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Utilization of liquid multi-microbe probiotics supplemented urea red palm sugar block as a potential improving balance of microorganisms in digestive tract for cattle economical production

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Abstract

Probiotics are live microbial feed supplements, providing beneficial effects on the host animal by improving balance of microorganisms in digestive tract. This study aims to examine the benefits of liquid multi-microbe probiotic supplementation on nutrient digestibility and economical production of beef cattle. The treatments were including ration control using fresh forage of King grass (*Pennisetum purpureum*) + 350 g of URPS-Block without liquid probiotic supplement (T0), fresh forage of King grass (*Pennisetum purpureum*) + 350 g of URPS-Block + liquid probiotic supplement of 2.0 cc/liter fresh water (T1), fresh forage of King grass (*Pennisetum purpureum*) + 350 g of URPS-Block + liquid probiotic supplement of 4.0 cc/liter fresh water (T2) and fresh forage of King grass (Pennisetum purpureum) + 350 g of URPS-Block + liquid probiotic supplement of 6.0 cc/liter fresh water (T3). Experimental design applied the Randomized Block Design involving 24 male cattle, divided into six groups of animal ages and four liquid multi-microbe probiotic doses. Parameters were feed dried matter consumption (DMC), average daily gain (ADG), feed conversion and income over feed costs (IOFC). The data were analyzed by variance analysis continued by Duncan test. Results showed that liquid multi-microbe probiotics supplementation of 2.0 to 6.0 cc/liter fresh water and URPS-Block of 350 g/day added into forages increased significantly (P<0.05) production of cattle indicated by significant higher ADG of 510 to 570 g (P<0.05) compared with those of control ration producing ADG of 380 g. The increase of animal production was supported linearly by the increasing drinking water consumption supplemented with liquid probiotic levels of 2.0 to 6.0 cc/liter fresh water of animals. Cattle supplemented with liquid probiotic of 2.0 to 6.0 cc/liter fresh water/animal/day also produced higher IOFC, equivalent with increases of 55.67 to 80.87 percents IOFC compared with the animals fed the control ration without liquid probiotics.

Keywords: Beef cattle; Liquid probiotics; Local ingredient feed supplement; Cattle economical production

1. Introduction

Generally tropical grasses contain quite high levels of lignin causing difficulty to be degraded by rumen microbes. To overcome the above problems, manipulation of the rumen ecosystem was carried out for efficient rumen fermentation by maximizing the digestibility of high fiber feed and microbial protein synthesis in the rumen [1]. The use of probiotics, a product containing one or a mixture of various microorganisms functioning as a fiber digester in feed could interact

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positively with rumen microbes in target animal livestock [1]. The specific function of probiotics could vary depending on the host animal and what is the most important characteristics of the probiotic [2].

The liquid probiotics contain live microbial cultures, desired and benefit for ruminant livestock by improving the microbial balance of the digestive tract playing a role in providing enzymes able to digest crude fiber (CF) and producing lactic acid bacteria able to increase the pH of the animal apparatus [2]. Animals with low digestion could produce anti-microbial substances (bacteriocins) so that they were able to inhibit the growth of unfavorable microbes [3].

Research has suggested the possibility of selective probiotics as feed additive producing several changes in livestock, reducing the risk of acidosis, increasing feed efficiency, increasing body weight (around 2.5%), milk production (0.75-2kg), reducing the content of *E. coli* in feces from infection, and improving the immune response in stress conditions [4]. The habitat in the rumen microbial ecosystem is a complex consortium of microbes, symbiotically related to the host, working in synergy to bio-convert lignocellulosic feed into volatile fatty acids (VFA) [5]. The total microbial content in liquid probiotics used in this study are high with contents of 1.5 x 10⁶ (*Colony Forming Unit, CFU/ml*) met the bacterial population standard with a minimum of 10⁷ cfu/g [6].

King grass is abundant and easy to grow in a variety of conditions and could be planted either by seeds or cuttings with the potential production of 200 tons/ha/year, making their worth encouraging as a source of forage protein for livestock feeds planted with combination by legume trees [7]. King grass is a ruminant feed plant that is very popular with farmers, due to its potential high product and other nutrients for ruminant animals as presented in Table 2.

Utilization of king grass (*Pennisetum purpureum*) as forage is better affordable choice with the potential production grass ranging from 2.7 to 8 ton/ha, although its low digestibility due to higher components of crude fiber including cellulose, hemicelluloses, lignin and indigestible ash [8]. Nutritional value of king grass (Table 2) could be enhanced by various processing methods including the use of nutrients by enzymes produced in the digestive tract and by probiotic bacteria able to convert several components of the feed into nutrients that are more easily utilized for the host animal needs [2].

Red palm sugar contained in the urea red palm sugar block (URPS-Block) is an easily digestible carbohydrate acting as a source of carbohydrate and carbon skeleton for protein process involved in synthesis of microbial cell walls [9]. URPS-Block is expected to increase the microbial population in the rumen increasing the feed digestibility and further enhanced the productivity of ruminants [10]. This study aims to examine the benefits of the efficient liquid probiotic supplementation levels mixed into drinking water on the performance of beef cattle, fed with king grass (*Pennisetum purpureum*) supplemented with the urea red palm sugar block (URPS-Block).

2. Materials and Methods

2.1. Animal location and measurement

The research was conducted at the household group farm at Purworejo Village, Modayang District of Boltim Regency, North Sulawesi of Indonesia. The research was carried out from May 2024 to September 2024. The research utilized type of the liquid multi-microbe probiotic supplements given daily to cattle.

The experimental animals were twenty-four local breed of Bali male beef cattle at the ages ranging from 18 to 33 months old. The duration of observation was 30 days, with an adaptation period of the first 10 days followed by an observation period of 20 days. Body weight measurements were carried out every 10 days. Livestock animals were grouped into six groups based on the age categories ($1 \ge 18$ months), ($2 \ge 21$ months), ($3 \ge 24$ months), ($4 \ge 27$ months), ($5 \ge 30$ months), ($6 \ge 33$ months). Each group involved 2 male animals. Body weights of male animals at the ages of 18 months old to 33 months old were ranging from minimum weight of 182 kg to maximum weight of 367 kg. Samples of ration (king grass) were collected at the initial and end of the study in each period of treatment. The left-over forage feeding was collected daily. Daily difference between feed consumed and the left-over forage feeding was defined as the animal feed consumption. Animal feed consumption was converted into dry matter consumption (kg unit) per animal per day.

2.2. Formulation of feed supplement

The feed used in the research consisted of urea red palm sugar block and king grass (*Pennisetum purpureum*). Liquid multi-microbe probiotics were prepared, then it was measured based on the needs of each cattle during the research. Feeding follows the instructions or habits carried out by local breeders. Feeding of animals were given in the morning,

at noon and in the afternoon. Amount of feed basal given to each animal (kg/day) with the treatments of liquid probiotics in the morning were king grass forage. The amounts of liquid probiotics were mixed in 10 liters/head/day (Figure 1).



Figure 1 Scheme for mixing the liquid probiotic solution containing 2.0 cc per liter of fresh water (T1), 4.0 cc per liter of fresh water (T2) and 6.0 cc per liter of fresh water (T3) into 10 liters of daily drinking water for the experimental animals.

The urea red palm sugar block (URPS-Block) supplementation (formulation form in Figure 2) was given constantly with a dose of 350 grams/head/day [11, 12] along with the forages feed in the morning. The remaining forage feed and drinking water without the treatments of liquid probiotics were given at noon and in the afternoon when animals run out of forages and drinking water containing the treatments given in the morning. Measurements of animals were made by measuring the chest circumference and body length, then these data were converted into body weight using formula [13] to determine the animal's body weight.



Figure 2 The composing technical process of the urea red palm sugar block (URPS-Block) supplement formulation using local ingredients from the agricultural waste products

The treatment given was to utilize basal feed with different levels of liquid multi-microbe probiotics. Feed and drinking were given in *ad libitum*. The method of giving the drinking water was by mixing homogeneously into the open bucket, described in Figure 1, and the forage feeds were given as the following composition:

T0: Free access to fresh basal forage of king grass (*Pennisetum purpureum*) *ad libitum* + 350 g of URPS-Block without liquid probiotic supplement;

T1: Free access to fresh basal forage of king grass (*Pennisetum purpureum*) *ad libitum* + 350 g of URPS-Block + liquid probiotic supplement of 2.0 cc/L fresh water;

T2 : Free access to fresh basal forage of king grass (*Pennisetum purpureum*) *ad libitum* + 350 g of URPS-Block + liquid probiotic supplement of 4.0 cc/L fresh water.

T3 : Free access to fresh basal forage of king grass (*Pennisetum purpureum*) *ad libitum* + 350 g of URPS-Block + liquid probiotic supplement of 6.0 cc/L fresh water.

The total bacteria (*Total Plate Count, TPC*) are high, reaching 1.0 to 1.5 x 10⁶ (*Colony Forming Unit, CFU/ml*). The provisions of liquid multi-microbe probiotics in water solution (T1, T2 and T3) were applied at 10 ml/animal/day (Figure 1) to the experimental cattle, all given firstly in the morning. The liquid multi-microbe probiotic was dissolved in water in a ratio of 1 cc liquid multi-microbe probiotic into 1 liter of water by procedures (Figure 1). The solution of the treatment liquid probiotic was mixed into the experimental animal's drinking water.

All treatments were given firstly to the unit of experimental animals in the morning. The forage feed and drinking water can be freely accessed by the experimental animals *ad libitum* at noon and in the afternoon. The remaining forage ration and drinking water were daily collected in the morning before the next feeding was given. The ingredients of the urea red palm sugar block (URPS-Block) with their composition were presented in Table 1. The nutritional contents of the forage king grass (*Pennisetum purpureum*), the urea red palm sugar block (URPS-Block) was presented in Table 2.

Table 1 Composition of urea red palm sugar block (URPS-Block)

Materials*	% (air' dry basis)		
Waste palm red sugar	50		
Urea	4		
Rice bran waste product	26		
Coconut meal waste product	9		
Animal bone meal waste product	6		
Salt Mineral	5		
Total	100		
*) [11]			

Table 2 Nutritional content of forage feeds and URPS-Block

Nutritional content*	King grass (P. purpureum)	URPS-Block	
Dry matter (%)	61.27	80.9	
Ash (%)	12.77	7.83	
Crude Protein (%)	13.2	15.32	
Crude fiber (%)	31.4	5.68	
Crude fat (%)	3.0	6.89	
Ca (%)	0.40	0.41	
P (%)	0.16	2.47	
TDN (%)	67.38	69.57	
Gross Energy (kcal/kg)	3300	3,260	
*) Results of analysis of the Manado industrial plant research institute laboratory, Indonesia (2024).			

Liquid multi-microbe probiotics were prepared from the Laboratory of Animal Product Technology, Faculty of Animal Science, Sam Ratulangi University. Then it was weighed based on the needs of each animal during the research. The total microbial contents in the liquid probiotics are presented in Table 3.

Table 3 Types and numbers of microbes in liquid probiotics used in the study

Types of Bacteria	Number of Bacteria in Liquid Probiotics (cfu/ml)	
Lactobacillus casei	1.5 x 10 ⁶ cfu/ml	
Saccharomyces cerevistae	1.5 x 10 ⁶ cfu/ml	
Rhodopseudomonas palustris	1.0 x 10 ⁶ cfu/ml	
Source: [14]		

2.3. Variables and Statistical Analysis

The variables observed in this research study were including: (1) dry matter consumption of ration (g/animal/day) and drinking water (L/animal/day), calculated as daily difference between feed consumption/drinking water and the

remaining ration/drinking water, and then converted into dry matter consumption, (2) average daily gain (ADG) (g/animal/day), calculated as difference between animal live weights at the end and at initial period of research divided by twenty days of data collection, (3) feed conversion, calculated as ratio between dry matter consumption of forage ration (g/animal/day) and the ADG (g/animal/day), and (4) income over feed cost (IOFC), calculated as difference between price of ADG in the unit of Indonesian rupiah (IDR) and daily feed consumption costs of king grass (*Pennisetum purpureum*) dry matter weight, UPSB supplement and liquid multi-microbe probiotics, all in the IDR per animal per day (IDR/animal/day).

The experimental design used was a randomized block design with three treatments and six replications. The three treatments in were doses of liquid probiotic mixed drinking water and rations mentioned above. Meanwhile, six groups of animals were replicated based on initial animal age groups with the initial average of their different body weights. The mathematical model of a randomized block design [15] was as follows:

 $Yij = \mu + Ai + Bj + Eij$

Yij : Observation value of the *j*-th experimental treatment unit that received by animals at the *i*-th age; μ : True average value; Ai: Influence of the initial animal weight group of the *i*-th age: (1=18 months), (2=21 months), (3=24 months), (4=27 months), (5=30 months), (6=33 months); Bj: Effect of the *j*-th probiotic treatments: (j.1) control, (j.2) liquid probiotic of 0.2% (v/w), (j.3) liquid probiotics of 0.4% (v/w); (j.4) liquid probiotics of 0.6% (v/w) and Eij: Effect of error from the experimental unit on the *i*-th group that received the *j*-th ration treatment.

The data obtained were analyzed using analysis of variance (ANOVA) to determine the effect of treatments on the observed variables and differences between treatments were tested using the Duncan distance test [15].

3. Result and Discussion

3.1. Characteristics of liquid probiotics in the feeds related to the sustainable environment

The impact of increasing ruminant animal production showed that beef cattle contribute to greenhouse gas emissions in the form of methane gas released through belching, which is the result of the natural process of animal feed fermentation [6]. The production of methane gas wastes and gives up energy of animal livestock and disturbs the sustainable environment [16, 17]. Therefore, beef cattle feed products based on environmentally friendly lactic acid bacteria need to be developed for two strategic reasons, including energy efficiency of beef cattle and reducing methane gas emissions in the environmental air [6, 14].

The liquid probiotics in this study are additional feed in the form of microorganisms with three types of microorganisms (Table 4), providing beneficial influence by maintaining and improving balance microorganisms in the digestive tract [18, 6]. The liquid probiotics are included in the functional feed category because it has health effects in its host [19, 8, 20].

Table 4 Liquid probiotic supplementation effect on the average of dried matter consumption (DMC), drinking water consumption (DWC), average daily gain (ADG), Metabolic Energy (ME), feed conversion (FC) and nutrient contents in animal feces of the experimental diets in each animal

Variables	Treatments				
	Т0	T1	Т2	Т3	
Consumption:					
DMC (kg/animal/day)	3.91 ± 0.92	4.68 ± 0.84	4.91 ± 0.53	4.98 ± 0.57	
DWC (liter/animal/day)	18.43±1.49 ^a	18.64 ± 1.02 ^a	21.85 ± 1.04 ^b	22.03 ± 1.08 ^b	
ADG and DWC:					
ADG (kg/animal/day)	0.38 ± 0.17 ^a	0.51 ± 0.27 ^b	0.56 ± 0.24 b	0.57 ± 0.29 ^b	
FC (DMC:ADG)	10.18±3.48 ª	9.12 ± 3.29 b	8.71 ± 4.14 ^b	8.73 ± 4.19 ^b	
^{<i>ab</i>} Means in the same row without common letter are different at p<0.05.					

Research study reports showed that the use of animal feed based on lactic acid bacteria produced by liquid probiotic supplementation could help to reduce methane gas emissions produced by beef cattle and make animal healthier [21]. The addition of liquid probiotic *Lactobacillus* sp. in the animal feed could provide a balance of microflora by increasing the number of lactic acid bacteria colonies, decreasing the colony numbers of *Salmonella* and *Eschericia coli*, increasing the activity of protease enzymes and lipase, as well as increasing the number and surface area of the intestinal villi [22].

Liquid probiotics in this study were containing the amount of total plate count (TPC) from liquid probiotics consumed of 1.5×10^6 cfu/liter for each bacteria type of the *Lactobacillus casei* and the *Saccharomyces cerevistae*, respectively. The amount of TPC from liquid probiotics consumed by each animal was 1.0×10^6 cfu/liter for bacteria type of the *Rhodopseudomonas palustris*. One of the criteria and characteristics of probiotics was that they survive in the high populations and were able to live in the digestive tract [14].

The liquid probiotics used during the study met the bacterial population standard with a minimum of 10^7 cfu/g [6]. In order to maintain viability and to be active in defending against stomach acid, hydrolytic enzymes and bile salts in the small intestine, the recommended dose of probiotics was 10^6-10^8 cfu/ml [14]. These bacteria were classified as probiotic microbes for ruminant livestock [23]. Bacteria type of the *Lactobacillus casei* improves intestinal microflora because it can live in the intestines [14].

Based on the results of the *in vitro* research, it showed the ability of *Rhodopseudomonas palustris* in inhibiting the growth of pathogenic microbes [14]. In addition, *Rhodopseudomonas palustris* was tolerant to bile salts and able to live up to temperatures of 100°C [24]. Therefore, the supplementation of liquid probiotics to ruminant animal, especially beef cattle in this study did not only provide solutions in terms of nutrition increasing beef cattle production, but also provided the benefits in terms of animal health and a sustainable green environment.

3.2. Effect of liquid probiotics on animal feed consumption

In general, the levels of liquid probiotic supplementation did not significantly affect the levels of the nutrient consumption of the forage feed and URPS-Block (Table 4). Factors influencing the animal consumption were feed characteristics, environment and animal condition [25]. Factors influencing feed consumption were palatability, energy level, protein level, forage composition and environmental temperature [26]. The previous research giving probiotics to ruminants had no real effect on feed consumption substances either at the start of the study or at the end of the study [27].

In this study, the liquid probiotic supplementation in the forage feed did not affect significantly the DMC (P>0.05) of the animals. However, the liquid probiotic supplementation in forage feed affected significantly the DWC (P<0.05) of the animals. This study might indicate that the increasing level of the liquid probiotic of 4 cc and 6 cc per liter of fresh water affected significantly DWC of 18.56 percents from DWC using forage feed without supplementation of the liquid probiotic. The amount of DWC by animals were very crucial factor in the digestion process of nutrients in rumen of ruminant animals [5].

Previous research showed that giving yeast of feeding animal did not have a real effect on dry matter consumption [28]. However, giving liquid probiotics to animal livestock tended to be better in increasing consumption of dry matter and drinking water compared with giving treatment of control without liquid probiotics in this study. This could reveal that liquid probiotics have a sour aroma, as the result of fermentation affecting the animal appetite [5].

Feed consumption was influenced by the taste and aroma of the feed [29]. In addition, drinking water consumption showed significantly different results (P<0.05) (Table 4). Animal's drinking water consumption was influenced by the trend of increasing level of dry matter consumption of 4 cc/L fresh water. In addition, drinking water consumption could be influenced by salt, sodium bicarbonate and feed protein content [30]. High feed fiber content will also trigger an increase of drinking water consumption due to water loss through feces [29]. Because the supplementation of liquid probiotics tended to have a real effect on increasing dry matter consumption, the level of drinking water consumption also showed significantly increasing results (P<0.05) as shown in Table 4.

3.3. Effect of liquid probiotic on average daily gain and feed efficiency

The results of the analysis of variance showed that the liquid probiotic supplementation treatment generally differed significantly in the average daily gain (ADG) of cattle body weight and there was a trend towards increasing body weight gain in the liquid probiotic supplementation treatment levels (Table 4). The results of analysis of variance showed that the liquid probiotic supplementation treatments generally differed significantly (P<0.05) in the average daily gain (ADG) of the animal and there was a trend towards increasing body weight gain in the liquid probiotic supplementation treatments generally differed significantly (P<0.05) in the average daily gain (ADG) of the animal and there was a trend towards increasing body weight gain in the liquid probiotic supplementation

treatment (Table 4). The ADG of T0 treatment feed without liquid probiotic was 0.38 kg/animal/day. However, the ADG of T1 treatment feed added by liquid probiotic of 2 cc/L fresh water was 0.51 kg/animal/day, and the ADG of T2 treatment feed added by liquid probiotic of 4 cc/L fresh water was 0.56 kg/animal/day. The supplementation of liquid probiotic of 2 cc/L and 4 cc/L fresh water supplemented in the forage feed increased the ADG of 34.21 and 47.37 percents, respectively in animals.

Higher average daily gain in liquid probiotic supplementation of 4 cc/liter fresh water were also indicated linearly by increasing the digestibility of feed nutrients and metabolic energy processes in the animal body gain optimally. The liquid probiotics containing good functional bacteria in this study were very effective in degrading the feed nutrients compared to bacteria in the rumen fluid and on the rumen walls. A ration that has a high nutritional content and a good level of palatability can quickly increase animal body weight gain during fattening. Body weight gain was influenced by several factors, including total protein obtained every day, breed of animals, age, genetic condition, environmental conditions, the condition of each individual and management [31].

Results of variance analysis showed that liquid probiotic treatments on animals used in this study performed feed conversion average values ranging from 8.71 to 10.18 of cattle animals showing high significant differences (P<0.05) with control treatment. In this study, increasing levels of the liquid probiotic supplement of 2 cc dissolved per liter of fresh water to 6 cc dissolved per liter of fresh water decreased significantly (P<0.05) animal's feed conversion, ranging from 9.12 to 8.71 in cattle animal compared with control of 10.18 (Table 4). This study indicated that the forage ration with liquid probiotic supplement of 2 cc to 6 cc, both dissolved in 1 liter of fresh water could change 9.12 to 8.71 kg forage feed of mixing king grass dry matter to produce 1 kg meat product. On the other hand, animals fed the ration without supplementation of the liquid probiotic of 2 cc to 4 cc dissolved per liter of fresh water were able significantly to increase the production of the cattle animals from 10.47 to 14.44 percents using forage feed with the T1, T2 and T3 treatments, respectively.

3.4. Effect of treatments on the income over feed cost (IOFC)

Economic analysis using the IOFC method was calculated based on the cost of rations during maintenance and the selling price of cattle. It is hoped that the use of liquid probiotics by breeders will be able to increase livestock productivity, both increasing meat production and increasing daily body weight gain. Apart from that, the use of the product by farmers will be sustainable if it provides high livestock production efficiency and provides very profitable economic benefits. The effect of feed used and the economic value expressed in IOFC are shown in Table 5. The factors that have an important influence in calculating IOFC are the average daily gain during period of fattening, ration consumption and ration prices [32].

Variables	Treatments			
	Т0	T1	T2	Т3
Total dried matter feed cost (IDR/animal/day)	10,270	11,910	12,470	12,710
Average daily gain (ADG) (kg/animal/day)	0.38	0.51	0.56	0.57
Price of animal live weight (IDR/kg)	50,000	50,000	50,000	50,000
Price of ADG (IDR/animal/day)	19,000	25,500	28,000	28.500
Income over feed cost (IOFC)	8,730	13,590	15,530	15,790

Table 5 Income over feed cost (IOFC) derived from consumption costs of King grass (*Pennisetum purpureum*), the urea red palm sugar block (URPS-Block), the liquid probiotic supplement and prices of animal live weight and average daily gain

Note: Basic prices of the ingredient feeds at the period of study using the Indonesian currency (IDR): Liquid Probiotics of 1 liter = IDR 50,000.- or Price of 1cc liquid probiotics = IDR 50.-; Ingredients and processing costs of 1 kg URPS-Block = IDR 7,000.-; Price of 20 kg king grass (P. purpureum) with 20% dried matter content = IDR 8,000.- or Price of 1 kg king grass dried matter = IDR 2,000.-.

IOFC is a metric that links nutrition to profitability [33]. The IOFC variable is a decision-making tool for feeding and evaluating changes in feeding programs. The basic definition of IOFC is the money left over for other expenses such as labor and maintenance after the cost of feeding the animals in production. This can be done by group, pen or even by individual animal [33]. The use of liquid multi-microbe probiotics in drinking water could increase the IOFC of the farmers. The increase in IOFC was a result of an increase in daily body weight gain of the animals. As explained in the

IOFC (Table 5), the liquid probiotic supplementation of 2 cc/liter, 4 cc/liter and 6cc/liter of fresh water could produce the IOFC value of IDR 13,590.-; 15,530.- and 15,790.-/animal/day, respectively. Meanwhile, in the control treatment, the IOFC value was IDR 8,730.- / animal /day.

The supplementation of liquid probiotics of 2 cc/liter, 4 cc/liter and 6 cc/liter of fresh water provided economic benefits of IDR 13,590.- to IDR 15,790.-.-/animal/day, equivalent to 55.67 to 80.87 percents greater than the IOFC of the control treatment without liquid probiotic supplementation provided economic benefits of IDR 8,730.- (Table 5). This means that low ration costs do not always provide large profits. Even though the cost of rations was high, with a high increase in animal body weight, the maximum benefits can be obtained. The liquid probiotic supplementation of 2.0 cc/L fresh water (T1), 4.0 cc/L fresh water(T2) and 6.0 cc/L fresh water(T3) showed a tendency of increasing nutrient consumption, especially dried matter consumption (Table 4) compared with the controls without liquid probiotic utilization (Figure 3).



Figure 3 Trends of the dried matter consumption (DMC) of forage feed, drinking water consumption (DWC), average daily gain (ADG), feed conversion (DMC/ADG) and income over feed cost (IOFC) in each treatment of the control (T0) without liquid probiotic, levels of liquid probiotic 2.0 cc/L fresh water (T1) and 4.0 cc/L fresh water(T2).

The supplemented levels of 4 cc and 6 cc liquid probiotics were more capable of increasing significantly (P<0.05) drinking water consumption (DWC) compared to controls. This case could be the fact that supplement liquid probiotics has greater digestibility of the forage feed compared with those without liquid probiotics supported by significantly increasing (P<0.05) the DWC. The prominent cases showed that the liquid probiotic supplementation produced significantly (P<0.05) average daily gain (ADG) and drinking water consumption (DWC) of the animals. These cases might reveal that DWC was needed more for supporting feed nutrient and metabolic energy digestion in the formation process of animals' daily weight gain [28].

The above cases indicated also that the increasing dose of 4 cc liquid probiotic (T2) in animal feeding could tend to increase consumption of the nutrients and drinking water consumption, resulting the significantly high increase of ADG in cattle animals. The increasing nutrients in dried mater consumption may indicate the animal ability to increase nutrient utilization in the process of tissue replacement and new formation in animal body metabolisms reflecting the animal higher growth and body weight gain [6, 28]. The results of this study were in agreement with the previous study reporting that feed characteristics influencing consumption were including the chemical content, feed form and feed nutrient digestibility [34].

4. Conclusions

Supplementation of the liquid multi-microbe probiotics of 2 to 4 cc/liter fresh water with the URPS-Block of 350 g/day increased significantly production of the cattle as shown by higher average daily gain (ADG) of 510 to 560 g, equivalent

to 34.21 to 47.37 precents increasing ADG under an environmentally friendly way, compared with those control forage ration without supplementation of the liquid probiotics producing ADG of 380 g. The increase of animal production was supported linearly by the predicted increasing digestibility of the nutrient contents in the forage feeds supplemented with the liquid probiotic levels of 2.0 cc/L to 4.0 cc/L in the drinking fresh water of animals. Cattle supplemented with the level of 2 cc to 4 cc liquid probiotic per liter fresh water per animal per day produced income over feed cost (IOFC) ranging from IDR 13,590.- to IDR 15,530.-/animal/day, equivalent to 55.67 to 77.90 percents increasing IOFC, compared with those control forage ration without supplementation of the liquid probiotics producing IOFC of IDR 8,730.- /animal/day.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

All authors declare and confirm that the version of the submitted manuscript have been read and approved.

References

- [1] Fuller, R. (1999). Probiotics for farm animals. Probiotics: A Critical Rev., 15-8: 15-22. ISBN: 1898486.
- [2] Zhang, L., Zhang, X. & Zhan, Z. (2016). Effects of dietary supplementation of probiotic, *Clostridium butyricum*, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with *Eschericia coli* K88. Journal of Animal Science and Biotechnology. <u>DOI 10.1186/s40104-016-0061-4</u>
- [3] Forte, C., Acuti, G., Manuali, E., Casagrande, P., Pavone, S., Trabalza, M., Moscati, L., Onofri, A., Lorenzetti, C. & Franciosini, M.P. (2016). Immunology, health and Disease. Effects of two different probiotic on microflora, morphology and morphometry of gut in organic laying hens. Poultry Science Association Inc.
- [4] Krehbiel, C.R., Rust, S.R., Zhang, G. & Gilliland, S.E. (2003). Bacterial direc feed microbials in ruminant diet: perpormance response and mode of action. *Journal of Animal Science* 81 (E.Sppl.2): E120-132. <u>https://doi.org/10.2527/2003.8114 suppl 2E120x</u>
- [5] Liong, M.T. & Shah, N.P. (2005). Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol coprecipitation ability of Lactobacillus strains. *International Dairy Journal. Vol.* 15: 391-398. https://doi.org/10.1016/j.idairyj.2004.08.007
- [6] Widyastuti, Y. (2008). Silage Fermentation and Probiotics Benefit of Silage to the Ruminants (*Fermentasi Silase dan Manfaat Probiotik Silase bagi Ruminansia*). Media Peternakan, 31 (3): 225-232. https://repository.ipb.ac.id/bitstream/handle/123456789/43188/Y.%20Widyastuti.pdf
- [7] Eryani, E., Fenti, F., Muswita, M. & Yelianti, U. (2020). Effect of Green Fertilizer of Gamal Plant (*Gliricidia sepium* (Jacq) DC.) on the Growth of Duku (*Lansium domesticum* Corr.). International Journal of Ecophysiology, Vol. 02, No. 01:55 61. <u>https://talenta.usu.ac.id/ijoep/article/view/3989/2745</u>
- [8] Broberg, A., Jacobsson, K., Ström, K. & Schnürer, J. (2007). Metabolite profiles of lactic acid bacteria in grass silage. Applied Environmental Microbiology, 73: 5547-5552. doi: 10.1128/AEM.02939-06
- [9] Preston, T.R. & Leng, R.A. (1985). Matching livestock systems to available feed resources. ILCA, Addis Ababa. <u>ISBN</u> 0958829012, 9780958829014

- [10] Elliot, R. & Amstrong, D. G. (1982). The effect of urea and urea plus sodium sulphate on microbial protein production in rumen of sheep given diets high in alkali-treated barley straw. *Journal of Agriculture Science, 99, 51-60.* DOI: <u>https://doi.org/10.1017/S0021859600055027</u>
- [11] Paputungan, U., Manopo, J.H., Agustinus, L. & Kartini, M. (2015). Study of Indonesian Ongole-crossbred cattle production and income over feed cost for supplements of agricultural waste product formulated in the urea palm sugar block. Livestock Research for Rural Development 27 (8): #167. https://www.lrrd.org/lrrd27/8/papu27167.htm
- [12] Kaunang, Ch. L., Pudjihastuti, E., Anis, S.D., Leke, J.R., Rumokoy, L.J.M. & Paputungan, U. (2023). Response of cattle fed with ammoniated maize stover, *Indigofera zollingeriana* leaves and Urea Palm Sugar Block (UPSB) supplementation. Livestock Research for Rural Development 35 (4): #34. http://www.lrrd.org/lrrd35/4/3534char.html
- [13] Paputungan, U., Manopo, J.H. & Utiah, W. (2018). Predicting live weight of Indonesian Local-Bali cattle using body volume formula. Livestock Research for Rural Development 30 (8): # 144. https://www.lrrd.org/lrrd30/8/umarf30144.html
- [14] Persada, S. (2022). Quality and Efficacy Testing of Bio feed Probiotics in Effective Microorganisms Feed Additive: A Liquid Multiple-microbe Probiotics for Animal Husbandry. Research Report. Songgolangit Persada (SLP).
- [15] Steel, R.G.D. & Torrie, J.H. (1993). Principles and Procedures of Statistics: Biometric Approach (Prinsip dan Prosedur Statistika: Pendekatan Biometrik). Sumantri B, Penerjemah. Jakarta (ID): Gramedia Pustaka Utama.
- [16] Savadogo, A., Quattara, C.A.T., Bassole, I.H.N. & Traore, A.S. (2004). Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. Pakistan Journal of Nutrition 3: 174-179. <u>https://scialert.net/fulltext/fulltextpdf.php?pdf=ansinet/pjn/2004/174-179.pdf</u>
- [17] Kumar, P. & M S Nain, M.S. (2010). Global warming and agriculture issues and strategies. Research Journal of Aanimal Science. Vol. 1 (3): 34-46.
- [18] Gollop, N., Zakin, V. & Weinberg, Z.G. (2005). Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. Journal of Applied Microbiology, 98: 662-666. <u>https://doi.org/10.1111/j.1365-2672.2004.02504.x</u>
- [19] Roberfroid, M.B. (2000). Prebiotics and probiotics: are they functional foods? American Journal of Clinical Nutrition, 71 (suppl): 1682S-1687S. DOI: <u>10.1093/ajcn/71.6.1682S</u>
- [20] De Vuyst, L. & Leroy, F. (2007). Bacteriocins from lactic acid bacteria: production, purifi cation, and food applications. Journal of Molecular and Microbiological Biotechnology, 13: 194-199. DOI: <u>10.1159/000104752</u>
- [21] Filya, I. (2003). The effect of *Lactobacillus buchneri,* with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. Journal of Applied Microbiology, 95 (5): 1080-1086. <u>https://doi.org/10.1046/j.1365-2672.2003.02081.x</u>
- [22] Izzuddiyn, M., Busono, W. & Sjofjan, O. (2018). Effects of Liquid Probiotics (*Lactobacillus* sp.) on Microflora Balance, Enzyme Activity, Number and Surface Area of the Intestinal Villi of Broiler. Jurnal Pembangunan dan Alam Lestari, Vol. 19, No.2. doi:10.21776/ub.jpal.2018.009.02.04
- [23] Sandine, W.E., Muralidhara, K.S., Elliker, P.R. & England, D.C. 1972. Lactic acid bacteria in food and health: a review with special reference to enteropathogenic Escherichia coli as well as certain enteric disease and their treatment with antibiotics and lactobacilli. Journal of Milk Food Technology. 35:691-702.
- [24] Nguyen, V.H., Maguyon-Detras, M.C., Migo, M.V., Quilloy, R., Balingbing, C., Chivenge, P. & Gummert, M. (2020). Rice Straw Overview: Availability, Properties, and Management Practices. In book: Sustainable Rice Straw Management. License. <u>CC BY 4.0</u> DOI: <u>10.1007/978-3-030-32373-8 1</u>
- [25] Fuller, R. (1992). The effect of probiotics on the gut micro-ecology of farm animals. The lactic acid bacteria, 1:171-192.
- [26] Cheeke, P.R. (1998). Applied Animal Nutrition. Volume ke-2, Feed and Feeding. New Jersey (USA): Prentice Hall, Upper Saddle River.
- [27] Hau, D.K., Nenobais, M., Nulik, J. & Katipana, N.G.F. (2005). Effect of Probiotics on the Digestibility of Rumen Microbes in Bali Cattle (*Pengaruh Probiotik Terhadap Kemampuan Cerna Mikroba Rumen Sapi Bali*). National Seminar on Animal Husbandry and Veterinary Technology. East Nusa Tenggara Agricultural Technology Research Center. Universitas Nusa Cendana, Kupang. Bali, Indonesia.

- [28] Antonius, A. (2009). Potential of Probion Fermented Rice Straw as a Feed Ingredient in Simmental Cattle Rations. National Seminar on Animal Husbandry and Veterinary Technology. Goat Research Workshop, Po Box 1 Sei Putih, Galang 20585, Sumatera Utara, Indonesia.
- [29] Lee, S.J., Shin, N.H., Oka, J.U., Jung, H.S., Chu, G.M., Kim, J.D., Kim, I.H. & Lee, S.S. (2009). Effects of Dietary Synbiotics from Anaerobic Microflora on Growth Performance, Noxious Gas Emission and Fecal Pothogenic Bacteria Population in Weaning Pigs. *Asian-Australasian Journal of Animal Science* 22(8):1202-1208.
- [30] Harris, B.Jr. & VanHorn, H.H. (2003). Water and Its Importance to Animals. Jurnal Media Peternakan 3(3):166-171.
- [31] National Research Council [NRC]. (2001). *National Research Council Nutrient Requirement of Dairy Cattle. 8th Edition*. Washington DC (USA): National Academic of Science.
- [32] Kasim, S.A. (2002). Performance of local sheep given a complete ration made from rice straw and agro-industrial waste product treated with rumen fluid (*Performa domba lokal yang diberi ransum komplit berbahan baku jerami dan onggok yang mendapat perlakuan cairan rumen*). Research Report. Fakultas Peternakan, Institut pertanian bogor, Bogor.
- [33] Hein, D. 2023. Reinventing ration cost evaluation: Utilizing income over feed cost. Copyright @ 2024 Progressive Publishing.
- [34] McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A. (2002). Animal Nutrition. 6th Edition. New York (USA): Ashford Colour Press Ltd.