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paper text:

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

24 **Elly Roza***, Salam N. Aritonang, Afriani Sandra,

Rizqan

5 **Faculty of Animal Science, Andalas University**, Jl. Raya Unand, **Kampus Limau Manis, Padang 25163**, West Sumatera, **Indonesia**, *email: elroz@ansci.unand.ac.id, Phone +628126757806 **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).

8 **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 reseawch of the research it can be concluded that cassava leaves, katuk leaves, and gliricidia leaves can be used as ruminant animal feed. Key words: cassava leaves, katuk, blood metabolites, milk production, buffalo INTRODUCTION Buffalo cattle family in Indonesia are swamp buffalo and river buffalo; river buffalo is only found in North Sumatera, while swamp buffalo spreads almost across all regions in Indonesia, especially in the

13 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 dadih, 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. 48 Cassava leaves (*Manihot esculenta*, Crantz) and dried cassava leaves (hay) are a source of protein 49 and

2 can be used as a feed supplement for ruminants

(Wanapat and Kang, 2015). A higher by-pass 50 protein of cassava leaves in rumen is a causative factor that causes of increasing of milk fat and 51 protein (Wanapat, 2009). Katuk leaves (*Sauropus androgynus* (L.) Merr) has been known to increase 52 breast milk production. Katuk leaves are expected to be a feed supplement to increase the quality and 53 quantity of dairy buffalo. Giving katuk leaves to the level of 170 grams/head/day increases milk 54 production to 45% and the quality of FH dairy cows, especially protein levels and milk fat levels 55 (Sutomo et al. 2019). 56 Gamal leaves (*Gliricidia sepium*) are available abundantly as a hedge plant in rubber plantations in 57 West Sumatra around the grazing area and not utilized yet as animal feed. Even though gamal leaves 58 is a tree legume plant containing high protein element with a crude protein level of 25.7% in a dry 59 material, crude fiber level of 23.9%, and in vitro digestibility of dry material is 48–77% (Herawati and 60 Royani, 2017) they are very suitable to be combined with grass feed for ruminant animal feed. Gamal 61 leaves are expected to be a feed supplement so that it can increase the productivity of buffalo. 62 Supplementation of local feed resources conducted in this research aimed to improve the availability 63 of nutrients in the blood to improve total protein, blood glucose, prolactin hormone, and milk 64 production of lactating buffalo. It is expected that the results of this research can provide information 65 on the benefits of fresh cassava leaves, katuk leaves and gamal leaves as feed supplements to improve 66 cattle productivity. 67 68

4 MATERIALS AND METHODS 69 This research was carried out

in Kanagarian Pamatang Panjang, Sijunjung, West Sumatra. It 70 was carried out in female lactating swamp buffaloes, which are milked and traditionally raised the age 71 of 4-6 years. The design used was Latin Square Design with four treatments, i.e., without additional 72 feed (A) cassava leaves (B), katuk leaves (C), and gamal leaves (D) and four replications per 73 treatments. 74 The linear model for Latin Square Design is: 75

$$19 Y_{ij}(t) = \mu + B_i + K_j + P(t) + \epsilon_{ij}(t)$$

76 77 Table 1. The Feed Composition of Buffalo of this Study (kg/ head/ day) Feed Composition Treatment (kg)

18 A (kg) B (kg) C (kg) D (kg) 1

2 3 Forage Concentrate - Tofu pulp - Bran - Corn - Palm kernel Cassava leaves Katuk Leaves Gliricidia Leaves Grass field 2.0 2.0 0.5 0.5 - - - Grass field 2.0 2.0 0.5 0.5 5.0 Grass field 2.0 2.0 0.5 0.5 - 5.0 - Grass field 2.0 2.0 0.5 0.5 - - 5.0 Note: Roza et al (2015) The data obtained

11 were analyzed using Analysis of variance (ANOVA). If there is a difference between treatments, the comparison of

treatment means was followed

4 by Duncan's Multiple Range Test (DMRT) Stell and Torrie

(2002). The observed variables were: 1. Total

26 Blood Protein: Determined by the biuret method

(Weichselbaum et al., 1946) 2. Blood Glucose Levels: Determined by the biuret method 3. Prolactin hormone: Determined by enzyme-linked immunosorbent assay (ELISA) method (DRG, 2009) 4. 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

10 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

10 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 adaption 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 113 Total Blood Protein 114 The average of the buffaloes total blood protein of this study could

20be seen in Table. 2 115 116 Table 2. Average of

Total Blood Protein, Blood Glucose and Prolactin Hormone of this study 117 118 119 Parameter Treatment A B C Total Blood Protein (g/dl) 6.04±0.40c 7.12±0.06a 7.01±0.39a Blood Glucose (mg/dl) 55.32±1.97c 62.12±1.48a 60.95±1.21a Prolactin (ng/ml) 12.91±0.12 13.20±0.26 12.98±0.18 D 6.34±0.67b 58.57±1.76b 13.06±0.20 Note: a,b,c Different superscripts within column is

5significant 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

23(P <0.01) compared to other treatments, treatment B and treatment C

was not

12different (P <.005) 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),

9condensed 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

22play 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.

3Iron 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 -

218.10 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,

4this 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

2converted 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 of prolactin hormone in dairy cows were 14.4 ng/ ml,

1511.8 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

14in the growth and development of the udder gland (mammogenesis), 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 because 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) Milk Production 7% FCM (kg/hr) $1.18 \pm 0.07a$ $2.17 \pm 0.15a$ $3.06 \pm 0.19b$ $2.91 \pm 0.32b$ $2.67 \pm 0.14b$ $1,56 \pm 0,4b$ $1.40 \pm 0.83b$ $1.35 \pm 0.42ab$ Note:

17a,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

16was 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 5 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

2in 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

6microbial feed supplements as natural growth promoters might play an important role

in enhancing the productive performance of ruminants, enhancing

6desirable 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 estrogenic hormones so that the amount

4of 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

7play 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),

1tannins 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

2can 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

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. REFERENCES Aritonang SN, Roza E (2014). Potensi Daun Ketela Pohon Segar Sebagai Anthelmentic Hayati Terhadap Hematologi dan Performans Produksi Pada Kambing. Laporan Penelitian. Universitas Andalas. Padang Azzaz HH, Morsy TA, Murad HA (2016). Microbial Feed Supplements for Ruminant's Performance Enhancement. 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