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Research Article

An Ammoniated Rice Straw Cow Diet Supplemented with Cassava Leaves and Synchronized Release of N-Protein and Energy in the Rumen

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Abstract

Background and Objective: The aim of this experiment was to examine the supplementation of cassava leaves in a cow diet utilizing ammoniated rice straw and synchronized the release of N-protein and energy in the rumen to obtain the best digestion nutrients and the best urine allantoin concentration, plasma progesterone and BUN (blood urea nitrogen) level in the blood of the cow. **Materials and Methods:** By a randomized block design of a 2×3 factorial, two diet treatments, either without (R1) or with the cassava leaves supplementation (R2), were offered to twelve cows of different breeds, namely, the local cow (Pesisir cow) and Bali and Simmental cows. **Results:** There was no significant effect of the interaction between the breed and diet treatments on all of the parameters. Even so, the R2 diet showed a relatively higher nutrient digestion and feed efficiency. It not only had a relatively lower BUN level but also had a lower urine allantoin concentration compared to that of R1, whereas the BUN level of the Bali and Pesisir cows was higher than that of Simmental cow ($p<0.05$). The Simmental cow, however, showed a relatively higher feed efficiency and allantoin concentration in the urine and a relatively lower nutrient digestion and BUN level than those of the Pesisir and Bali cows. **Conclusion:** It is concluded that the supplement of 3.0% cassava leaves in the cow diet with ammoniated rice straw, having a synchrony index of 0.567, produced a good production performance and had no effect on the plasma progesterone level in the cow. Moreover, the highest plasma progesterone level was in the Bali cow followed by the Pesisir cow and Simmental cow.

Key words: Ammoniated rice straw, cassava leaves, cow diet, progesterone, synchrony index

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Based on field observations, many farmers who receive Simmental cows in the West Sumatra province do not understand how to feed them and do not know what the feed should be. This situation can cause nutrition deficiency, especially related to energy or protein. Low nutrition affects the body weight and body condition, delays the onset of puberty, increases the postpartum interval to conception, interferes with the normal ovarian cyclicity by decreasing gonadotropin secretion and increases infertility[1]. Excess energy causes obesity, while excess protein causes ammonia poisoning. In these cases, it also causes retained placenta, more uterine infections, more cystic ovaries, impaired maturation of oocytes and subsequent fertilization or maturation of the developing embryos[1,2]. In addition, the offering of rice straw, as a substitute for native grass, has a low nutrition value. Therefore, it can lead to reproductive disorders, i.e., low fertility, conception failure, small birth weight or abortion[3,4].

Ammonia (N-proteins) rumen can be utilized for rumen microbial protein synthesis if the energy for the process is available from the fermentation of organic matter (OM)/carbohydrate. The rumen microbial protein synthesis is the main protein source of livestock ruminants. Ammonia utilization, as the result of the degradation of feed protein into the ruminal microbe protein synthesis, is limited by the availability of energy[5]. To increase the efficiency in synthesizing microbial proteins in the rumen, it should be synchronized with the release of the N-protein and energy feed in the rumen. The process results in protein degradation and OM in the feed in the rumen. The asynchronous diet produces more rumen ammonia compared to a synchronous diet and furthermore, the blood urea nitrogen (BUN) formed reaches its highest level, approximately 300 μmol L^{-1}, in dairy cattle[4]. Therefore it can cause ammonia poisoning as a result of low energy yield in the rumen[4].

A study on effect of a synchronous diet and fertility performance in cows shows that the plasma progesterone of cows consuming a synchronous diet was higher than those on an asynchronous diet. The study also showed that the highest plasma progesterone produced was in the Bali cow followed by the Pesisir and Simmental cows[6].

The ruminal microbes also require branches chain amino acids (BCAA) for protein synthesis and growth, especially cellulolytic microbials[7-9]. The use of cassava leaves as a supplement in a cow's diet increases the branches chain of volatile fatty acids (BCVFA) in the rumen[10] due to the high composition of BCAA in the leaves.

The BCVFA used as carbon skeletons for rumen microbial protein synthesis, including cellulolytic bacteria, which is used as an N source, come from ammonia as the result of the protein degradation in the rumen[9]. Thus, the use of cassava leaves, combined with the ammoniated rice straw and synchronized with the release of N-protein and energy in the rumen, has the highest possibility to increase the efficiency of microbe protein synthesis in the rumen. Thus, this increase the effectiveness of ammoniated rice straw usage in a cow's diet and it decreases the BUN content, without causing reproduction disorders.

The objective of the research was to assess the effect of cassava leaves as a cow diet supplement and the supplement was combined with ammoniated rice straw and was also synchronized with the releasing of N-protein and energy in the rumen to assess the physiological performance of the cow breed.

MATERIALS AND METHODS

The rumen-fistulated cattle were used to determine the ruminal degradability coefficient of the organic matter (OM) and protein of the feedstuff, namely, by means of the equation:

$$p = a+b\,(1-e^{-ct})$$ [11]

Where

p = The amount degraded at t time
a = The rapidly soluble fraction
b = The potentially degradable fraction
c = The rate of degradation of the fraction b
t = Time (h)

From the hourly quantity of the OM and the protein degraded, the synchrony index of the released nitrogen and energy in the rumen was then calculated by the following equation:

$$\text{Synchrony index}^{12} = \frac{25-\sqrt{\dfrac{\sum_{1-24}(25-\text{hourly}N/OM)^2}{24}}}{25}$$

where, 25 = 25 g N kg^{-1} of organic matter truly digested in the rumen. The synchrony index of the feedstuffs was then used to formulate two treatment diets. A synchrony index of 1.0 represented a perfect synchrony between the nitrogen and energy supply throughout the day, while values <1.0 indicated the degree of asynchrony. The synchrony index and chemical compositions of every feedstuff that was as an ingredient in two diet treatments are shown in Table 1.

Table 1: Chemical composition (%) and synchrony index of the feedstuff

Feedstuff	DM[a]	Protein	CF[a]	Ash	TDN[a]	I_{25}[b]
Ammoniated rice straw	89.9	10.9	33.5	11.4	61.5	0.739
Rice brand	89.9	13.0	8.3	10.3	66.8	0.621
Corn meal	87.8	7.0	2.3	13.6	81.9	0.528
Palm kernel cake	90.7	18.0	13.8	12.4	63.1	0.403
Cassava leaves	83.3	24.1	22.1	12.1	61.8	-0.650
Mineral	100.0	-	-	100.0	-	-

[a]DM: Dry matter, CF: Crude fiber, TDN: Total digestible nutrients, [b]I_{25}: Synchronization index[12]

Table 2: Feed and chemical composition (%) and the synchronic index of the diet

	R1	R2
Ammoniated rice straw	27.00	27.000
Rice brand	31.45	30.160
Corn meal	30.79	29.510
Palm kernel cake	10.56	10.130
Cassava leaves	-	3.000
Mineral	0.20	0.200
Dry matter	89.36	89.370
Organic matter	87.98	88.160
Protein	11.09	11.490
Crude fiber	13.83	14.310
Crude fat	4.40	4.390
Ash	11.81	12.040
TDN	69.58	69.400
Synchronic index	0.60	0.564

The process of urea-ammoniation of rice straw was based on what was recommended previously[13]. The experiment was conducted based on a random block design of a factoria 2×3, with 2 replicates. The first factor was the diet treatment, namely, the R1 = The diet without the cassava leaves supplementation and the R2 = The diet with cassava leaves supplementation. The second factor was the cow breeds, which consisted of the local cow (Pesisir cow), the Bali cow and the Simmental cow.

The average body weights of the Simmental cow, Bali cow and Pesisir cow were 209±8.84, 160±24.14 and 136±5.43 kg, respectively. The Simmental cows were below 2 years old, whereas both of the other cows were approximately 3 years old. Every cow breed was divided into two groups and each group consisted of two cows, which received a different diet treatment and were placed randomly in individual cages (2.5×1.5 m). The diet was given two times a day, with the same amount, at 8:00 am and 4:30 pm. Fresh water was available at all times (ad libitum).

This study was carried out over 30 days. The adaptation period of the diet treatment was 7 days and 20 days was used to determine the diet consumption and for taking of blood to measure the concentration of the hormone progesterone, by means of an RIA (Radio Immuno Assay). Blood samples (10 mL) were collected from the cows via a jugular vena puncture into vacutainer plain tubes (lithium heparin) after the morning feeding, which were immediately centrifuged within 10 min (3000 rpm for 15 min) to separate the blood plasma and were stored at -20°C until the analysis of the plasma progesterone concentration or the BUN concentration. The blood was taken six times in the period of 20 days. The last blood collection was also used for measuring the BUN. An intramuscular injection of prostaglandin PG2α was carried out to synchronize estrus prior to the start of the 20 days. Over three days, before the end of the study, urine was collected to measure the concentration of allantoin. The data on the consumption and digestion of nutrients, the average daily gain (ADG), the feed efficiency, the BUN and the allantoin concentration in the urine were analyzed statistically.

RESULTS AND DISCUSSION

Table 3 shows that the allantoin concentration in the urine of the cows that consumed the R2 diet (88.72 mg mL^{-1}) was lower compared with the cows that consumed the R1 diet (100.11 mg mL^{-1}) but almost all of the parameters of the consumption and digestion of nutrients served the R2 diet tend to be higher than those in the R1 diet. The low allantoin content in the urine of the cows fed the R2 diet was mainly caused by a low index of synchronization, i.e., 0.564 (Table 2). It might also be caused by the tannins contained in the cassava leaves, which might reduce protein degradability in the rumen and furthermore, there would be a lack of N-ammonia for bacteria protein synthesis in the rumen[14-16]. However, the use of cassava leaves led to more availability of BCAA for microbial protein synthesis, in particular cellulolytic bacteria[7]. Consequently, the cellulolytic bacteria were the predominant bacteria in the rumen of cows fed the R2 diet. Therefore it would cause a lot of cell wall fraction (fiber fraction) of feed digested and then followed by the digestion of cells contents or other nutrients[17]. Table 3 shows that the nutrients in the R2 diet tend to have a higher digestion level compared to the R1 diet, which does not use the cassava leaves.

A similar situation was found when examining the content of allantoin in the urine of the Bali cow, which was relatively lower than that in the Pesisir cow or Simmental cow. However, the digestibility of crude fiber in the Bali cow was

Table 3: Effect of the diet treatment and cow breed on the consumption and digestibility of nutrients and the allantoin concentration in the urine

	Diet (R)		Cow (S)				
	R1	R2	Simmental	Pesisir	Bali	SE	R vs S
Allantoin (mg mL^{-1})	100.11	88.72	95.27	94.88	93.10	178.37	ns
Consumption (kg)							
Dry matter (DM)	2.98	3.53	3.44	2.93	3.38	0.70	ns
Crude fiber (CF)	0.39	0.49	0.46	0.41	0.46	0.01	ns
Protein	0.33	0.40	0.39	0.33	0.38	0.01	ns
Digestibility (%)							
Dry matter (DM)	57.27	63.97	57.35	61.78	62.74	86.22	ns
Crude fiber (CF)	30.46[b]	41.52[a]	30.34[b]	39.44[a]	38.18[ab]	51.42	ns
Protein	56.09	63.20	55.16	61.01	62.76	112.67	ns

[ab]Significant difference in the row between the diet and between the cow breeds ($p<0.05$), ns: Non-significant

Table 4: Effect of diet treatment and cow breed on the average daily gain (ADG), feed efficiency, BUN and plasma progesterone

	Diet (R)		Cow (S)				
	R1	R2	Simmental	Pesisir	Bali	SE	R vs S
ADG (kg)	0.37	0.57	0.59	0.45	0.37	0.06	Ns
Feed eff. (%)	11.56	17.10	16.55	15.61	10.83	38.58	Ns
BUN (mg dL^{-1})	9.50		6.30[b]	10.00[ab]	10.90[a]	4.82	Ns

[ab]Significant difference between the cow breeds ($p<0.05$), ns: Non-significant

higher than that of the Simmental cow. This might indicate that there is a predominance of cellulolytic bacteria in the rumen of the Bali cow, which furthermore had a higher digestibility of other nutrients. Moreover, the high feed digestion in the diet with cassava leaves might be caused by the contribution of feed digestion, especially feed protein, in the abomasum and the small intestine, as a result of releasing the feed protein, which is bound by condensed tannin in the cassava leaves. The cleavage between the feed protein and the condensed tannin in the post-ruminal is caused by the low pH, of approximately 2, in the post-ruminal. The precipitation of fraction 1 protein by condensed tannins is virtually complete at a pH between 5 and 8[14].

The high digestibility of the diet accelerates the rate of gastrointestinal emptying and, then, increases nutrient consumption. The high digestibility of the nutrients and the rapid rate of passage and emptying of the stomach encourages the consumption of food that contains the nutrients[18]. This is the reason for the higher nutrient consumption for R2 compared to R1. However, the different consumption of the nutrients among the cow breeds might be caused mainly by the different body conditions among them. The Simmental cow is of the heaviest cow among them and thus, the nutrient consumption of the Simmental cow was the highest followed by the Bali cow and the Pesisir cow. The larger animals consume greater quantities of food. However, the relationship is not iso-metric but scales allometrically with body mass and intake is commonly expressed on the basis of the metabolic body weight or live weight ($LW^{0.75}$)[19].

By increasing digestion and consumption of the R2 diet, there was much greater nutrients availability for animal production that were consumed in the R2 diet. Table 4 shows that the ADG of the R2 diet was higher compared to that of the R1 diet (Table 3). The Simmental cows generally consumed more feed than the Bali and Pesisir cows did. This is in accordance with a previous study, showing that Simmental cows have the highest to consumption of feed compared to the other cows. This occurs because of the difference in the cow breed or in the body weight, such that cows of a large breed consume much more feed than those of small breeds, such as the Bali and Pesisir cows. The magnitude of the energy consumption is equivalent to that of the feed consumption, which is influenced inter/within the cattle breeds, body weight, dry matter digestibility, ratio of concentrate and forage, feedstuff processing, moisture content and temperature[20]. The Simmental cows, therefore, had the highest ADG followed by the Pesisir and Bali cows. The Pesisir cows had a higher ADG compared to the Bali cows, which actually had a heavier body weight than the Pesisir cows. This might presumably be caused by an effect of compensatory growth in the Pesisir cows, as a result of the lack of feeding. Before conducting the experiment, the Pesisir cows seemed slightly under weight, whereas the Bali cows were fleshy. This corresponded to a previous report[21].

As mentioned above, the R2 diet showed a high amount of nutrient consumption and that was followed by digestion and therefore, the R2 diet had the high availability of nutrients and energy for production. This indicated that the feed efficiency of R2 was higher than that of R1, which might be caused primarily by the nutrient digestion of R1, which was lower than that of R2. This corresponded to a previous study, showing that the feed efficiency is associated with the digestibility of the diet[21].

Table 4 shows that the feed efficiency of the Simmental cows was higher than that of the other cows, whereas that of the Pesisir cows was higher than the Bali cows. This result was in accordance with a previous study[6]. In addition, the Simmental cow is classified into bos taurus and has a higher feed efficiency compared to bos indicus[22]. The higher feed efficiency of the Pesisir cows compared to the Bali cows might be due to the fact that the Pesisir cows had the smaller body condition and a higher crude fiber digestibility. Feed efficiency is affected by the digestibility of ration, the forage quality, the body condition of the cattle, the feed additive and environmental factors[21].

The lowest feed efficiency was in the Bali cows compared to the other cow breeds, indicating that the energy requirement of a one unit gain for the Bali cow was higher than for the other cow breeds. If the cow's dietary energy is not adequate to meet its requirements, it can be supplied by the breakdown of body fat and muscle[2]. Thus, in the Bali cows, much protein is available to be oxidized to yield energy and this would mean that more ammonia is produced and converted to urea in the liver. The formed urea, furthermore, increases the urea concentration in the blood, which was called the blood urea nitrogen (BUN). The excess rumen ammonia is absorbed from the rumen and the ammonia from tissue metabolism is transported in the blood to the liver and kidneys where it is converted to urea. Table 4 shows that the treatment of either diet or cow breed that showed a high feed efficiency produced a low BUN. This might be caused by balanced nutrients, especially between energy and protein. In this case, the energy requirement in the diet corresponded to the amount of protein available and vice versa. This is in accordance with a previous report, showing that cattle offered high grain diets, as an energy source, need more protein to meet their growth potential and to improve their feed efficiency[21].

Although, the feed efficiency of the Pesisir cows was high enough compared to that of the Bali cows (15.61 vs 10.83 %, respectively), the different BUN content between the Bali cows and Pesisir cows was considerably small, i.e., 10.00 and 10.90 mg dL^{-1}, respectively (Table 4), indicating that there was BUN over production in the Pesisir cows, as a result of the metabolism of the protein nutrients available in order to obtain more energy to encourage their growth. As shown above, the Pesisir cows underwent compensatory growth and this, furthermore, caused more BUN formation. Severe nutrition depletion, as a result of a prolonged lack of nutrition or disease, causes protein catabolism and results in a high BUN concentration[23]. However, high dietary protein intake, resulting in a BUN of greater than 19-20 mg dL^{-1}, is associated with an altered uterine environment and decreased fertility (reduced conception rate and decreased pregnancy rate) in lactating dairy cows and heifers.

Figure 1 shows that the progesterone levels in both diet treatments are relatively low but still show a normal pattern associated with the estrus cycle phases. The progesterone concentrations were below 2 nmol mL^{-1} on collection day 2, showing that the cows were in the stages of the follicle phase. After that, the concentration increased, corresponding with the development of the corpus luteum at the luteal phase. The minimum level of the progesterone concentration in the condition of estrus has a range of 0.46-0.55 ng mL^{-1} [24]. The fluctuation in the progesterone levels between day 2 up to day 20 of the blood sample collections indicated that there was the development of a follicle in the luteal phase but it did not produce follicle de Graaf. The progesterone profiles that were obtained also showed that all of the cows had two waves of the follicle development within once the estrus cycle. The levels of cow progesterone in the luteum phase (day 7-9 of the estrus cyclic) range from 1.58±0.11 to 2.21±0.18 ng mL^{-1} [25].

The supplement of cassava leaves in the R2 diet caused the development of the corpus luteum faster as well as the development of its follicles, as seen by decreasing progesterone concentration at blood collection day 8. A high concentration of progesterone occurred at blood collection day 17 and then decreased at blood collection day 20, which was an indication of corpus luteum regression in order to undergo the next estrus cycle. Figure 1 shows that the

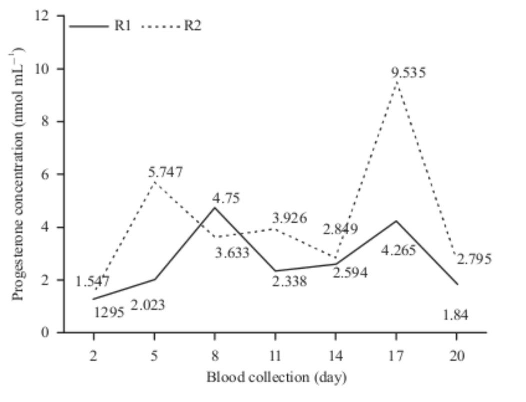

Fig. 1: Effect of the diet treatments on progesterone concentration

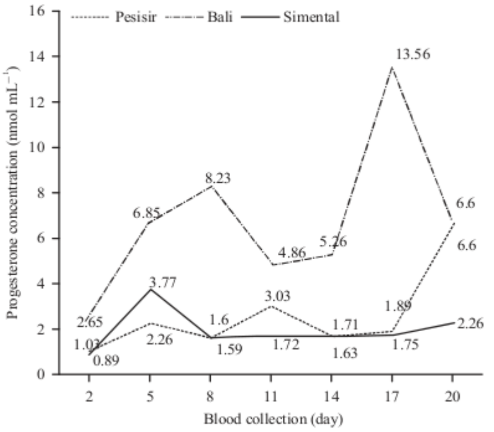

Fig. 2: Effect of cow breed on progesterone concentration

progesterone concentration in the R2 diet was higher than that of the R1 diet. As mentioned above, the digestion and consumption of the R2 diet, which was supplemented with cassava leaves, were higher than that of the R1 diet. In other words, more nutrients and energy are available in the R2 diet and there was more progesterone hormone compared to R1.

The progesterone concentrations in the Bali cows were higher compared to the other cow breeds. While the progesterone concentrations between the Pesisir cows and the Simental cows were not significantly different at almost all phases (Fig. 2). In general, the progesterone hormone profiles of the three cow breeds showed almost the same pattern, in which the value of the first blood collection, at day 2 of the estrus cyclic, showed the lowest value, indicating that all the cows were still at the follicle phase. Furthermore, the progesterone concentration of every cow breed, at blood collection day 5, was above average, because it was in the luteal phase. A significant decline in the progesterone concentration in the Bali cows on blood collection day 20 showed that the Bali cows began to enter the next estrus phase, whereas the Pesisir and Simmental cows were still in the luteal phase. Compared to *Bos Taurus*, *Bos Indicus* have a higher concentration of steroid hormones, including progesterone[26]. This is assumed to be due to the increase in the metabolism of the steroid hormone in the liver and the lack of steroid production at follicle and corpus luteal phases in *Bos Taurus*. A previous study also shows that insulin and IG-1 acts as stimulators of granulosa cell proliferation and steroidogenesis in cattle[27] and that IGF1 works synergistically with FSH in steroidogenesis[28]. These correlated each other because the bos indicus cow has a greater oocyte quality[26,29]. As mentioned above, the digestion and consumption of the Bali cow were higher than that of Pesisir and Simmental cows. In other words, the Bali cow has more available nutrients and energy and then also forms much more progesterone hormone compared to the Pesisir cow and the Simmental cow.

CONCLUSION

In conclusion, supplementation with 3% cassava leaves in a cow diet of ammoniated rice straw and having a synchrony index of 0.566 produced a good production and reproduction performance, namely, in terms of the production of the hormone progesterone in the cow, while the highest plasma progesterone was produced by the Bali cow followed by the Pesisir and Simmental cows.

SIGNIFICANCE STATEMENT

This study discovered that addition of 3% cassava leaves in cow diet could be lower the efficiency of microbial protein synthesis in the rumen but could increase the availability of energy and protein for the formation of the plasma progesterone hormone; whereas the highest concentration of the plasma progesterone hormone during the estrus cycle was Bali cow followed Pesisir cow and Simmental cow. This study is expected to overcome fertility problems of cow that is with the addition of cassava leaves in diet of rice straw ammoniated basis and with synchronizing release of N-protein and energy in the rumen.

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