glucose of buffalo compared to feeding with only field grass. The highest blood glucose level was in treatment B (cassava leaves). The lowest was in treatment A (field grass), blood glucose level is considered an indicator of energy status for ruminants. The glucose is the main raw material for milk

synthesis, Due to milk fat and milk lactose synthesis. Blood glucose levels from this study were in the normal range i.e. 55.32 - 62.12 mg/dl. The results obtained are still following the research of Pande et al. (2016) where blood lactose levels in buffaloes ranged from 48.57 - 71.57 mg/dl, and the results obtained were higher compared to research by Das et al. (2017) where buffalo blood lactose levels in early lactation (40.67 mg / dl), mid lactation (42.5 mg / dl) and late lactation (46.37 mg / dl).

Blood glucose levels in treatment B and C did not show a significant difference, this was due to the crude protein and crude fiber content of cassava leaves (CP 24.8% and CF 23.8%) and *katuk* leaves (CP 15.0% and CF 31 , 2%) at the same level. Furthermore, the main source of glucose is obtained from feed carbohydrates, but feed proteins can form glucose through gluconeogenesis. In the process of gluconeogenesis, amino acids are converted into glucose. Gluconeogenesis is the process of glucose formation from sources other than non-carbohydrate components, such as protein. Where carbohydrates are the main source of acetate, butyrate, and propionic in ruminal fermentation, the main end product of fermentation in the rumen is VFA, which is used as the main energy source of ruminants (Rahman et al. 2013). Propionic acid as part of VFA after being absorbed from the rumen, is converted into glucose in the liver through gluconeogenesis. Yusuf (2010) stated that propionic acid further undergoes gluconeogenesis in the liver. Therefore blood glucose is formed. Glucose and galactose arrive in the small intestine mixed with pancreatic sap containing α -amylase so that both processes will increase glucose levels and then enter the blood stream which thereby increasing blood glucose levels

PROLACTIN HORMONE LEVELS

Statistical analysis in this study showed that the feeding of cassava leaves, *katuk* leaves and gliricidia leaves does not have a significant effect ($P>0.05$) on prolactin hormone levels. This means that supplementary feeding did not effect on the levels of prolactin hormone. The prolactin hormone's average level in this study ranged from 12.91-13.20 ng/ml. This study's results differ from Muftah et al. (1987), which found that the early lactation, mid-lactation, and late lactation prolactin hormone in dairy cows were 14.4 ng/ ml, 11.8 ng/ ml, and 10.5 ng/ ml, respectively. Therefore, it can be said that supplementary feeding does not affect the prolactin hormone, this is following the study of Freeman et al. (2000) which found prolactin plays a role in the growth and development of the udder gland (mam-mogenesis), milk synthesis (lactogenesis), and in maintaining the persistence of milk production. The specific effect of prolactin stimulates milk protein synthesis, including lactobumin and carbohydrate. Prolactin is very important in the beginning and maintenance of milk secretion be-

cause it maintains part of the enzyme for milk formation. Therefore, the feeding of cassava leaves, *katuk* leaves, and *gamal* leaves do not affect prolactin levels.

THE EFFECTS OF THE TREATMENT ON MILK PRODUCTION

Production of buffalo milk given with feed supplements of cassava leaves, *katuk* leaves, and gliricidia leaves is presented in Table 3.

Table 3: Average of of Buffalo Milk Production

Variable	Treatment			
	A	B	C	D
Milk Production (l/hr)	1.18 ± 0.07 ^a	1.56 ± 0.4 ^b	1.40 ± 0.83 ^b	1.35 ± 0.42 ^{ab}
Milk Production 7% FCM (kg/hr)	2.17 ± 0.15 ^a	3.06 ± 0.19 ^b	2.91 ± 0.32 ^b	2.67 ± 0.14 ^b

Note: ^{a,b,c} Different superscripts within column is significant different ($P < 0.01$).

Statistical analysis showed that treatment B (Table 3) was the highest buffalo milk production. It was significantly different ($P < 0.01$) with treatment A, but not significantly different with treatment C and D. whereas treatment D was not significantly different with treatment A ($P > 0.05$). This showed that the feeding of cassava, *katuk* and gliricidia leaves feed supplements significantly increases buffalo milk production. Increased production of buffalo milk along with the feeding of cassava leaves, *katuk* leaves, and *gamal* leaves as feed supplements for the buffalo was cause of the high protein content in the feed supplements. Cassava leaves have crude protein of 25 - 27%, *katuk* leaves have crude protein of 20 - 23% and *gamal* leaves have crude protein of 18 - 24% (Gohl, 1981). Protein is a precursor in the formation of NH_3 in the rumen. The NH_3 is used as a source of nitrogen for the growth of microorganisms, so the microorganisms' activity in the rumen in fermenting polysaccharides into volatile fatty acids (VFA) also increases. VFA was used as an energy source by cattle for production. Higher VFA production in rations made from cassava, *katuk*, and *gamal* leaves, makes buffalo get the greater energy source; therefore, their productivity is better; this is proven by higher milk production. This is consistent with Azzaz's (2016) statement that microbial feed supplements as natural growth promoters might play an important role in enhancing the productive performance of ruminants, enhancing desirable microbial growth in the rumen environment and stabilization of ruminal pH.

Cassava leaves are suspected of containing steroid compounds that play a role in the prolactin reflex or stimulate alveoli to produce milk, and stimulate the oxytocin hormone to spur milk production and delivery. Steroid compounds are thought to have affected the increase in

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed to conducting research, data processing and writing this manuscript

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estrogenic hormones so that the amount of milk production increases. Suprayogi et al. (2001) reported that active compounds present in cassava leaves simultaneously play an important role in milk synthesis in the secretory glands. Besides that, *katuk* leaves contain a high protein, steroids, and beta carotene. Efficacious protein stimulates increased milk secretion, while steroids and vitamin A play a role in stimulating the proliferation of the alveolar epithelium, so that new alveoli will form, thereby increasing the number of alveoli in the udder gland.

Furthermore, increasing milk production by feeding cassava, *katuk*, and gliricidia leaves supplements was caused these forages to contain tannin. Tannins can bind the protein, cellulose, and hemicellulose so that protease and cellulase enzymes' activity is inhibited (by pass protein). According to Huang et al. (2018), tannins are the major research subject in developing a natural alternative to in-feed antibiotics; strong protein affinity is the well-recognized property of plant tannins, which has successfully been applied to ruminant nutrition to decrease protein degradation in the rumen and thereby improve protein utilization and animal production efficiency. This is due to the high quality protein that can be protected by tannins from the degradation of rumen microorganisms to be more available in the post rumen digestive tract. Besides that, tannins' ability to form complexes with proteins negatively affects rumen fermentation in the nutrition of ruminant animals.

Treatment B was not significantly different from treatment C and D was due to the relatively similar content of crude protein and crude fiber, so that NH₃ as a nitrogen source and VFA as an energy source of the cattle to produce are relatively the same. The results of this study higher than the study of Wirdahayati and Bamualim (2007), which showed milk production of buffalo supplemented with gliricidia leaves was 1.1 lt/ day, and Roza (2013) found that supplementing cassava leaf supplements in the form of pellets improve the production of milk buffalo 1.1 - 1.35 l/ day. This study's conclusion is Cassava leaves, *katuk* leaves, and gliricidia leaves can be used as feed supplements for buffalo. It significantly increased protein levels, blood glucose levels, and milk productions, but it does not affect prolactin hormone.

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