



Concentration of Rumen Parameters in Vitro Complete Pellet Feed Contains Different Alternative Energy Source Materials

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ABSTRACT: This research aims to determine the effect of using alternative energy sources in the form of banana tubers and rejected cassava in complete pellet feed with different levels on VFA, NH₃ and pH levels in vitro. The method used in this research is an experimental method using a completely randomized design (CRD) with 4 treatments and 4 replications, the treatments in this research are: R₀; complete feed pellets 40% banana weevil + 60% concentrate, R₁; complete feed pellets 60% banana weevil + 40% concentrate, R₂; pelleted complete feed (40% cassava + 60% concentrate, R₃; pelleted complete feed 60% cassava + 40% concentrate. The data obtained were analyzed using analysis of variance. Based on the results of statistical analysis, it showed that the treatment had a significant effect ($p < 0,05$) on VFA, NH₃ and rumen fluid pH levels. The conclusion of this research is that based on the discussion above, it is concluded that the use of different energy source materials in complete pelleted feed is able to increase VFA production and NH₃ concentration and rumen fluid pH in vitro to the best level. 40%.

KEYWORDS: alternative energy sources, complete feed, in vitro, pellets, rumen parameters.

INTRODUCTION

Continuous availability of feed in terms of quality and quantity is one of the factors in supporting livestock productivity throughout the year. In the rainy season, the availability of feed is abundant with sufficient nutritional content to meet basic living needs and production, while in the dry season, the availability of feed in pastures, forage cultivation gardens and other sources decreases drastically as a result of heat stress and the minimal availability of water for forage for livestock.

This has an impact on the low quantity of feed and a decrease in the nutritional value of the feed produced to meet livestock needs, especially energy to be able to carry out their activities in the production process so that livestock will use their energy reserves in the form of fat in the body to maintain their productivity, this has a very significant impact on weight. livestock body.

Based on these problems, a solution is needed to maintain and increase livestock productivity by optimizing the potential of agricultural and plantation waste, this waste can be in the form of stem leaves and tubers. One of the waste components which so far has not been able to be utilized optimally as feed is banana tubers which have a carbohydrate content of 78.69% and gross energy of 3981.47 kcal, [1], apart from that there are also rejected cassava tubers which contain carbohydrates amounted to 74.81% and gross energy amounted to 3421.62 kcal so that these two ingredients have the potential to become energy source feed ingredients for ruminant livestock to replace rice bran and ground corn in the ration.

Seeing the potential of these two wastes, their use and administration to livestock needs to be processed first and combined with other feed ingredients in the form of concentrate feed and formulated in the form of complete feed. According to [2] providing complete feed has been proven to be able to meet nutritional needs, especially for cows that are lactating. Complete feed ensures even distribution of daily ration intake, so that fluctuations in ecosystem conditions in the rumen can be minimized. Meanwhile, when given to livestock, it is served in pellet form so that it is more palatable and improves the physical quality of the ration. According to [3], the advantage of pelleted feed is that it makes the feed formula more efficient, because the pellets contain the same nutrients, palatability and minimizes wastage of feed due to waste or spillage.

However, before giving it directly to livestock, it is necessary to carry out an in vitro test to determine the effectiveness of the feed and the ability of microbes to digest which is described through the concentration of Volatile Fatty Acid (VFA), NH₃ and the pH of the rumen fluid. So this research aims to determine the effect of its use. Alternative energy source materials in the form of banana tubers and rejected cassava in complete pelleted feed with different levels of VFA, NH₃ and pH levels in vitro.



RESEARCH MATERIALS AND METHODS

This research was conducted at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Marine and Fisheries, Undana for 7 weeks. This research activity is divided into 3 stages, namely: 1 preparation stage including the stages of collecting, chopping, drying and grinding the feed ingredients used. The tools used in this research are: a machete used to chop banana weevils, a hanging scale with a capacity of 50 kg with a sensitivity of 10 gram used to weigh chopped banana weevils before and after drying, a digital scale with a capacity of 5 kg with a sensitivity of 0.5 gram used to weigh feed samples, a grinding machine The FFC disk mill brand is used to grind banana tubers, cassava, moringa leaves, corn and coconut cake.

Research methods

The research method used is an experimental method using a completely randomized design (CRD) with 4 treatments and 4 replications, the treatments are:

- R₀ = complete pellet feed (40% banana weevil + 60% concentrate)
- R₁ = complete pellet feed (60% banana weevil + 40% concentrate)
- R₂ = complete pelleted feed (40% cassava+60% concentrate)
- R₃ = complete pellet feed (60% cassava + 40% concentrate)

Table 1. Composition of ingredients and percentage of concentrate ingredients

Ingredients	Presentase
Ground Corn	15
Rice Bran	30
Moringa Leaves Meal	15
Soybean Meal	17
Fish Meal	5
Coconut Meal	10
Salt	4
Urea	2,5
Probion	0.5
Premix	1
Toxin Binder	0,5
Total	100

Table 2. Complete pellet feed formula

Ingredients	Treatment			
	R ₀ %	R ₁ %	R ₂ %	R ₃ %
Banana Corm Meal	40	60	0	0
Rejected Cassava	0	0	40	60
Concentrate	60	40	60	40
Total	100	100	100	100

Working procedures for in vitro digestibility experiments

This in vitro digestibility test procedure refers to a two-level modification method. [4] as follows:

1. The samples are weighed at 0.5 g each then put into 15 centrifuge tubes which have been numbered according to the treatment and 2 blank tubes containing buffer solution and rumen fluid.
2. 50ml of buffer solution and rumen fluid (4:1) is added into each tube. Before the tubes were covered with rubber, they were supplied with CO₂ to ensure anaerobic conditions in the tubes, then the tubes were placed in a water heater at 390C for the first 48 hours and shaken twice every day.



3. After 48 hours, the bacteria were killed by adding hydrochloric acid (HCl) at pH, then given pepsin HCl solution and incubated for a second 48 hours. This second period occurs in the post-rumen organ (Abomasum).
4. After 48 hours, the two tubes were removed from the water heater, then soaked in cold water, sometimes shaking.
5. Next, the tube was spun in a centrifuge at 2000 rpm for 15 minutes, then the supernatant was taken to further measure Rumen NH₃ and VFA in vitro.

Rumen Fluid Collection Technique

Rumen fluid is taken from livestock that has just been slaughtered from the slaughterhouse. Fill it in a thermos which is previously filled with hot water, take it to the laboratory, then the rumen fluid is filtered using glass wool with layered gauze, then mixed with saliva at a rate of 1:4.

Parameters measured:

1. Substrate pH

Substrate pH data collection was carried out using a digital pH meter Hanna brand type H198107.

2. Volatile Fatty Acid (VFA) Levels

Determination of VFA production was carried out by steam distillation of 5ml of rumen fluid supernate into a special tube then 15% 1mIH₂SO₄ was added, the tube was closed and distillation was carried out immediately. The tube is connected to a cooling flask and a flask containing heated distilled water. The distillation results were placed in an Erlenmeyer containing 5ml of 0.5N NaOH. The distillation process ends until the distillate collected reaches a volume of approximately 250ml. then add 1-2 drops of phenoptalen indicator and titer with 0.5N HCl until color occurs. VFA production is calculated using the formula:

$$\text{Total VFA} = (a-b) \times N \text{ HCl} \times (1000/5 \text{ mM})$$

Information :

a = ml HCl blank titration (5ml NaOH)

b = MI sample titration

3. NH₃ concentration

Determination of NH₃ production was determined using the modified Conway technique, 1 ml of supernatant was placed in one of the partitions of the Conway cup. On the other side, 1 ml of saturated Na₂CO₃ solution is placed, in the middle it is filled with boric acid with methyl red indicator and 1 ml of green bromine cresol. Na₂CO₃, then left for 24 hours at room temperature. The ammonia bound to boric acid is titrated with 0.005N H₂SO₄ until the color changes from blue to reddish-red.

$$\text{Formula: ml titration} \times N \text{ H}_2\text{SO}_4 \times 14 \times 100 \text{ (mg/100ml)}$$

Data analysis

The data analysis used was *Analysis Of Variance* to see the effect of treatment and if there was a real difference between treatments, Duncan's further test was carried out according to the instructions of [5].

RESULT AND DISCUSION

Composition of Food Substances complete pelleted feed

The composition of the food substances of complete pellet feed from various treatments can be seen in Table 3. Based on the results of laboratory analysis, it can be seen that there is no difference in the content of Dry matter (DM), Organic matter (OM), Crude protein (CP), Crude Fat, Crude fiber (CF), carbohydrate (CHO) and energy in the four treatment rations. Based on the results of proximate analysis of the substrate and the digestibility of DM and OM in vitro are presented in Tables 1 and 2 below.

Table 3. Chemical composition of treatment diets.

Ingredients	%DM	DM	Crude Protein	Crude Fat	Crude Fiber	CHO	NNFE	Energy	
		(%DM)	(%DM)	(%DM)	(%DM)	(%DM)	(%DM)	MJ/kg DM	Kkal/kg DM
Pellet R ₀	81,52	80,15	17,26	3,06	13,36	59,83	46,47	15,58	3.709,01



Pellet R ₁	82,27	81,59	17,49	3,42	13,68	60,68	47	15,9	3.786,54
Pellet R ₂	80,35	79,76	16,36	3,09	15,26	60,31	45,05	15,45	3.679,28
Pellet R ₃	79,82	79,12	15,84	2,99	15,67	60,29	44,62	15,29	3.640,33

Note: analysis results from the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Maritime Affairs and Fisheries, Nusa Cendana University

Effect of Treatment on the concentration of VFA, NH₃ and pH of Rumen Fluid In Vitro

The production of VFA flying fatty acids and NH₃ ammonia can be used as a measure of feed fermentability, and VFA production is the fermentation of organic matter. VFAs act as carbon skeletons to form microbial proteins. The following average levels of VFA, NH₃ and pH of rumen fluid in vitro are presented in Table 4.

Table 4. Mean levels of VFA, NH₃ and pH of rumen fluid in vitro.

Parameters	Treatment				P-Value
	R ₀ ±SD	R ₁ ± SD	R ₂ ± SD	R ₃ ± SD	
Concentration VFA (mM/L)	58,01±1,23 ^a	65,97±4,15 ^b	93,60±7,34 ^c	97,47±1,98 ^c	0.02**
Concentration NH ₃ (mM/L)	5,59±0,20 ^a	8,07±0,80 ^b	10,36±0,31 ^c	10,22±0,51 ^c	0.02**
pH rumen fluid	5,67±0,31 ^a	6,40±0,10 ^b	6,73±0,06 ^b	6,77±0,06 ^b	0.04**

Note: different superscripts on the same line show a significant effect $P < 0.05$

Based on the calculation results of VFA production of complete pelleted feed, the average R₀, R₁, R₂, R₃ were obtained respectively, namely 58.01 ± 1.23 mM/L, 65.97 ± 4.15 mM/L, 93.60 mM/L, 93.60 ± 7.34 mM/L and 97.47 ± 1.98 mM/L, then NH₃ production obtained an average of R₀, R₁, R₂, R₃ respectively, namely 5.59 ± 0.20 mM/L, 8.07 ± 0.80 mM/L, 10.36 ± 0.31 mM/L and 6.77 ± 0.06 mM/L, while the pH obtained was the average of R₀, R₁, R₂, R₃ respectively also 5.67 ± 0.31 mM/L, 6.90 ± 0.10 mM/L, 6.73 ± 0.06 mM/L, 6.73 ± 0.06 mM/L and 6.77 ± 0.06 mM/L.

The results obtained are still within the normal range of VFA production as stated by [6] that the VFA concentration for optimal microbial growth ranges between 70-150 mM and the amount is influenced by the type of feed, while the NH₃ levels needed to support Microbial protein synthesis is between 4-12 mM [7].

The results of statistical analysis showed that the use of different alternative energy sources in complete pelleted feed showed a very significant effect ($P < 0.01$) on the production of VFA, NH₃ and rumen fluid pH in vitro. This is caused by differences in the use of energy source materials in complete pellet feed which is a source of easily digestible carbohydrates so that they can be converted into simple sugars in the form of organic acids (acetic, lactic, propionic and butyric) as an energy source and carbon skeleton (C), which is able to influence increased VFA production.

Increased VFA production can indicate the ease with which nutrients in feed, especially carbohydrates and proteins, are degraded by rumen microbes, so that VFA production in the rumen can be used as a measure of feed fermentability which is closely related to rumen microbial activity and population [8]. [9] added that the NH₃ concentration is determined by the level of feed protein, the degree of degradability and the length of time the feed is in the rumen can determine the ammonia concentration. This is also reinforced by the opinion of [10] that protein degradation is faster than microbial protein synthesis, so NH₃ will accumulate and exceed its optimum concentration. Therefore, the NH₃ content of the rumen fluid depends on the amount and nature of the protein in the feed ingredients.

Based on the Duncan test, it can be seen that the R₀-R₁, R₀-R₂, R₀-R₃, R₁-R₂, and R₁-R₃ treatments are very significantly different ($P < 0.01$), while the R₂-R₃ treatments are not significantly different ($P > 0.05$) on VFA and NH₃ production. This is due to differences in levels of energy source ingredients in complete pelleted feed, thus providing opportunities for microbes to break down nutrients in the substrate in the form of easily digestible carbohydrates in the form of CHO and BETN which are then metabolized into VFA as the main energy source for ruminants. This is in accordance with what was reported by [7] that high total VFA production reflects



that the organic material of the ration is easily broken down by microbes in the rumen. The differences in VFA produced in each experimental ration indicate that the organic feed ingredients used in the rations are different in nature. [11] added that the concentration of ammonia in the rumen also determines the efficiency of microbial protein synthesis which ultimately influences the fermentation of organic feed materials in the form of volatile fatty acids (VFA) which are the main energy source for livestock. [12] added that the amount of VFA formed is greatly influenced by the digestibility and quality of the feed added.

Meanwhile, the pH of the rumen fluid based on Duncan's test results showed that the pH values in the R₀-R₁, R₀-R₂, R₀-R₃ treatments were very significantly different (P<0.01), while in the R₁-R₂, R₁-R₃, and R₂ treatments R₃ was not significantly different (P>0.05) to rumen fluid pH in vitro. This is due to differences in the composition of energy source materials, thereby increasing the production of VFA and NH₃ in the rumen, making them more easily digested by rumen microbes which have optimal activity at these pH conditions, which is characterized by increased digestibility of dry matter and organic matter. According to [13], one of the factors that influence rumen pH is the physical characteristics, type and chemical composition of feed. It was further stated that if the feed contains a lot of fiber or structural carbohydrates, the pH tends towards alkaline (alkaline; value 7.5), whereas if the feed contains more starch or soluble carbohydrates, the pH tends towards acid (value 5). A different view was put forward by [6] that if the levels of starch or propionate and butyrate in feed increase, the pH will decrease to 4.5-5. In such low pH conditions, it will inhibit the growth of cellulolytic bacteria, thereby inhibiting the digestion of forage. However, it can be said that the pH resulting from the treatment in this study was in the range of 6.85-6.98 which can still be said to be a pH that is conducive to the growth and development of cellulolytic bacteria.

CONCLUSION

Based on the discussion above, it can be concluded that the use of different energy source materials in complete pelleted feed is able to increase VFA production and NH₃ concentration and rumen fluid pH in vitro to the best level of 40%.

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