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In vitro digestibility and fermentation ruminant of buffalo ration based on *Neptunia plena* L. Benth and *Leersia hexandra* Swartz as local resources

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ABSTRACT: Utilization of local resource (*Neptunia plena* L. Benth and *Leersia hexandra* Swartz) as feed ration for buffalo fattening could make cost efficiency, fulfil primary life needs and production. The objectives of study is to determine the Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD), NH_3 ruminant fermentation and Volatile Fatty Acid (VFA). The conduct of the study was in Laboratory of Animal Husbandry Nutrient Science, Faculty of Animal Science and Agriculture, Diponegoro University, Semarang by using in vitro method with a Complete Randomized Design (CDR) of five treatments and five replicates: (1) T_1 =100% *Leersia hexandra* Swartz; (2) T_2 = 100 % *Neptunia plena* L.Benth*;* (3) T_3 = Ration (15% *Neptunia plena* L.Benth + 15% *Leersia hexandra* Swartz + 70% other feedstuffs); (4) T_4 = Ration (20% *Neptunia plena* L.Benth + 20% *Leersia hexandra* Swartz + 60% other feedstuffs); and (5) T_5 = Ration (25% *Neptunia plena* L.Benth + 25% *Leersia hexandra* Swartz + 50% other feedstuffs). Data analysis used analysis of variance with a significance level of 95% and then followed by Duncan Multiple Range Test (DMRT). The results showed that T_3 and T_4 treatments produced the highest DMD at ($P < 0.05$), i.e. 43.65% and 43.26%, respectively. T_2 treatment (47.66%) significantly produced the highest OMD ($P < 0.05$) compared to T_4 (46.81%) and T_1 (45.36%). T_5 treatment (5.28 mM) significantly produced in the highest NH_3 ($P < 0.05$) compared to T_2 (4.88 mM); T_3 (4.73 mM); and T_1 (4.43 mM). T_5 treatment (145.4 mM) significantly produced the highest VFA ($P < 0.05$) compared to T_4 (140.0 mM); T_3 (135.4 mM); T_2 (134.8 mM); and T_1 (123.6 mM). In vitro digestibility and fermentation ruminant of buffalo ration based on *Neptunia plena*, L. Benth, and *Leersia hexandra* Swartz as local resources can buffalo improved ruminant fermentation so that it is capable of increasing the buffalo productivity.

Keywords: Fermentation; Ruminant; Digestibility; Ration; Buffalo

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INTRODUCTION

Food production system consists of several components, including livestock which importantly contributes to quality and variety of meat supply (Wanapat *et al.*, 2013). Ruminant production is more competitive and oriented towards sustainability, a decent and economical production system (Goes *et al*., 2019). Buffalo (*Bubalus bubalis*) also has the potential to produce meat in addition to other livestock such as cattle, goats and sheep (Nanda and Nakao, 2003). The population of buffalo in Indonesian in 2018 was 1.356.390 heads, with meat production reaching to 31.30 thousand tons. East Kalimantan Province with geographic potential (climate and land), as well as the carrying capacity of its feed, has the potential to become a buffalo development area. The population of buffalo in East Kalimantan Province in 2018 was 7.124 heads with a level of meat production reaching to 59 tons (Ministry of Agriculture, 2018).

The interaction between genetic, feed and fattening management determines livestock productivity, including reproductive productivity. A feed is the largest component in production cost. The high cost of feed ingredients causes an increase in production cost, so it is necessary to do efficiency (Goes *et al.*, 2019). Utilizing local feed resources can help to reach the efficiency of feed cost that are available continuously throughout the year, not competing with humans, the quality and quantity can meet the needs of livestock production and reproduction (Wanapat and Rowlinson, 2007). Buffalo, with their existing digestive system, can adapt well to the extreme environment and feed quality with high crude fibre content (Wanapat *et al.*, 2013). Supan-supan legumes (*Neptunia plena* L. Benth) and Kolomento grass (*Leersia hexandra* Swartz) are local feed resources which capable for the feed ingredient for buffalo ration. Evaluation of feed ingredient aims to determine the best type of feed, optimize the provision of ration, and find out the potential feed digested by livestock.

Digestion is an early indication of the availability of various nutrients contained in the feed. Digestibility and fermentation rate test can be carried out by in vitro method (Khanum *et al.*, 2007). The in vitro method is an alternative method to estimate feed degradation in livestock's digestive apparatus, especially ruminants without involving the animal (Mohamed and Chaudhry, 2008).

Addition of *Neptunia plena* L. Benth and *Leersia hexandra* Swartz aimed at providing cheap feedstuff because of abundant availability and has a nutrient composition which capable of fulfilling needs of buffalo. Utilization of local feed resources composed in the form of the ration with a combination of other feed ingredients becomes an alternative way in providing buffalo ration, so it needs further study at the testing stage on livestock on an experimental scale.

MATERIALS AND METHODS

The study was in at the Laboratory of Animal Husbandry Nutrition Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang (December 2018). The research used several stages as a series of activities.

Preparation of sample and rumen inoculum

Preparation of feed material sample used physical treatment, namely: cutting, drying, and grinding, so that the sample was mash-shaped. Samples test is using proximate analysis (Table 1) to determine their nutrient content. Local feed resources (*Neptunia plena* L. Benth and *Leersia hexandra* Swartz), and other feed ingredients composing ration (rice bran, maize, palm oil cake, and calliandra) came from wild grasslands, agricultural by-products, and plantations in Samarinda City, East Kalimantan Province. Buffalo rumen liquid was originally from Slaughterhouse

Boestaman Semarang collected at dawn, then filtered and put into a thermos that had been filled with warm water beforehand to a temperature of 39°C, then closed the lid to maintain an anaerobic atmosphere, then taken to the laboratory for analysis. The nutrition content of feedstuff ration was analyzed used proximate analysis (AOAC,1990) which includes moisture content (MC), ash, crude protein (CP), ether extract (EE), crude fibre (CF), and material extract without nitrogen (NFE). The result of the proximate analysis of feedstuff showed in Table 1.

Tabel 1. Nutrient content of feedstuff

Feedstuff	Nutrient Content (%)					
	DM	Ash	OM	CF	CP	NFE
Leersia hexandra Swartz	85.09	9.57	90.43	49.23	11.28	27.93
Neptunia plena L. Benth	86.89	4.82	95.18	54.76	15.49	21.73
Maize	89.97	0.77	99.23	0.38	8.14	89.13
Rice Bran	88.91	5.49	94.51	24.75	9.97	53.82
Palm oil cake	92.27	1.37	98.63	48.78	14.03	15.17
Calliandra	93.54	11.35	88.65	55.84	23.86	6.72

Source: Proximate analysis result from Laboratory of Animal Nutrition Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang (2017).

Research materials

Buffalo ration ingredients were *Neptunia plena* L. Benth, *Leersia hexandra* Swartz, rice bran, maize, palm oil cake and calliandra. The ration was made based on DM and already met the lower and upper limit of CP needs for livestock, ranging from 10 - 14% with TDN 60% (Sunarso, 2003). Protein and energy balance is significantly needed by rumen during fermentation (Rodriguez *et al*., 2015). Materials used for in vitro analysis were Mc Dougall's solution (artificial saliva), pepsin-HCL solution, ice water, distilled water, CO_2, methyl red and bromocresol green indicator, saturated sodium carbonate (Na_2CO_3), sulfuric acid (H_2SO_4) 15%, boric acid solution, HCl 0,5%, sulfuric acid 0,0055N, phenolphthalein indicator 1%, vaseline, NaOH 0,5N, and Whatman filter paper 41.

Experimental design

The experimental design uses a Complete Randomized Design (CRD) with five treatments and five replicates. The main consideration in ration projection followed the balance of CP content of 11% - 12%, with the ration energy content calculated based on Total Digestible Nutrient (TDN) ±60%. The balance limit of ration was in the range between the lowest limit of CP for ruminant, i.e. 10% and the highest limit of CP for ruminant, i.e. 14% and the energy requirement (TDN) was ±60%. Addition of leguminous calliandra to ration aimed to achieve the balance of CP and utilize sufficiently feedstuff in East Kalimantan, particularly in Samarinda.

The treatments consisted of T_1=100% *Leersia hexandra* Swartz; T_2=100% *Neptunia plena* L. Benth; T_3, T_4, and T_5 ration which formulated from *Leersia hexandra* Swartz, *Neptunia plena* L. Benth, maize, rice barn and palm oil cake with a composition of T_3=12% CP and TDN 60%; T_4=11.92% CP and TDN 59.80%; T_5= 11.68% CP and TDN 59.39% (Table 2).

In vitro method

In vitro method of Tilley and Terry is a well-known method to evaluate the utilization amount of ruminant feed nutrients. This method had two stages, namely fermentative digestion using rumen fluid buffer for 48 hours and enzymatic digestion with a pepsin-HCL solution for 48 hours (Tilley and Terry, 1963; Mabjeeshh *et al*., 2000; Makkar, 2004). In vitro analysis was started with the preparation of sample and research materials, then poured 10 ml of buffalo rumen liquid and added with 40 ml of Mc Dougall solution into a 50 ml

measuring cup until obtaining a homogeneous mixture. Then, the mixture put into a fermenter tube that had been filled with treatment samples as much as 0.55-0.56 g. The fermentor cylinder was then closed. The little amount of CO_2 gas added into the fermentor in order to get anaerobic condition inside the fermentor tube. Fermenter tubes incubated the containing treatment sample with the help of a water bath (temperature 39ºC). Microbial fermentation happened for 48 hours, well-shaken for every six hours. The microbial fermentation process stopped after 48 hours by moving the fermenter tube from the water bath into a container filled with ice

Table 2. Chemical composition of each ration (% dry matter)

Composition	Treatment				
	T_1	T_2	T_3	T_4	T_5
			.....(%).....		
Feedstuff:					
Leersia hexandra Swartz	100.00	-	15.00	20.00	25.00
Neptunia plena L.Benth	-	100.00	15.00	20.00	25.00
Maize	-	-	34.00	39.00	42.00
Rice barn	-	-	14.00	9.50	1.00
Palm oil cake	-	-	14.50	3.00	2.00
Calliandra	-	-	7.50	8.50	5.00
Total	100.00	100.00	100.00	100.00	100.00
Feed nutrient:					
DM	85.09	86.89	89.92	89.65	88.69
OM	90.43	95.18	94.30	94.27	94.42
CP	11.28	15.49	12.00	11.92	11.68
TDN*	40.88	38.38	60.00	59.80	59.39

Source: Proximate analysis result from Laboratory of Animal Nutrition Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang (2017).
*Calculation result, according to Sutardi (2001)

water, then centrifuged at a speed of 3000 rpm for 15 minutes. The clear liquid (supernatant) had separated from the residue before carried out an enzymatic digestive process on the sediment. Enzymatic digestion happens by adding 50 ml pepsin-HCL solution into the fermenter tube containing centrifuge precipitate. The fermentor tube was then incubated into the water bath at a temperature of 39ºC for 48 hours and shake every six hours. The next process was taking residue and filtering it using Whatman 41 filter paper assisted with a vacuum pump. The produced residue was put into crucible porcelain and put into an oven at 105ºC for 12 hours. The sample heated inside the oven was cooled using a desiccator for 15 minutes and weighed it using analytical scales.

Statistic calculation and analysis

Parameters of Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD), NH_3 fermentation level and Volatile Fatty Acid fermentation (VFA) level uses these following equations (Mayulu *et al.,* 2018):

1. DMD equation:

$$\text{DM Digestibility} = \frac{\text{DM weight of the sample} - (\text{DM contained in residue} - \text{blanco})}{\text{DM weight of the sample}} \text{x } 100\% \quad \text{.....(1)}$$

2. OMD equation:

$$\text{OM digestibility} = \frac{\text{OM weight of sample} - (\text{OM contained in residue} - \text{blanco})}{\text{OM weight of the sample}} \text{x } 100\% \quad \text{.....(2)}$$

Remarks:
DM sample = sample weight x % DM
DM residue = weight after oven-CP-filter paper
Blanco = weight after oven-CP- filter paper
OM sample = weight of DM sample x % OM
% OM = 100% DM- (% ash inside DM)
OM residue = weight after oven – weight after tenure – filter paper

3. Ammonia (NH_3) production equation:
NH_3 production (mM) = (ml titrant x N H_2SO_4 x1000) .....(3)
Remarks: N= H_2SO_4 solution normality

4. VFA production equation:
VFA Production (mM) = (a–b) x N HCl x1000/5.....(4)
Remarks:
a = Titran volume of the blanco (ml) b = Titran volume of the sample (ml)

Statistical analysis

The result obtained from in vitro was then analyzed by using analysis of variance (ANOVA) at significance level 95% and then followed by Duncan Multiple Range Test (DMRT) by using CoStat program approach.

RESULTS AND DISCUSSION

Buffalo is a ruminant that capable of changing low-quality feedstuff (high fibre) to becomes quality of food products (meat) which has rich in nutrition. Buffalo is more efficient in digesting crude fibre, ruminant nitrogen ammonia (NH_3-N), recycling nitrogen (N), degrading dry matter and crude protein compared to cattle (Sarwar *et al*., 2009; Wanapat *et al*., 2013). The efficient digestion in buffalo happens due to the diversity of rumen microorganisms including cellulolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* species) which are dominant and contribute in fibre degradation and are supported by the presence of protozoa (*Holotrichs*, *Entodiniomorphs*) which role in digesting fermentable carbohydrate, and capable of fermenting sugar (sugar, starch) (Wanapat *et al*., 2013). Ruminants are very dependent on forage as the main source of feed, but their availability fluctuates and depends on the season, so the quality and quantity are uncertain (Makkar, 2004; Wanapat and Rowlinson, 2007). Biological evaluation of feed on a laboratory scale through a quantitative approach (in vitro method) is to predict feed quality. Digestion is a parameter in determining the number of feed nutrients that can be utilized in the body of livestock and absorbed by the digestive tract (gastrointestinal tract) in supporting maintenance, production, and reproduction (Makkar, 2004; Dijkstra *et al*.,2005; Mould *et al.*, 2005; Mayulu *et al.*, 2018).

Dry matter and organic matter digestibility

A good quality ration depends on the nutrient content and the level of nutrient utilization in the animal's body. Dry matter digestibility (DMD) is the ability of animals to digest and utilize dry matter contained in the ration given. As seen in Table 3, means of in vitro DMD of buffalo ration based on local feedstuff, based on ANOVA, showed the highest DMD average value, i.e. T_3=43.65%; T_4 = 43.26%; T_5= 39.87%; T_1=37.81%; and T_2= 36.96%. DMRT results showed that T3 produced the highest DMD, but it was not significantly different from T_4. Treatment T_3 was significantly higher ($P<0.05$) compared to T_5, T_1, and T_2.

Table 3. Means of in vitro dry matter and organic matter digestibility of buffalo treatment ration

Parameter	Treatment				
	T_1	T_2	T_3	T_4	T_5
	----------(%)----------				
DMD	37.81^{bc}±3.4	36.96^{c}±0.8	43.65^{a}±0.4	43.26^{a}±0.8	39.87^{b}±1.7
OMD	45.36^{c}±0.02	47.66^{a}±0.22	47.12^{ab}±0.13	46.81^{b}±0.08	47.36^{ab}±0.01

Remark: Different superscript shows significant difference in the same line (P<0.05), T_1 =100% *Leersia hexandra* Swartz; T_2 = 100 % *Neptunia plena* L.Benth*;* T_3 = Ration (15% *Neptunia plena* L.Benth + 15% *Leersia* Swartz *hexandra* + 70% other feedstuffs); T_4 = Ration (20% *Neptunia plena* L.Benth + 20% *Leersia hexandra* Swartz + 60% other feedstuffs); T_5 = Ration (25% *Neptunia plena* L. Benth + 25% *Leersia hexandra* Swartz + 50% other feedstuffs).

The result recommends the utilization of local feed e of 15 and 20 in the ration, based on the results of the study showed that DMD of T_3 and T_4 was 43.65% and 43.26%, respectively. Those values were better when compared to the utilization of single feed T_1 (100% *Leersia hexandra* Swartz) and T_2 (100% *Neptunia plena* L. Benth) with the value of 37.81% and 36.96%, respectively (Table 3). However, the results of DMD were lower when compared to studies conducted by Sugoro*et al*. (2015) who found that in vitro buffalo ration composed of local feed (50% sorghum straw silage) and 50% concentrate where the highest DMD was 62.93%. This low digestibility value happens due to crude fibre contained in the feedstuffs, according to the opinion of Mayulu (2014) which stated that low digestibility because of alkaloid and crude fibre content. The difference in digestibility values depends on several factors including the physical form of feedstuff, nutrient content, feedstuff, composition comparison among the feedstuffs, treatment of the feed and the period of stay inside the rumen (Mabjeesh *et al.,* 2000; Sugoro *et al.,* 2015; Mayulu *et al.,* 2018).

The activity of rumen microorganism can determine the level of feed digestibility because rumen microbial activity depends on nutrient contained in feedstuffs (Mayulu *et al*., 2018). The feedstuffs that have high crude fibre content can cause low digestibility, while feedstuffs with low crude fibre generally have higher digestibility, this is because the cell walls of the feedstuff are thin so that they can be penetrated by digestive sap (Mayulu *et al*., 2018).

Organic Matter Digestibility (OMD) is a percentage of the amount of organic matter in a feed or ration that can be digested by the digestive tract and subsequently will be utilized by the livestock body and rumen microorganisms to produce energy or Volatile Fatty Acid (VFA) (Mayulu, 2015). The organic matter digestibility is closely related to DMD because some DM is OM which consists of crude protein, crude fat, crude fibre and Nitrogen Free-Extract (NFE). Organic matter acts as a source of energy in supporting metabolic processes in the body (Mayulu, 2015).

As seen in Table 3, means of in vitro OMD of buffalo ration based on local feedstuff, based on ANOVA, showed the highest OMD average value of T_2 = 47.66%; T_5= 47.36%; T_3= 47.12%; T_4 = 46.81% and T_1 = 45.36%. Duncan, multiple range test results, showed that T_2 produced the highest DMD, but it was not significantly different from T_3 and T_5. Treatment T_2 was significantly higher (P<0.05) compared to T_4 and T_1. The result showed that the utilization of single feed T_2 (100% *Leersia hexandra* Swartz) produced the highest OMD of 47.66%. However, this result was lower when compared to studies conducted by Sugoro *et al*. (2015) who found that the highest DMD to in vitro buffalo ration composed of local feed (50% sorghum straw

silage) and 50% concentrate was 59.97%. This low OMD value happens due to the activity of rumen microorganisms, nutrient content of feed ingredients (high crude fibre content) and too small of feed particle size which causes decreasing of feed flow rate leaving the rumen and having an impact on reducing the chance of rumen microbes to degrade feed particles. Mayulu (2015) stated that the low OMD value could cause by the high content of neutral detergent fibre (NDF) cell walls in the ration.

Parameter of NH_3 ruminant fermentation and VFA

Pure protein and non-protein nitrogen (NPN) are feed protein that enters the rumen and degraded into peptide and amino acid. The degradation results will produce the final product in the form of ammonia (NH_3) and other products such as VFA and carbon dioxide (CO_2) (Christiyanto *et al*., 2005). Ammonia is the main nitrogen source for protein synthesis of rumen microorganism, NH_3 formation in the rumen is highly dependent on the chemical structure of the protein contained in the ration material (Christiyanto *et al.*, 2005; Mayulu, 2015; Aderinboye *et al.*,2016; Phesatcha dan Wanapat, 2016). NH_3 level needed to support the maximum rumen microbial biosynthesis is 3.57-7.14 mM (Sunarso, 2003; Mayulu, 2015). Buffalo rumen microbes have high efficiency in utilizing NPN for microbial protein synthesis. The means of NH_3 production of buffalo ration based on local feed in-vitro (Table 4), based on ANOVA shows the average value of the highest NH_3 production wasT_5= 5.28 mM; T_4= 5.20 mM; T_2= 4.88 mM; T_3= 4.73 mM and T_1= 4.43 mM. Duncan's multiple range test results showed that T_5 produced the highest NH3 production, but it was not significantly different from T_4. T_5 treatment showed significantly higher results ($P<0.05$) compared to T_2, T_3, and T_1.

The highest NH_3 production was produced by T_5 (5.28 mM) treatment, which contained crude protein 11.68% and TDN 59.39%. These results indicate that T_5 treatment ration is in the range of optimum NH_3 concentration (3.57-7.14 mM) so that it can support the biosynthesis of rumen microbes. The difference in NH_3 production in this study probably happens because of the amount of protein in the ration, protein solubility, and the degradation rate of feed protein. Low NH_3 production can cause rumen microbial growth to be slow, resulting in inhibited carbohydrate degradation.

Table 4. Means of in VFA and NH_3 production in vitro of buffalo treatment ration

Parameter	Treatment				
	T_1	T_2	T_3	T_4	T_5
	----------(mM)----------				
NH_3	4.43^{d}±0.02	4.88^{b}±0.21	4.73^{c}±0.08	5.20^{a}±0.02	5.28^{a}±0.04
VFA	123.6^{d}±3.79	134.8^{c}±3.79	135.4^{c}±3.79	140.0^{b}±0.00	145.4^{a}±3.79

Remark: Different superscript shows significant difference in the same line ($P<0.05$), T_1 =100% *Leersia hexandra* Swartz; T_2 = 100 % *Neptunia plena* L.Benth*;* T_3 = Ration (15% *Neptunia plena* L.Benth + 15% *Leersia* Swartz *hexandra* + 70% other feedstuffs); T_4 = Ration (20% *Neptunia plena* L.Benth + 20% *Leersia hexandra* Swartz + 60% other feedstuffs); T_5 = Ration (25% *Neptunia plena* L.Benth + 25% *Leersia hexandra* Swartz + 50% other feedstuffs).

Volatile Fatty Acid is the final product of the fermentation process by rumen microbes and acts as an energy source (about 80%) for livestock. Buffalo rumen fluid has VFA with the proportion of acetic acid (C_2) 66.9-73.8%, propionate (C_3) 16.2-28.8%, butyrate (C_4) 4.7-6.6% (Hart *et al*., 2007; Wanapat and Rowlinson, 2007; Mayulu, 2015). The means of in vitro VFA production of buffalo ration based on local feed (Table 4), based on ANOVA shows that the means value of the highest VFA

production was T_5 = 145.4 mM; T_4 = 140.00 mM; T_3= 135.40 mM; T_2= 134.80 mM and T_1= 123.60 mM. Duncan multiple range test shows the production of VFA from T_5 (145.40 mM) was significantly higher ($P < 0.05$) compared to T_4, T_3, T_2, and T_1. VFA concentration produced in this study was in the normal range (80-160 mM) to support optimum microbial growth. VFA concentration is affected by the quality of the ration, the type of feed carbohydrate, the physical form of the feed, the level of fermentability of feedstuff, and the number and types of bacteria present in the rumen. High VFA concentration indicates increased rumen microbial activity because more organic matter is fermented in the rumen and indicates that the fermentation process is more effective than the ordinary one, however too high VFA concentration can disrupt the rumen system balance (Madrid *et al*., 2002; Mayulu, 2015).

CONCLUSION

In vitro digestibility and fermentation ruminant of buffalo ration based on *Neptunia plena* L. Benth and *Leersia hexandra* Swartz as local resources can buffalo improved ruminant fermentation so that it is capable of increasing the buffalo productivity.

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