In vitro: The Increase of The Quality of Sakura Block As a Dietary Supplement to Increase The Concetration Branched Volatile Fatty Acids (BCVFA) and total bacteria

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Abstract

The enrichment of sakura block by utilizing palm kernel cake as a substitute for corn and optimal use of earthworm flour in increasing branched volatile fatty acids and rumen bacteria population needs to be undertaken to support the cattle-palm oil integration system in Indonesia. This study used a completely randomized design with 6 treatments and 3 replications and it consisted of commercial Sakura block block treatment as a control (P0), Sakura block enriched with palm kernel cake (P1) and sakura block enriched with palm kernel cake and 2% earthworm flour (P2). 4% (P3), 6% (P4) and 8% (P5). The results indicated that there was a significant increase in the concentration of total branched volatile fatty acid, isobutyrate, isovalerate, valerate and total bacteria in the sakura block treatment enriched with palm kernel cake and earthworm flour. From all parameters, the highest increase was obtained in the treatment of sakura block enriched with palm kernel cake and 6% earthworm flour (P4).

Keywords: commercial sakura block, palm kernel cake, earthworms, branched volatile fatty acids, total of bacteria.

Introduction

The effort to increase the value of ruminant feed such as beef cattle are carried out by paying attention to nutrient requirements for rumen microbial growth and livestock production needs [1]. The contribution of microbial protein to the needs of rumnans reaches 70-100% and the energy supply from fermentation is 70-85% [2]. Therefore, it is not enough to take into account the nutritional content in it, but it is also necessary to take into account the ability of bacteria to utilize the nutrients contained in the feed. Sakura block is a supplement feed made of low quality coconut sugar, bran, corn, sago, urea, salt, TSP, mineral mix and top mix [3]. Sakura block contains 20.39% crude protein, 3.73% crude fiber and 72.59% TDN. Provision of 10% sakura block supplementation based on dry matter rations can increase dry matter consumption, weight gain and organic matter digestibility of local beef cattle in Kaur district, Bengkulu province fed with palm midrib basalt and Setaria sp grass [3]. Sakura block has been produced commercially in the Department of Animal Husbandry, Faculty of Agriculture, Bengkulu University and has been widely used by beef cattle and dairy farmers in Bengkulu Province.

The growth of the palm oil industry in Indonesia can be an opportunity for the development of beef cattle [4,5,6]. Although the production of forage decreases with increasing age of oil palm planting [7,8,9]. On the other hand, there is potential for waste from oil palm processing that is used as a source of animal feed [10,11,12]. Palm kernel cake is one of the palm oil processing wastes whose production is quite large, in every 1 tonne of palm bunch processing an average of 120 kg of palm oil is produced [13]. The average chemical composition of palm kernel cake was 89-95% for dry matter, 12-15% for crude protein, 6-24.9% for crude fiber, Also, acid detergent fiber being 43.7%, neutral detergent fiber being 66.7% and lignin being 21.1% [14,15,16]. Palm kernel cake can be used as a substitute for corn and its use is up to 28% of ruminant concentrate material [17], even the use of palm kernel cake protected with molasses in the concentrate material can reach 40% [18].

Besides, utilizing palm kernel cake, the introduction of earthworms has the potential to be developed to optimize the development of beef cattle in the oil palm area. Cow dung waste is very good for earthworm growth media [19,20]. Earthworms are also good decomposers to produce organic fertilizer for plants [20,21,22]. The chemical content of earthworms is 63.08% protein, 18.51% crude fat, 1.08% crude fiber, 5.81% ash and 12.41% BETN [23]. The protein contained in earthworms is a source of Branched Chain Amino Acid (BCAA) [24,25,26] which is needed for the growth of fiber-destroying microbes in the rumen [27,28,29,30,31].

Dealing with the description above, it is necessary to enrich the sakura block by utilizing oil palm meal as a substitute for corn and the optimal level of use of earthworm flour in increasing branched volatile fatty acids and rumen bacterial population.

Materials and Methods

Ethical approval

This research did not use any live animals so, ethical approval is not needed.

Research Procedure

Making Sakura Blocks

Prepare the ingredients, including: rejected brown sugar, rice bran, corn, sago, urea, salt, mashed TSP, mineral mix and topmix respectively. The ingredients in the form of flour were mixed first starting from the least amount to the most homogeneous. water soluble ingredients such as rejected brown sugar, urea and salt are dissolved in water as much as 7% of the total ingredients, then heated over low heat until dissolved. Furthermore, the dry ingredients were mixed with liquid ingredients until evenly distributed and printed. Sakura blocks that have been printed were dried for 2-3 days, then they were stored in plastic wrap or used immediately.

Treatment

This study used a completely randomized design with 6 treatments and 3 replications consisting of commercial product sakura blocks as control (P0), Sakura blocks enriched with oil palm meal (P1) and sakura blocks enriched with oil palm meal and 2% earthworm flour (P2), 4% (P3), 6% (P4) and 8% (P5). The composition composition of the control block and treatment is presented in Table 1.

Table 1. Ingredient and Nutrient Sakura Block

Ingredient (%)	Treatment					
	P0	P1	P2	P3	P4	P5
Rejected brown sugar	32.0	32.0	32.0	32.0	32.0	32.0
Rice barn	28.0	28.0	26.0	24.0	22.0	20.0
Corn	15.0	0.0	0.0	0.0	0.0	0.0
Palm kernel cake	0.0	15.0	15.0	15.0	15.0	15.0
Earthworm flour	0.0	0.0	2.0	4.0	6.0	8.0
Sagoo	15.0	15.0	15.0	15.0	15.0	15.0
Urea	5.0	5.0	5.0	5.0	5.0	5.0
Salt	2.0	2.0	2.0	2.0	2.0	2.0
Triple superphospate	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix	1.0	1.0	1.0	1.0	1.0	1.0
Topmix	1.0	1.0	1.0	1.0	1.0	1.0
Total Nutrient	100	100	100	100	100	100
Dry matter (%)	89.06	89.81	89.80	90.07	90.78	89.19
Organic matter (%)	93.55	93.65	93.60	93.61	93.85	93.61
Crude Protein (%)	17.83	20.37	21.95	23.50	25.28	25.72
Crude fibber (%)	3.67	5.25	4.97	4.53	4.62	4.58
Extract eter (%)	3.00	3.27	2.91	3.92	3.05	3.21
Total Digestible Nutrient (%)	78.87	81.90	84.35	86.96	87,76	88.27

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In vitro

In vitro analysis was carried out to determine Branvhed Volatile Fatty Acid (BCVFA), isobutirat, isovalerat and valerat and of each feed component using the Tilley and Terry [32] method, performed for 48 h for forage and 24 h for concentrate. Rumen liquor was obtained from slaughterhouse from local goat fed with native grass and concentrate. Incubation was stopped by immersing the Erlenmeyer flask into ice water to stop the microbial activity, after which pH measurement was carried out using a pH meter. Next, the supernatant was separated from the residue. To do this, the mixture obtained from in vitro analysis was put into a centrifuge tube and then centrifuged for 30 min, 3000 rpm, at 4°C. The supernatant was stored in bottles and then frozen until subsequent BCVFA, isobutirat, isovalerat and valerat. Concentration BCVFA, isobutirat, isovalerat and valerat was determined using gas chromatography. Rumen bacteria population was analyzed through Ojimoto and Imail method developed in Nutrition Laboratory of Livestock Research Center Bogor, Indonesia.

Statistical Analysis

The obtained data were analyzed using an analysis of variance [33]. If there were differences, they were then tested Duncan's multiple range test.

Results and Discussion

The characteristics of rumen fluid observed in vitro analysis are shown in table 2. There were significant differences in the parameters of total branched volatile fatty acid, isobutyrate, isovalerate, valerate and total bacteria between treatments ($P<0.05$).

Table 2. Concentrations Of BCVFA, Isobutyrate, Isovalerate, Valerate And Total Bacteria

Parameter	Treatment					
	P0	**P1**	**P2**	**P3**	**P4**	**P5**
BCVFA (mM)	11.44 ± 0.74^{a}	13.48 ± 0.15^{b}	13.50 ± 0.74^{b}	13.89 ± 0.17^{b}	15.03 ± 0.83^{c}	14.51 ± 0.77^{b}
Isobutyrate (mM)	4.32 ± 0.37^{a}	4.88 ± 0.20^{ab}	5.00 ± 0.71^{ab}	4.44 ± 0.26^{ab}	5.47 ± 0.25^{b}	5.17 ± 0.37^{ab}
Isovalerate (mM)	3.96 ± 0.39^{a}	4.45 ± 0.19^{b}	4.53 ± 0.12^{b}	4.17 ± 0.46^{ab}	4.97 ± 0.34^{c}	4.17 ± 0.09^{b}
Valerate (mM)	3.16 ± 0.13^{a}	4.15 ± 0.31^{b}	3.97 ± 0.14^{b}	4.28 ± 0.05^{b}	4.29 ± 0.69^{b}	4.17 ± 0.32^{b}
Total Bacteria (kol x 10^{9})	2.47 ± 0.06^{b}	2.45 ± 0.38^{b}	2.60 ± 0.13^{bc}	2.65 ± 0.45^{bc}	2.81 ± 0.55^{c}	1.79 ± 0.26^{a}

BCFVA; branched volatile vatty acid, source: Laboratory of Animal Research Institute, Ciawi. Bogor

Treatment of palm kernel cake substituted with corn and earthworm flour on commercial sakura blocks was able to significantly increase branched volatile fatty acids (BCVFA) ($P<0.05$). The average concentration of BCVFA in the treatment of palm kernen oil with corn substitution (P1) was 13.48 mM, an increase of 17.83% from the commercial sakura block (P0) of 11.44 mM. The concentration of BCVFA (P1) did not show any difference ($P>0.05$) with the concentration of BCVFA produced by P2, P3 and P5. Meanwhile, the highest increase in BCVFA concentration ($P<0.05$) was produced by the sakura block which was treated with palm kernel cake as a substitute for corn and 6% earthworm flour (P4), which increased by 31.29% from the commercial sakura block (P0). Other parameters such as isobutyrate and isovalerate also showed the same results, where those who received oil palm meal as a substitute for corn and 6% earthworm flour (P4) experienced the highest increase compared to other treatments (P1, P2, P3 and P5). The concentrations of isobutyrate, isovalerate and valerate of sakura block (P4) were 5.47 nM, 4.97 nm and 4.29 nM or increased by 26.62%, 25.50% and 35.75%, respectively, from commercial sakura block (P0). In line with the results obtained by the parameters of BCVFA, isobutyrate, isovalerate and valerate, a significant increase in bacterial population was produced in the sakura block treatment enriched with 6% earthworm flour (P4) compared to other treatments. The total rumen bacteria P4 was 2.81x109 col significantly larger than the population of P0, P1, P2, P3 and P5.

The increase of the concentration of BCVFA, isobutyrate, isovalerate and valerate in liquid was due to the high content of branched amino acids (valine, leucine and isoleucine) in earthworms [25,34]. Branched amino acids (BCAAs included: valine, leucine and isoleucine) are degraded and decarboxylated to produce Branched Chain Fatty Acids (BCVFA) such as isobutrirate, isovalerate and valerate [35]. BCVFA is then used as a cofactor for the formation of rumen bacterial cells [28,36,37], especially cellulolytic bacteria such as Ruminoccocus flavifaciens and Bacteriodes succinogenes which have an important role in degrading lignin in cell walls. plants [27]. Tylutki and Fox [30] stated that deficiency of branched amino acids, especially lysine in ruminant diets with high fiber content can inhibit the formation of rumen bacterial cells.

However, the addition of branched amino acids to the diet has limitations, this is due to lysine, leucine and isoleucine are more difficult to synthesize by rumen microbes [29, 38]. In this study, the optimal limit for using earthworms in sakura block to increase BCVFA and bacterial population was 6%. Besides, the increase of the concentration of BCVA and the population of rumen bacteria, sakura block enriched with 6% earthworms can increase ammonia and volatile fatty acids which were the main sources of nitrogen and energy for the synthesis and growth of rumen bacteria [39] while Zhang et al. [40] revealed that the optimal limit for the addition of valine, leucine and isoleucine in fibrous rations to increase rumen fermentation products and nutrient digestibility was 2 mmol/L. Sihombing et al. [41] pointed out that the supplementation of earthworm flour to a level of 6% in the ration did not significantly improve the performance of sheep in the period of growth.

Conclusion

Synchronization between protein and carbohydrates which balanced from palm kernel cake substitution treatment for corn and earthworm flour to a level of 6% in commercial sakura blocks was able to optimally increase the concentration of branched volatile fatty acids, isobutyrate, isovalerate, valerate and total rumen bacteria for growth and protein synthesis of rumen microbes. In the process of cell formation, generally, the rumen microbes used volatile fatty acids from carbohydrate synthesis and ammonia (NH3) as the result of protein synthesis and non-protein nitrogen (NPN) animal feed. Earthworm flour was a protein-rich feed ingredient and it contained of branched amino acids (valine, leucine and isoleucine) which will be degraded and decarboxylated to free isobutyrate, isovalerate and valerate.

Author Contribution

JJ, LW, MZ and KK formulated experimental designs and experimental work in the laboratory. JJ compiled the manuscript and performed data analysis under the supervision of LW, MZ and KK. All authors read and agreed to the final version of the manuscript.

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