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Dynamics of rumen microbial population in goats (*Capra hircus*) supplemented with buffered banana (*Musa balbisiana*) blossom pellets

Cañete, Roberto Jr., N.[1], Constantino, Mark James M.[1], Delmonte, Meg Aui O.[1]
[1]*College of Agriculture and Allied Sciences, Northern Negros State College of Science and Technology*
Corresponding email: rncanete@nonescost.edu.ph

ABSTRACT

Increased efficiency in ruminant production through rumen manipulation have been of great interest to animal nutritionist, practitioners, and researchers. This study sought to understand the effect of Buffered Banana Blossom Pellets (BBBP) on rumen pH and population dynamics of ruminal bacteria and protozoa. Four treatments with four replications were randomly allocated to sixteen goats aged 4 to 6 months old. Data were analyzed using Analysis of Variance (ANOVA) in Randomized Complete Block Design (RCBD) in Statistical Tool for Agricultural Reaserch (STAR) version 2.0.1.. Treatment 1 (control), Treatment 2 (1% BBBP + basal diet), Treatment 3 (2% BBBP + basal diet), and Treatment 4 (3% BBBP + basal diet) were subjected to a 35 days experiment with the first 7 days establishment period. The result shows an increase in rumen pH. T4 with 3% BBBP obtained the highest increase in pH. The highest reduction in the ruminal bacterial count was observed in T1 with -95%. But an increase in the bacterial count was observed in T2 with 1% supplementation of BBBP. A decrease in the ruminal protozoal count was also observed in T1 with -3.01%. In comparison, there is an increase in protozoal count in treatments with the supplementation of BBBP. The nutrient content of Buffered Banana Blossom Pellets revealed a high content of minerals that neutralize the rumen ecology. It has protein (5.4%), fiber (24.8%), fat (1.5%), and carbohydrates (31.8%). It was therefore concluded that supplementation of BBBP, which is high in minerals, could buffer the rumen environment and a subsequent improvement in the population of ruminal protozoa and improvement of ruminal bacteria at 1% BBBP supplementation.

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INTRODUCTION

Ruminant meat and milk are key agricultural commodities and vital protein source for humans. Ruminant farming has a high economic value and is essential for food security across many regions in the world (Morgavi et al., 2013; Mizrah et al., 2021). Ruminants' gut like goat, sheep, buffalo and cattle are built different from monogastric animal like swine and horse. The first compartment in the digestive system of the ruminant is the rumen (Sauer et al., 2012), where it is occupied by microorganisms which comprises bacteria, protozoa, fungi, and archaea (Enjalbert et al., 2017). Ruminants do not manufacture cellulolytic or hemicellulolytic enzymes to break down the plant matter they consume, like all mammalian herbivores. Instead, they depend on symbiotic relationships with bacteria, fungus, and protozoa in the rumen to carry out this role (Gruninger et al., 2019). The host eats plant fibers that can only be broken down by rumen-associated microorganisms through a series of intricate metabolic pathways (Morais & Mizrahi, 2019). Host animal energy is dependent on this microbiota as they are responsible to carbohydrate fermentation, protein breakdown and microbial protein synthesis in an anaerobic condition (Malmuthe & Guan, 2017; Enjalbert et al., 2017). The host ruminant's immune system, general production effectiveness, and maintenance depend on the complex network of symbioses that make up the rumen microbiome (Cammack et al., 2018).

Furthermore, anaerobic fermentation of low-quality fibrous plant material performed by microorganisms produces input metabolites to synthesize volatile fatty acids. The volatile fatty acids (VFAs) are absorbed by the host animal and utilized as an energy while excess compounds like ammonia, carbon dioxide and molecular hydrogen are being utilized by other microbes (Calsamiglia et al., 2007). Such rumen methanogens use carbon dioxide and hydrogen to obtain their own energy, which produces methane as a waste by-product (Llonch et al., 2017). This methane compound will be expelled from the animal's gut via respiration that contributes to the atmospheric buildup of greenhouse gasses (Doyle et al., 2019). Dietary intervention may influence the dynamics and abundance of microbes in the rumen, and these different types of microbiota may inhibit or favor the generation of methane (Gruninger et al., 2019). The possibility exists to improve ruminants' lifetime performance and welfare while simultaneously reducing their environmental effect through dietary intervention that extend their productive lifespan (McGrath et al., 2018). Continued increases in production efficiency are necessary as the livestock business struggles to satisfy the demands of an expanding human population by producing extra pound of milk and meat. Rumen microbiome research has entered a new phase with the introduction of new, large-scale genetic tools (Cammack et al., 2018). Therefore, we must deepen our comprehension of the fundamental mechanisms at play in order to properly regulate and fully utilize the ruminal microbiota for the benefit of mankind as well as ruminant animals in the long run (Mackie & White, 1990). In this study, the researcher explored the mechanisms limited only on the rumen microbial dynamics in response to the buffered banana blossom pellets.

OBJECTIVES

This research seeks to analyze the population dynamics of rumen microorganisms in goats (*Capra hircus*) in response to the varying level of banana blossom buffered pellets. Specifically, the goal of this study is to determine the rumen pH; ascertain the protozoal and the bacterial count as colony-forming units (CFU) from the rumen sample. Ruminal pH may dictate what microorganisms can thrive, as their growth and survival may be influenced by the rumen's ecology's acidity or alkalinity. (Franzolin & Dehority, 2010).

METHODS

Together with diet, host genotypic structures of animal may influence the dynamics of rumen microorganisms. Genes of different breed could either inhibit or favor the growth of certain microorganism (Pararillo et al., 2014). Different stages of gut development may also modify the abundance nof microorganisms. Hence, this study utilized sixteen (16) goats of the same breed aged four (4) to six (6) months of which the same method was adapted from Delmonte and Bestil (2017), ensuring an unbiased result.

The feeding trial was timed for thirty-five (35) days. Buffered pellets (pelleted banana blossom) were used as a treatment fed on the ruminant plus the basal diet. The basal diet or the dry matter requirement (DMR) of goat is 3% of their body weight as described by the United States Department of Agriculture (2010). Basal diet comprised a 50% forage, 30% sugarcane tops, 10% pollard feeds, 5% fresh leaves of Madre de Cacao (*Gliricidia sepium*), and 5% molasses. Preparation of banana blossom meal was adapted from the procedure of Pillones (2018).

Experimental Design

The goats were divided into four treatments at random with four goats per treatment, considered as four (4) replications. Randomized Complete Block Design (RCBD) was employed in the experimental design, where age was used as a block. Trt 1 (Basal diet or control), Trt 2 (Basal diet plus 1% buffered pellets of DMR), Trt 3 (Basal diet plus 2% buffered pellets of DMR), and Trt 4 (Basal diet plus 3% buffered pellets of DMR).

Statistical Tools

All date obtained was analyzed using Analysis of Variance (ANOVA) in RCBD using Statistical Tool for Agricultural Research (STAR). To test the level of significance in comparing means among treatments at 0.05 level of significance was used.

Procedure

The buffered pellets were given to the animals twice each day as fed, at 8:00 am in the morning and 3:00 pm in the afternoon.

The schedule of activities are as follows:

Days	Activities
1	The measure of the initial weight of goats. Collect rumen fluid to measure the initial rumen pH.
1-7	Adaptation period. Establishing *ad libitum* intake of buffered pellets.
8	Evaluate of goat weight.
21	Collect rumen fluid. Measure the rumen pH.
35	Evaluate the goat weight. Collect the rumen fluid. Determining the rumen pH.

Feeding the Experimental Animals

Experimental animals were fed two times daily with buffered pellets. Feeding was done at 8:00 a.m. and 3:00 p.m. Period of adaptation to the diet will last for seven days wherein feed supplied and feed refused were counted to determine voluntary *ad libitum* consumption. The experiment proper was conducted after the seven-day establishment period which is included in the 35-day experiment. It was excluded in the 35-day trial the preparation of the materials needed in the conduct of the study. Clean drinking water was consistently made available to the animals throughout the course of the experiment at their discretion.

Collection of rumen fluids

Rumen fluid was collected using a 50 ml plastic syringe inserted to the mouth and into the stomach through the esophagus. Prior to insertion, tube was lubricated using oil to minimize gut stress to the animal. Immediate measurement of rumen pH was made using digital pH meter.

Bacterial Counting

Using a 5 ml pipette, 1 ml rumen sample were taken and diluted into a series of dilution up to 1:1 000 000. Standard Plate Count (SPC) each milliliter of the test sample was calculated applying the "pour plate technique", performed by aspirating 1 milliliter of sample for every dilution into sterile Petri dish. Petri plate contained 15ml of liquefied MRS Agar growth medium heated at 45°C, to culture any bacterial cell present in the sample. Petri plates were then put in the incubator under the anaerobic conditions at 40°C with a BD GasPak anaerobic container bag for twenty-four hours to ensure anaerobic condition. This is to mimic the anaerobiotic ecology of the digestive tract of the animal. Duplicate analyses of each treatment replicate will be performed. A colony counter will be used to count any bacterial colonies that have grown after 24 hours. The following equation was used to calculate colony-forming units (CFU)/ml:
Cfu/ml = number of bacterial colonies x dilution rate.

Protozoal counting (dilution, sample reduction and staining)

Using a 5 ml pipitte, 1 ml rumen sample were taken into the test tube and added with 3 drops of Lugol's solution and let stand with at least 15 minutes. This is to stain any protozoal structure present in the rumen sample. Afterwards, it is diluted with 9 ml distilled water containing a 30% glycerol solution. Because of its high viscosity, the 30% glycerol solution was chosen to prevent protozoa from rapidly settling whenever subsamples are pipetted for counting. The 1ul of the diluted test sample was pipetted into a Sedgewick rafter counting chamber by a wide-orifice pipette and were analyzed in a digital microscope connected to a monitor that uses digital camera instead of using an eye piece. At a 10x magnification, the number of protozoa were tallied. On the whole chamber surface, all grids that are evenly spaced apart were tallied. The total protozoa per millimeter of diluted rumen liquid were calculated by multiplying the total protozoa count by the constant number of 10 and the dilution factor, which is 20. All procedures were adopted from Reproto & Bestil (2016).

Data gathered

1.Dry Matter Intake (DMI) of Buffered Pellets in grams. Using the formula: DMI= Voluntary Feed Intake x % of Dry Matter in feed.
2.The pH of rumen samples extracted from the ruminant.
3.Bacterial count (cfu/ml). Determined as colony forming units per ml rumen fluid (cfu/ml) using the formula: cfu/ml= number of bacterial colonies x dilution rate.
4.Protozoal count. Population of protozoa per milliliter of rumen fluid were measured using the equation. Protozoal count= Number of Protozoa Counted x Total magnification x Dilution Factor.

Total Magnification= Ocular lens x objective lens
Where: 67= ocular lens
40= objective lens
10= dilution factor

RESULTS AND DISCUSSION

Change in rumen pH

Table 1 shows that the average rumen pH in goats per treatment at day zero (0) ranges from 5.45 to 5.675, which is statistically not significant (0.6132). On day twenty-one (21), the average rumen pH in goats per treatment ranges from 6.325 to 6.55, which is statistically not significant (0.4071). Although the result in

treatment 3 (2% BBBP of DMR) is not significant, it obtained the highest increase in pH on day 21. On day thirty-five (35), the mean rumen pH in goats varies from 6.175 to 6.45, which showed no significant difference (0.3573). Treatment 4 (3% BBBP of DMR) obtained the highest increased in rumen pH on day 35. Regarding rumen pH fluctuations, the measured rumen pH ranged from 6 to 7, which is favorable for rumen microbial development and activity (Adebayo et al., 2017). The observed rumen pH was due to the buffering effect of BBBP (Wanapat et al., 2018). According to the findings of Kang and Wanapat (2013), the increased rumen pH caused by BBBP administration causes an increase in bacterial, protozoal, and fungal community in ruminants particularly, buffalo and cattle.

Table 1: Changes in rumen pH in goats supplemented with Buffered Banana Blossom Pellets (BBBP) on different days of the feeding experiment

Rumen pH	T1 CON	T2 1% DMI	T3 2% DMI	T4 3% DMI	cv (%)	P-Value
Day 0	5.45	5.625	5.675	5.625	3.44	0.6132^{ns}
Day 21	6.4	6.4	6.55	6.325	3.55	0.4071^{ns}
Day 35	6.175	6.175	6.325	6.45	3.83	0.3573^{ns}

CON= control
DMI= dry matter intake
ns- not significant
cv- coefficient variance

The study observed an enhanced rumen pH in goats after the supplementation of BBBP. Previous investigations showed that ruminants could benefit from using banana blossom as a rumen enhancer (Kang & Wanapat, 2013). Moreover, the authors reported that the effect of banana blossom to neutralize the rumen ecology could be seen more in ruminants fed on high concentrates. A abrupt decreased in the pH of rumen to below the ideal level occurs when animals are fed an excessive amount of quickly fermentable carbohydrates due to the buildup of volatile fatty acids or acid-associated microbes in the paunch (Kang et al., 2017). During the seven (7) days adaptation period, all treatments were exposed to the 20% inclusion of Buffered Banana Blossom Pellets (BBBP) of DMR. That being the case, alterations in rumen pH over 6 were noticed, which is also in line with the conclusions of Wanapat et al.(2018). However, treatment one (1) or the control group at day twenty-one (21) were able to maintain pH above 6 despite the absence of BBBP after the establishment period of seven days. Franzolin and Dehority (2010) concluded that feeding pattern, frequency and diet preference will have a important affect on rumen pH and a subsequent influence on the rumen microbiota. In addition, the current investigation has a high ratio of roughage than concentrates and is a probable reason why pH was maintained. This is because a high roughage ratio limits the amount of rumen-degradable organic matter and, as a result, slows down the synthesis of volatile fatty acids (VFAs), which raises the rumen pH (Jiang et al., 2017). The ruminal pH is represented by a collective relative levels of buffers, bases, and acids. Raising ruminal intake of basic mineral or neutralizers from feed or saliva will stop the rumen's pH from dropping (Kang et al., 2014). In general, BBBP incorporation enhance ruminal pH to ideal range, which enhanced rumen function and nutrient digestibility (Kang & Wanapat, 2013, Kang et al., 2014, 2015, 2017).

Nutrient composition of BBBP

Buffered Banana Blossom Pellets (BBBP), as shown in Table 2, contains protein (5.4%), fiber (24.8%), carbohydrates (31.8%), and fat (1.5%). It has a moisture content of 15.3% and is high in ash at 21.3%.

Table 2: Nutrient composition of Buffered Banana Blossom Pellets, analyzed in Negros Prawn Producers Cooperative Analytical And Diagnostic Laboratory (2022).

	Percent (%)	Grams (g.)	Milligrams (mg.)

Protein	5.4	-	-
Fiber	24.8	-	-
Fat	1.5	-	-
Carbohydrates	31.8	-	-
Sugar	-	3	-
Sodium	-	-	98
Potassium	-	-	1650
Moisture	15.3	-	-
Ash	21.3	-	-

High in ash indicates that the BBBP was rich in minerals (Adebayo et al., 2017). The present study observed that BBBP contains minerals like sodium and potassium, and the conclusions of Wanapat et al.(2018) concurred with this, that banana blossom is high in Ca, Mg, K, and Na as basic cations which could neutralized and maintained the rumen pH leading to improved ruminal fermentation efficiency. BBBP, that is rich in ratio of mineral elements, has been integrated as a rumen buffering agent source in the early investigation of Kang and Wanapat (2013) and Kang et al. (2014, 2015, 2017). Kang et al.(2015) concluded that the chemical supplements have a harmful aftereffect and were outlawed globally at the beginning of 2006 for safety grounds. As a result, plants with high mineral content are used like banana blossom as a rumen neutralizing agent as an option to replace bicarbonates in ruminants.

Changes in population dynamics of ruminal bacteria and protozoa

Table 3 shows that there were changes in both bacterial and protozoal counts. Initial and final bacterial count in all treatments was found statistically not significant, with a p-value of 0.0725 and 0.1763, respectively. Initial protozoal count in all treatments was highly significant, with a p-value 0.0033 lower than the 0.01 level of significance. At the same time, the final protozoal count was found to be statistically significant, with a p-value of 0.0421 lower than the 0.05 level of significance.

Table 3: Changes in ruminal bacteria and protozoa counts in goats supplemented with BBBP during and after the feeding experiment.

	Bacteria (1x 10^6 CFU/ml)			Protozoa (1 x 10^4/ml)		
	Initial (D 21)	Final (D 35)	Percent Change	Initial (D 21)	Final (D 35)	Percent Change
T1	3.900	2.250	-95	3.752	3.752	-3.01
T2	2.750	2.875	5.00	3.819	5.159	25.52
T3	2.375	3.000	-24.88	2.412	5.427	56.42
T4	2.750	2.750	-49.58	2.211	5.159	56.48
P-value	0.0725ns	0.1763ns	0.0926ns	0.0033**	0.0421*	0.0002**

***-highly significant*
** - significant*
ns- not significant

The microbial population's activity enables the ruminants to get their energy from plant sources (Nur-Atikah et al., 2018). Bacteria make up around 95% of the entire biomass of rumen microbes and are the most numerous and varied group of rumen microbes (Bainbridge et al., 2016) While the rumen ecosystem contains more varied bacteria than protozoa, (10^{10}-10^{11} cells/mL vs. 10^4-10^6 cells/mL, respectively), but for the reason that they are larger (protozoa: 10–200 μm, bacteria: 0.5–2 μm), half of the rumen's microbial biomass is composed of protozoa (Bainbridge et al., 2018). The function that bacteria play in ruminant nutrition is crucial since these particular microbes are essential for helping animals thrive on low-quality fibrous forages as well as using diets that are inappropriate for monogastric animals (Nur-Atikah et al., 2018). In addition, protozoa also

account for the degradation of 1/4 of the total fiber (Lee et al., 2000), and are accountable for the significant bacterial protein turnover caused by rumen bacteria's predation (Patra & Saxena, 2009). Lee et al. (2000), observed that the interaction effect of bacteria and protozoa have a subsequent influence and increased cellulose digestion. As a result, protozoa and bacteria worked together in a beneficial way as evidenced by the discovery of greater enzyme activity that support enhanced rumen function.

As seen in Figure 2, the percent change in bacterial count in T1 is -95, T2 with 5.00, T3 with -24.88, and T4 with -49.58, which is statistically not significant with a P-value 0.0926 higher than the 0.05 level of significance. It was observed that treatment supplemented with 1% BBBP gained an increase in bacterial colony counts.

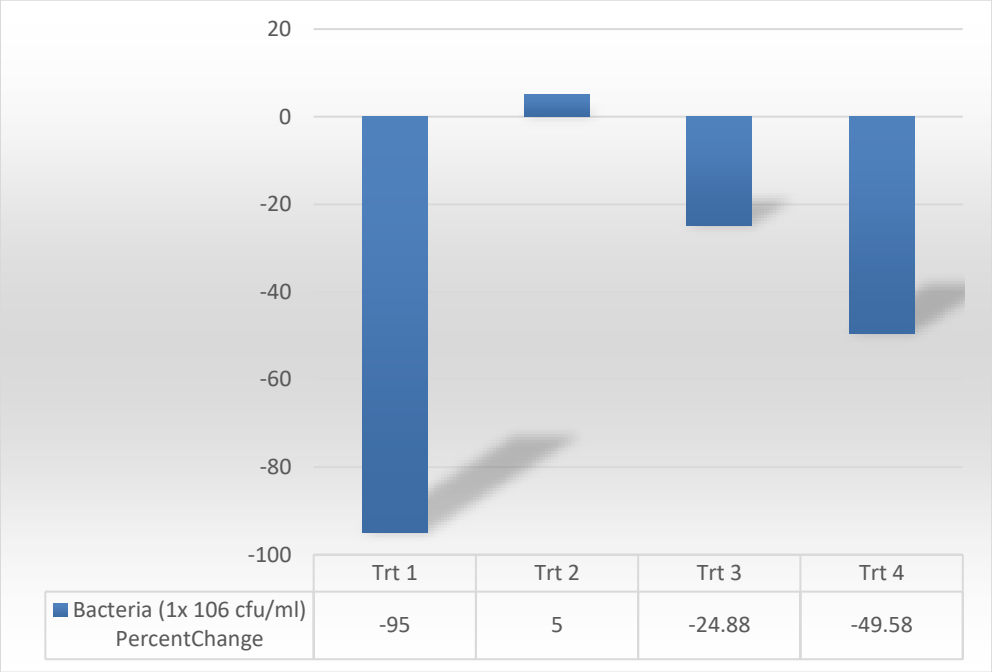

Figure 1. Percent change in bacterial count in all treatments between day 21 and day 35

In addition, the percent change in protozoal count in T1 is -3.01, T2 with 25.52, T3 with 56.42, and T4 with 56.48, which is statistically highly significant with a p-value 0.0002 lower than the 0.01 level of the significance. It was observed that all treatments supplemented with BBBP increase in protozoal count excluded in T1 with negative percent change. Adebayo et al. (2017) found that the bulk of rumen microorganisms are bacteria, which are the primary agents for digesting the carbohydrates in plant cell walls. *Ruminococcus albus*, *Bacteroides succinogens*, and *Butyrivibrio fibrosolvens* are the common fiber eater. These three species of rumen bacteria increased with the supplementation of BBBP (Kang & Wanapat et al., 2013). Regardless of how, rumen cellulolytic digestibility subsides when rumen pH falls below 6.00 pH (Santra et al., 2003) due to the intolerance of rumen bacteria to an acidic environment (Kang et al., 2015). The present expirement shows that the control group has the highest decreased in bacterial count. Factors like low protein cause protozoa to digest bacteria, causing a reduction in bacterial biomass (Adebayo et al., 2017). Furthermore, bacterial protein degradation by protozoa happened every hour, based on the findings of Belanche et al. (2012).

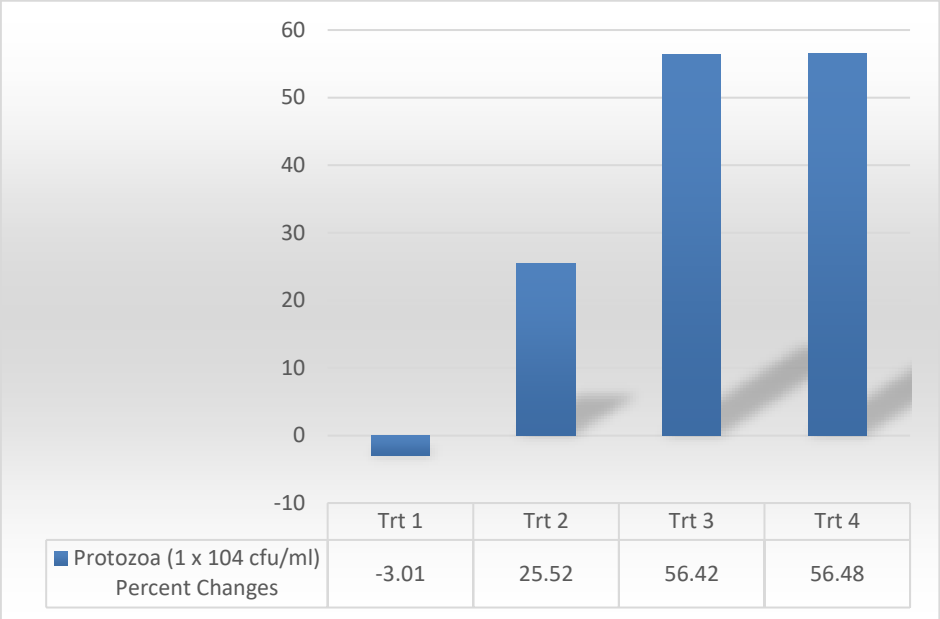

Figure 2. Percent change in protozoal count in all treatments between day 21 and day 35

Franzolin and Dehority (2010) reported that protozoal populations tend to decline after high concentrate feeding because the rumen lacks a fibrous floating mat, which is where the protozoa remain attached to grow. However, the present experiment has a high ratio of roughage where there is a supply of fibrous floating mat where protozoa multiply and a probable reason for the increase of protozoal count. In general, rumen protozoal activity and survival is favored by a slightly alkaline pH, but is disrupted when the pH decreases below 6 going to acidic environment (Santra et al., 2003). The enhancement in rumen pH after supplementation of BBBP increases the number of protozoa (Kang and Wanapat et al., 2013). Moreover, the increase in protozoa also has a stabilizing effect by ingesting starch that slows down the fermentation process and the subsequent low production of organic acids (VFA), which prevents to decrease in the pH (Franzolin & Dehority, 2010). The most important ruminal element influencing the population of microbes and their activity is likely the pH of the ruminal content (Adebayo et al., 2017). Enhanced pH led to increasing dry matter digestibility, rumen fermentation, VFA production, and microorganism growth (Kang et al., 2015, Kang and Wanapat, 2013).

SUMMARY

Supplementation of Buffered Banana Blossom Pellets (BBBP) in goats increases the average final rumen to 6.31 from the average initial rumen pH of 5.64. The nutrient composition of Buffered Banana Blossom Pellets revealed a high content of minerals with potassium (1650 mg.), sodium (98 mg.), and ash (21.3%) that neutralizes the rumen ecology. In the composition of ruminal bacteria, only treatment 2 with 1% supplementation of BBBP increased in percent change while treatment 1 gained the highest decrease in percent change. The composition of ruminal protozoa in treatment with no BBBP decreases in percent change, but treatments with BBBP supplementation increases the protozoal count.

CONCLUSION

The dynamics of rumen microbes determine the degradation of feeding materials and the utilization of nutrients that will be used by the host ruminants. The area of concern was the creation of a way to improve rumen efficiency by neutralizing rumen pH and manipulating rumen microorganisms through the supplementation of buffered banana blossom pellets. In conclusion, supplementation of buffered banana

blossom pellets (BBBP) in goats which is high in minerals enhances the rumen pH and a subsequent improvement in the population of ruminal protozoa while improvement of ruminal bacteria at 1% BBBP supplementation.

RECOMMENDATIONS

Three recommendations are made based on the study's findings: the first is to experiment the BBBP at a high concentrates ratio. Concentrate feeds is a readily available carbohydrates that causes a rapid fermentation which accelerate the increase in pH. This is to challenge the capacity of BBBP to buffer and neutralize the rumen pH and to further validate the results. The second is to find out how BBBP affects the production of volatile fatty acids (VFAs) and inclusion of fungi in the microorganisms of interest. And the third is to identify the bacterial and protozoal species in the rumen and determine which ones have good effect in ruminant animal. Identification of the microorganisms at the genera or species level could be attainable by integration microbial metagenomics and microbial metabolomics. And understand what microbes are linked to the production performance (feed efficiency, high milk yield or health status) of the ruminant, particularly in the goats.

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