RESEARCH ARTICLE

REVISED *In vitro* evaluation of ruminal digestibility and fermentation characteristics of local feedstuff-based beef cattle ration [version 3; peer review: 1 approved]

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Abstract

Background: Consumption of local feedstuff *Neptunia plena* L. Benth and *Leersia hexandra* Swartz as a ration by the animal subject is expected to promote cost efficiency and production, as well as provide essential nutrition needs. Therefore, this study aimed to evaluate ruminal dry matter digestibility (DMD), organic matter digestibility (OMD), ammonia (NH_3) production, and volatile fatty acid (VFA) in beef cattle. **Methods:** Feed and rumen inoculum samples were prepared and analyzed for their proximate contents. There were five treatment groups based on the diet received by beef cattle, namely: T_1 (*Leersia hexandra* Swartz 100 %); T_2 (*Neptunia plena L. Benth* 100%); T_3 (*Leersia hexandra* Swartz 15% + (*Neptunia plena L. Benth* 15% + 70 % Other Feedstuffs); T_4 (*Leersia hexandra* Swartz 20% + (*Neptunia plena L. Benth* 20% + 60% Other Feedstuffs); T_5 (*Leersia hexandra* Swartz 25% + (*Neptunia plena L. Benth* 25% + 50% Other Feedstuffs). *In vitro* approaches were used to determine the DMD, OMD, NH_3 production, and VFA in beef cattle. **Results:** The results showed that the highest DMD (P<0.05) was derived from T_5 (56.47%), followed by T_4 (56.45%) and T_3 (55.90%). T_5=62.40% significantly (P<0.05) generated the highest OMD followed by T_4=61.95% and T_3=60.82%. This treatment had the highest NH_3 value, namely 5.02 mM, compared with T_3=4.55 mM, T_4=4.50 mM, T_2=4.22 mM, and T_1 =3.99 mM. Furthermore, T_5 had the highest VFA (P<0.05) compared with T_4, T_3, T_2, and T_1 with the value of 150.5, 133.0, 130.5, 130.0, and 123.5 mM, respectively. **Conclusions:** The local feedstuff-based ration can be used to ensure the sustainable production of beef cattle

Keywords

digestibility, fermentation, ration, beef cattle, in vitro, Functional Feed, Nutrition

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Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 2**

1. Adding the word "ruminal" before the word "dry matter digestibility...."
2. Eliminate the sentence "The data were analyzed using ANOVA at a significance level of 95%, and a Duncan Multiple Range Test", in the abstract.
3. Reconstruct the word "derived" from the sentence "The highest NH3 production, i.e. 5.02 mM, was from T5 which contained 11.68% CP and 59.39% TDN".
4. Reconstruct the sentence "The highest NH3 concentration produced from T5 was compared to the report by Al-Arif *et al.* (2017)".
5. Removing the word "contributed" in the abstract and replacing it with the word "can be used to ensure the sustainable production of beef cattle".
6. Adding the following feed sample drying procedure: natural drying (utilizing indirect sunlight by spreading Neptunia plena L. Benth, Leersia hexandra Swartz, and calliandra in a greenhouse).
7. Changing the word "nutritional contents" with "chemical composition" (Table 1).

Any further responses from the reviewers can be found at the end of the article

Introduction

Livestock, particularly ruminants, is an integral part of the agricultural sector and represents a significant impact on the national economy (Beigh *et al.* 2017). Ruminants are produced at a more competitive rate than poultry to enhance business sustainability (Silva *et al.* 2019). They form nutritious food material (meat) from plant fiber (Krizsan *et al.* 2012). Global food demand for animal protein has been rising significantly, hence some efforts are needed to ensure adequate supply. One of these efforts is to increase livestock productivity through more efficient use of available resources, which are 98% natural (Andriarimalala *et al.* 2019). Feed is the main constraint faced by breeders in Indonesia to boost beef cattle productivity. Feed deficiency becomes a dominant threat during the dry season (Al-Arif *et al.* 2017), specifically for forages (Al-Masri 2010). Wild grass and agriculture biomass are consumed as an alternative during the dry season. However, these feedstuffs contain high fiber and low nutrients such as protein, energy, mineral, and vitamin that affect the ruminal microbe fermentation process (Andriarimalala *et al.* 2019). The maintenance and production needs of beef cattle cannot be fulfilled from a single feed source such as forages (Al-Arif *et al.* 2017), therefore a balanced or quality ratio is needed (Ramaiyulis *et al.* 2018).

The beef cattle population in the East Kalimantan Province has reached 119,675 heads (Indonesian statistics 2020). This needs to be increased through some efforts which include enhancement of the feed sector. The optimum productivity is achieved with adequate feed supply, both in terms of quality and quantity (Daru and Mayulu 2020). Local feedstuffs are accessible for breeders due to being available in abundance (Hasan *et al.* 2020), hence their exploitation is expected to increase feed production sustainability. The local feedstuff sources in East Kalimantan Province, such as *Supan-Supan Leguminosae* (*Neptunia plena* L. Benth) and *Kolomento* grass (*Leersia hexandra* Swartz) are essential factors in creating a balanced ration for beef cattle (Mayulu *et al.* 2019).

Neptunia plena L. Benth is a semi-aquatic legume from the Fabaceae family, with compound leaves and a stem that forms a fibrous sponge and taproots to support growth on the water surface, known as floating (Mayulu *et al.* 2020, 2021). Also, *Leersiahexandra* Swartz is annual in nature, easily grown (Liu *et al.* 2011) in inundated wetlands, known as swamps (Lin *et al.* 2018), tolerant to heavy metal chromium (Cr) (Zhang *et al.* 2007), and can be cultivated artificially (Ning *et al.* 2018). This plant possesses the potential for copper phytoextraction on contaminated soil (Lin *et al.* 2019) and is harvested several times during the growing period. It has dry matter production up to nine tons/ha within 60 days and is used as feed ingredients for the beef cattle ration (Liu *et al.* 2011).

Knowledge of the potential nutrition contained in local feedstuff ration is expected to increase breeders' willingness to adopt their respective sources. *Neptunia plena* L. Benth and *Leersia hexandra* Swartz tend to be developed into a sustainable feedstuff ration for beef cattle due to being abundant throughout the year, specifically during feed scarcity. It is important to measure ruminant digestibility and fermentation level with the feedstuffs, as well as compose these to formulate a perfect ration. Various feedstuffs need to be evaluated in ration formulation (Hasan *et al.* 2020; Peiretti 2020) because the chemical content presents quality-related information (Forejtová *et al.* 2005; Al-Arif *et al.* 2017). Determination of feed nutrient quality requires a fast and accurate method such as chemical and biological analysis (Baran *et al.* 2017). An *in vitro* method is a digestibility and fermentation rate test (Mayulu *et al.* 2020) that provides animals' biological attributes in a simpler way (Fondevila and Espés 2008). This can be used in daily feeding evaluation which is performed to achieve feed optimization and usage efficiency as well as to minimize nutrient excretion into the environment (Dijkstra *et al.* 2005). Ideally, the ruminant feed is evaluated *in vivo* to obtain more accurate results, particularly for nutrient quality, but the method is not practical and cost-effective. Therefore, alternative evaluations need to be performed in laboratory conditions using *in vitro* methods (Dijkstra *et al.* 2005; Daru and Mayulu 2020).

Advantages of evaluating ruminal feed digestibility using *in vitro* methods include testing several feed samples simultaneously to ensure cheaper cost and less time consumption (Dijkstra *et al.* 2005; Mayulu *et al.* 2019; Zewdie 2019; Daru and Mayulu 2020). Hence, this research aimed to evaluate the beef cattle ration biologically on a laboratory scale through quantitative assessment or *in vitro* method.

Methods

This research was carried out in the Laboratory of Feed Nutrient Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang. Some of the materials used were feed ration which consisted of *Neptunia plena* L. Benth and *Leersia hexandra* Swartz, as well as rice bran, palm cake, and calliandra. The *in vitro* analysis used beef cattle rumen fluid derived from the Boestaman Semarang animal slaughterhouse, pepsin-HCl solution as the protein-degrading enzyme, McDougall solution (artificial saliva), saturated sodium carbonate (Na_2CO_3), 15% sulfuric acid (H_2SO_4), and 0.5N NaOH, boric acid solution, 0.5% HCl, 1% phenolphthalein indicator, 0.0055N sulfuric acid, vaseline, methyl red and Bromocresol Green, Whatman filter paper 41, Aquadest, CO_2, and ice for stopping the fermentation process.

Preparation of feed sample

Feedstuff sample materials were prepared through physical treatment consisting of cutting, natural drying (utilising indirect sunlight by spreading Neptunia plena L. Benth, Leersia hexandra Swartz and calliandra in a greenhouse), and milling process, until they were mashed (Fondevila and Espés 2008). These were tested through proximate analysis (Acland 1985), to determine their nutritional content. Local feed resources such as *Neptunia plena* L. Benth and *Leersia hexandra* Swartz (used whole stems and leaves), and other rations, namely rice bran, maize, palm oil cake, and calliandra, were obtained from wild grasslands, agricultural by-products, and plantations in Samarinda, East Kalimantan Province.

Preparation of rumen inoculum sample

The rumen fluid was obtained from the Boestaman Semarang Slaughterhouse from an Ongole Peranakan beef cattle with a slaughter weight of 296.4 kg. Cattle are kept conventionally and given forage-based feed with a frequency of twice a day. The rumen fluid was collected in the morning after slaughter. The rumen liquid obtained was then filtered and put into a thermos that had previously been filled with warm water at a temperature of 39°C. This was closed to maintain an anaerobic atmosphere and brought to the laboratory for research observation.

Proximate analysis

The Association of Official Agricultural Chemists (AOAC) procedure (Acland 1985) was applied to determine the observed feedstuffs' nutritional content, namely dry matter (DM), crude fiber (CF), crude protein (CP), ether extract (EE), ash, and nitrogen-free extract (NFE) (Evan *et al.* 2020). The proximate analysis results were presented in prior research (Mayulu *et al.* 2020, Table 1).

Experimental design

In this research, a completely randomized design with five treatments was used. The main consideration in ration formulation used was 11%-12% crude protein balance, with ration energy calculated based on the total digestible nutrient (TDN) ±60%. The ration CP balance was in the range of 10% minimum and 14% maximum, and the energy needs TDN was ±60%. The treatments consisted of T_1 (*Leersia hexandra* Swartz 100%); T_2 (*Neptunia plena* L. Benth. 100%); T_3 (*Leersia hexandra* Swartz 15% + (*Neptunia plena* L. Benth 15% + 70 % Other Feedstuffs); T_4 (*Leersia hexandra* Swartz

Table 1. The chemical composition of the feedstuff ration.

Feedstuffs	Chemical composition (%)						
	DM	Ash	OM	CF	EE	CP	NFE
Neptunia plena L. Benth	86.89	4.82	95.18	54.76	3.20	15.49	21.73
Leersiahexandra Swartz	85.09	9.57	90.43	49.23	1.99	11.28	27.93
Calliandra	93.54	11.35	88.65	55.84	2.23	23.86	6.72
Maize	89.97	0.77	99.23	0.38	1.68	8.14	89.13
Rice bran	88.91	5.49	94.51	24.75	5.97	9.97	53.82
Palm oil cake	92.27	1.37	98.63	48.78	9.57	14.03	15.17

Source: Proximate analysis result, Laboratory of Feed Nutrient Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University.
DM = Dry matter; OM = Organic matter; CF = Crude fiber; EE = Ether extract; CP = Crude protein; NFE = Nitrogen-free extract.

Table 2. Feedstuff ration percentage and nutritional value.

Composition	Treatment (% DM)				
	T_1	T_2	T_3	T_4	T_5
	(%)				
Feedstuffs:					
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 bran	-	-	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
Nutritional value:					
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, Feed Nutrient Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang (2017).
*Calculation result according to Sutardi (2001).

20% + (*Neptunia plena* L. Benth 20% + 60% Other Feedstuffs); T_5 (*Leersia hexandra* Swartz 25% + (*Neptunia plena* L. Benth 25% + 50% Other Feedstuffs) (Mayulu *et al.* 2020, Table 2).

In vitro analysis

Tilley and Terry's (1963) *in vitro* analysis is an alternative method to specifically evaluate ruminants' feed nutrient usage amount to determine the DMD, OMD, NH_3 production, and VFA in a laboratory setting (Gosselink *et al.* 2004; Banakar *et al.* 2017). The *in vitro* analysis employed rumen fluid as microbial inoculum (Tufarelli *et al.* 2010), and two stages were involved: fermentative digestion by using a buffer of rumen fluid for 48 hours and enzymatic digestion by using a pepsin-HCl solution for another 48 hours (Hristov *et al.* 2019; Daru and Mayulu 2020). Fermentation levels of NH_3 was carried out by the Conway microdiffusion technique. Measurement of NH_3 production begins with: weighing a sample weighing 0.55-0.56 g, then put into a fermenter tube and added 40 ml of McDougall's solution and 10 ml of rumen fluid. The fermenter tube which has been filled with the sample is then filled with CO_2 gas and closed (for anaerobic conditions). The fermenter tube is then put into a rack that has been provided in a waterbath with a temperature of 39°C to be incubated for three hours and shaken every 30 minutes. the fermentation process will be stopped after three hours by moving the fermenter tube from the water bath into a container containing ice cubes, then centrifuged for 15 minutes to separate the residue and supernatant. The supernatant liquid as much as 1 ml was then put into a Conway dish (sterilized and the lips of the cup and the lid were smeared with Vaseline) on the left side of the screen and on the right side of the bulkhead dripped with saturated sodium carbonate (Na_2CO_3) and the middle of the cup was dripped with methyl red and indicator green bromcresol. The filled cup is then closed tightly and shaken (forming a figure eight) slowly until the supernatant and sodium carbonate are homogeneous and allowed to stand for 24 hours at room temperature with the aim that the resulting NH_3 can be bound with boric acid, after 24 hours the titration is carried out with H_2SO_4 0.0055 N until the color changes from green to pink. Measurement of VFA using Steam Distillation technique. VFA measurements were carried out by taking and inserting 5 ml of supernatant and 1 ml of 15% H_2SO_4 using a pipette into a distillation tube and inserting it into a 1000 ml Erlenmeyer flask with 800 ml of distilled aquadest, then preparing a 100 ml Erlenmeyer flask to which NaOH solution was added 5 ml of 0.5 N (useful as a catcher for hot steam from the distillation) and tightly closed and heated with Bunsen. The hot steam will push the VFA through the condensed cooling tube and accommodated in a 100 ml Erlenmeyer flask containing 15 ml of 0.5 N NaOH solution until the volume reaches 100 ml, then the Bunsen is turned off. The captured steam is then added with 2 drops of 1% phenolphthalein indicator and titrated with 0.5% HCL solution until the color changes from red to clear (colorless).

Calculation and statistical analysis

Parameters of DMD, OMD, NH_3 fermentation level, and VFA fermentation level were calculated by using the following equations (Hristov *et al.* 2019; Daru and Mayulu 2020).

DMD equation:

$$\text{DMD} = \frac{\text{DM weight of the sample} - (\text{DM contained in residue} - \text{blank})}{\text{DM weight of the sample}} \times 100\% \quad (1)$$

OMD equation:

$$\text{OMD} = \frac{\text{OM weight of sample} - (\text{OM contained in residue} - \text{blank})}{\text{OM weight of the sample}} \times 100\% \quad (2)$$

Remarks:

M sample = sample weight × % DM

DM residue = weight after oven-CP-filter paper

OM sample = weight of DM sample × % OM

% OM = 100% DM − (% ash contained in DM)

OM residue = weight after oven-tanur-filter paper

Blank = weight after oven-CP-filter paper

NH_3 production equation:

$$NH_3 \text{ production (mM)} = (\text{mL titrant} \times \text{N } H_2SO_4 \times 1000) \quad (3)$$

Remarks: N=H_2SO_4 solution normality

VFA production equation:

$$\text{VFA production (mM)} = (a - b) \times \text{N HCl} \times 1000/5 \quad (4)$$

Remarks:

a = Titrant volume of the blank (mL)

b = Titrant volume of the sample (mL)

The *in vitro* method-derived results were analyzed using ANOVA at a significance level of 95%, followed by Duncan Multiple Range Test (DMRT) which applied the Costas program approach.

Results and discussion

Dry matter and organic matter digestibility

Beef cattle convert low-quality feed (high fiber) into products containing high nutritional value and quality, such as meat (Deutschmann *et al.* 2017; Mayulu *et al.* 2020; Daru and Mayulu 2020). This ability is promoted by a complex digestive system, particularly the stomach which consists of four compartments, namely the rumen, reticulum, omasum, and abomasum (Mayulu *et al.* 2021). The rumen, sometimes called reticulum-rumen, accommodates about 80% of the total digested amount and contains microbes that digest fibers effectively. Therefore, it enables ruminants to survive with poor nutritional quality and conditions (Mohamed and Chaudhry 2008). Feed deficiency elevates ruminal microbes' degradation rate and increases the metabolic capacity to use energy, both of which lead to an OMD increase (Al-Masri 2010).

Digestibility is defined as the number of nutritional feedstuffs absorbed or used by livestock to satisfy their needs such as production, growth, reproduction, and other functions (Abbasi *et al.* 2018). It is also an important indicator in measuring the nutritional quality of feed (Al-Arif *et al.* 2017). Low quality of feed or rations is caused by high crude fiber content, including ADF and NDF (Gülşen *et al.* 2004). Dry matter consists of all nutrients, while organic matter comprises all

Table 3. Means of *in vitro* DMD and OMD of beef cattle ration formulated from local feedstuffs.

Parameter	Treatment				
	T_1	T_2	T_3	T_4	T_5
	(%)				
DMD	$41.30^{b}\pm3.96$	$42.94^{b}\pm1.51$	$55.90^{a}\pm0.73$	$56.45^{a}\pm1.88$	$56.47^{a}\pm0.31$
OMD	$52.89^{b}\pm4.22$	$49.31^{c}\pm1.17$	$60.82^{a}\pm1.02$	$61.95^{a}\pm1.40$	$62.40^{a}\pm0.28$

Remarks: Different superscripts show a significant difference ($P < 0.05$), where T_1 = 100% *Leersiahexandra* Swartz and T_2 = 100 % *Neptunia plena* L. Benth. T_3 = Ration of 15% *Neptunia plena* L. Benth + 15% *Leersia hexandra* Swartz + 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).

Table 4. Means of *in vitro* N-NH_3 and volatile fatty acid (VFA) of beef cattle ration based on local feedstuffs.

Parameter	Treatment				
	T_1	T_2	T_3	T_4	T_5
	(%)				
N-NH_3	$3.99^{c}\pm0.20$	$4.22^{bc}\pm0.34$	$4.55^{b}\pm0.25$	$4.50^{b}\pm0.28$	$5.02^{a}\pm0.17$
VFA	$123.5^{c}\pm4.18$	$130.0^{bc}\pm0.00$	$130.5^{bc}\pm7.58$	$133.0^{b}\pm8.37$	$150.5^{a}\pm7.58$

Remarks: Different superscripts show significant difference ($P < 0.05$), where T_1 =100% *Leersiahexandra* Swartz and T_2 = 100 % *Neptunia plena* L. Benth. T_3 = Ration 15% *Neptunia plena* L. Benth + 15% *Leersia hexandra* Swartz + 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.

nutrients excluding ash. DM digestibility in beef cattle plays an important role in evaluating feed nutrients absorbed by the digestive tract (Al-Arif *et al.* 2017). A decrease in this parameter is affected by the ratio of stems and forage leaves (Kamal *et al.* 2020). Table 3 shows the *in vitro* DMD and OMD of beef cattle rations formulated from local feedstuffs.

ANOVA results showed that T_5 = 56.47% was the highest DMD mean, followed by T_4 = 56.45%, T_3 = 55.90%, T_2 = 42.94%, and T_1 = 41.30%. According to DMRT results, T_5 produced the highest DMD but was not significantly different from T_4 and T_3. T_5 treatment resulted in a significantly higher DMD ($p < 0.05$) than T_1 and T_2. Local feedstuff usage in the ration with percentages of 15, 20, and 25 produced T_5 = 56.47%, T_4 = 56.45%, and T_3 = 55.90%. These values were higher compared with single feedstuff T_1 (100% *Leersiahexandra* Swartz) and T_2 (100% *Neptunia plena* L Benth) which had a DMD of 42.94% and 41.30% respectively, as presented in Table 3. Based on Table 1, the low digestibility of single feedstuff in T_1 and T_2 is due to high crude fiber content *i.e.*, 49.23% and 54.76% respectively. This is in line with the results of Mayulu *et al.* (2021) who stated that high CF contained in the feedstuffs causes low digestibility.

Crude fiber is part of the nutritional components of feedstuffs which is difficult to digest but is needed in the digestive tract for promoting peristalsis, specifically to support ruminal performance (Adesogan *et al.* 2019; Andriarimalala *et al.* 2019; Mayulu *et al.* 2019). This is composed of lignin which causes low feedstuff digestibility due to being hard to degrade enzymatically by ruminal microbes. It also increases along with the plant's age and maturity (Andriarimalala *et al.* 2019). Different digestibility values are caused by several factors including nutritional content, composition ratio, and duration of feedstuffs inside the rumen (Mayulu *et al.* 2019). The DMD value produced from all treatments was higher compared with Al-Arif *et al.* (2017) results, *i.e.* 23.76% obtained from single feedstuff and 49.96% from the *in vitro* ration. This indicates that in terms of quantity, the local feedstuff-based ration contributes to beef cattle productivity.

Organic matter (OM) acts as the energy source for building substances to promote the body's metabolic processes (Mayulu and Sutrisno 2010). OMD is defined as a proportion of OM digested by the digestive tract, which is used to measure available energy, and estimate protein synthesis by ruminal microbes (Al-Arif *et al.* 2017). This is closely related to DMD since the part of DM consists of OM which contains CF, CP, EE, and NFE (Mayulu *et al.* 2020).

Based on the ANOVA results, *in vitro* OMD means of beef cattle ration based on local feedstuffs from the highest to smallest value were T_5 = 62.40%, T_4 = 61.95%, T_3 = 60.82%, T_1 = 52.89%, and T_2 = 49.31%. The DMRT results showed that the highest OMD was derived from T_5, but it wasn't significantly different from T_3 and T_4. T_5 treatment had a significantly higher OMD ($P < 0.05$) compared to T_1 and T_2. Organic matter digestibility derived from T_1, T_3, T_4, and T_5 had a higher value than the report by Al-Arif *et al.* (2017) who obtained an *in vitro* OMD of 24.98% from a single feed

forage and 49.70% from the ration. The low T_2 OMD value of 49.31% was probably due to ruminal microbes' activity or feedstuff nutritional content and extremely small particle, causing a lower rate of feed leaving the rumen and smaller chances of proper degradation (Mayulu *et al.* 2020).

Production of NH_3 and VFA

In addition to the digestibility value, feed nutritional content was calculated from the fermentation variable, *i.e.* NH_3 and VFA concentration. Protein is an essential nutrient that determines the economic success of the beef cattle industry (Chathurika *et al.* 2019). The beef cattle rumen degrades low biological protein and low-quality fiber into a microbial protein with high biological value (Liu *et al.* 2019; Chathurika *et al.* 2019). Ammonia serves as a primary nitrogen source for most ruminal microbes (Imsya *et al.* 2013), which is responsible for carrying out higher microbial protein synthesis (Supapong *et al.* 2019; Mayulu *et al.* 2021). The measurement of this element is employed to estimate protein degradation and usage by ruminal microbes; hence OMD has a strong correlation with microbial protein synthesis (Imsya *et al.* 2013). NH_3 production reflects the amount of feedstuff protein degraded, and the rate at which this process occurs is an important characteristic for determining protein value (Liu *et al.* 2019). Ammonia nitrogen is an essential nutrient in promoting microbial growth. High NH_3 production is needed to reach maximum fermentation level and increases feed digestibility (Al-Arif *et al.* 2017). NH_3 concentration in the rumen is a balance between the produced and absorbed amount, known to be optimal for microbial needs once ranging from 3.57-7.14 mM (Mayulu *et al.* 2019).

The *in vitro* NH_3 means of beef cattle ration based on local feedstuffs obtained from ANOVA were T_5 = 5.02 mM, T_3 = 4.55 mM, T_4 = 4.50 mM, T_2 = 4.22 mM, and T_1 = 3.99 mM. The DMRT result showed that the highest NH_3 was produced from T_5. A high value of NH_3 concentration from T_5 is probably due to the ration's carbohydrate structure and remnant retention duration inside the rumen (Mayulu *et al.* 2019). The result of T_5 was significantly higher ($P < 0.05$) compared with T_3, T_4, T_2, and T_1. The highest NH_3 production, *i.e.* 5.02 mM, was from T_5 which contained 11.68% CP and 59.39% TDN. The highest NH_3 concentration was produced from T_5 was compared to the report by Al-Arif *et al.* (2017) who produced an *in vitro* NH_3 concentration of 3.95 mM with single forage feedstuff and 2.88 mM with the ration. This result was in the optimum range between 3.57-7.14 mM, hence it was expected to promote ruminal microbial biosynthesis. Higher NH_3 concentration reflects more protein decomposition during *in vitro* fermentation, and this is associated with higher CP content (Wang *et al.* 2021). The different NH_3 derived in this research tended to be initiated by the amount of feedstuff crude fiber, as well as protein solubility and degradation rate. Low NH_3 production causes slow growing rate of ruminal microbes which leads to decreasing population and inhibited carbohydrate degradation (Mayulu *et al.* 2020; Sarnataro and Spanghero 2020).

VFA is the end product of carbohydrate metabolism by ruminal microbes (Supapong *et al.* 2019) and acts as an energy source (80%) (Mayulu *et al.* 2020). VFA is developed through hydrolysis of polysaccharide carbohydrates which are converted into monosaccharides, specifically glucose. These are then converted into acetate (C_2), propionate (C_3), butyrate (C_4), isobutyrate, valerate, isovalerate, methane (CH_4), and CO_2 (Abbasi *et al.* 2018; Kongphitee *et al.* 2018). OM in a ration that is easily degraded by ruminal microbes is indicated by a high VFA concentration (Mayulu *et al.* 2019). VFA concentration depends on nutrient digestibility (particularly that of carbohydrates), VFA absorption rate, the ruminal microbial community activity, and degradation rate (Tilahun *et al.* 2022).

The *in vitro* VFA means of beef cattle ration based on local feedstuffs obtained from ANOVA were T_5 = 150.5 mM, T_4 = 133.0 mM, T_3 = 130.5 mM, T_2 = 130.0 mM, and T_1 = 123.5 mM. The DMRT results showed that T_5 had a significantly higher value *i.e.* 150.5 mM ($P < 0.05$) compared with T_4, T_3, T_2, and T_1. A high VFA concentration indicates an increased ruminal microbes' activity because more OM is being fermented inside the rumen (Hasan *et al.* 2020). The result of T_4 was not significantly different once compared to T_3 and T_2 values. The obtained VFA concentration was normal, ranging from 70-150 mM (Tilahun *et al.* 2022) and 80-160 mM (Mayulu *et al.* 2019, 2021), with a tendency to promote optimum microbial growth. This is in line with the report by Mayulu *et al.* (2019, 2021) and Tilahun *et al.* (2022) who stated that VFA concentration promotes ruminal microbe biosynthesis. Increasing VFA concentration within the optimum range reflects an effective fermentation process, but an extremely high value causes a balance disorder inside the rumen (Mayulu *et al.* 2019). VFA concentration is influenced by the ration's carbohydrate content (Supapong *et al.* 2019), inoculum collecting duration, incubation time, particle size, and inoculum preparation (Patra and Yu 2013), and fiber digestibility.

Conclusions

The results of the study and through the approach of analysis of variance, evaluation of the digestibility value (DMD and OMD) and fermentation level (NH_3 and VFA) of beef cattle consuming local feedstuff-based ration *in vitro*, it can be concluded that the use of local feed ingredients in quantity is able to can be used to ensure the sustainable production of beef cattle and further research needs to be done both from the author and other researchers, especially by expanding the variables and the stage of direct testing on cattle (*in vivo* and *in sacco*).

Data availability

Underlying data

Figshare: RAW Data for in vitro Evaluation of Ruminal Digestibility and Fermentation Characteristic of Local Feedstuff-Based Beef Cattle Ration, https://doi.org/10.6084/m9.figshare.20089154.v1 (Mayulu 2022).

This project contains the following underlying data:

- RAW of HAMDI MAYULU in vitro Sapi Potong.xlsx

Acknowledgments

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Open Peer Review

Current Peer Review Status: ✔

Version 3

Reviewer Report 06 July 2023

https://doi.org/10.5256/f1000research.148436.r182265

✔ **Yosra Ahmed Soltan**
Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

The manuscript can be accepted for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Animal nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 17 March 2023

https://doi.org/10.5256/f1000research.139994.r154197

? **Yosra Ahmed Soltan**
[1] Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt
[2] Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

In the abstract, please add the word "ruminal" before the word "dry matter digestibility...."

You don't need to put this sentence in the abstract "The data were analyzed using ANOVA at a significance level of 95%, and a Duncan Multiple Range Test", please remove it.

Remove the word "derived", and write this sentence again.

"This treatment had the highest NH 3...", this sentence has to be rewritten. You cannot start the sentence with this treatment, put its name directly and make the necessary changes.

In the conclusion of the abstract, please remove "contributed" and replace it with "can be used to ensure the sustainable production of beef cattle".

For the "Preparation of feed sample", how were the samples dried?

For the "Proximate analysis", please replace the words "nutritional contents" with "chemical composition". The same observation is in the title of table 1.

Table 2, what are the T1, T2, etc? Tables have to be stand-alone, and all abbreviations in tables have to be inserted in full names.

Please write the statistical analysis in a separate part, and include the experimental unit, number of repetitions/treatment, and the model used to analyze these data.

Table 3, and 4 where are the p values? and what do these letters mean?

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Animal nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 27 September 2022

https://doi.org/10.5256/f1000research.135260.r149682

?

Yosra Ahmed Soltan

[1] Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt
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Please insert the full name of all abbreviations when first mentioned (e.g., in the methods of the abstract, NH3-N, VFA...).

In the abstract, what are these five treatments?

In the results, T1, T2... refer to what?

Keywords, 'Functional feeds' in place of 'Functional foods'.

In the introduction you must mention the experimental plants that you used, and why you selected them? Their advantages as alternative feed resources for cattle.

For the methods part, you should insert the number of animals that you used their rumen from the slaughterhouse, mention their breed, weight, feed, and life stage.

Which part of *Leersia hexandra* did you use? Leaves or grains? How did you collected these plants?

The treatments are not clear.

Table 1, was the chemical composition DM based?

Table 2, what are T1, T2...?

How did you measure NH3-N and VFA?

May you insert the fiber content and NFC for your treatments in table 2? These parameters can be used to explain the obtained results, as NFC is composed mainly of starch, in addition to simple sugars and soluble fiber. Thus, each fraction can be fermented differently while providing various energy sources.

For ruminal microbial growth, and consequently the OMD (Please see Soltan *et al*., 2021[1]).

The conclusion needs to be rewritten again, why did you insert the data again? Just refer to their meaning and promote advice from this study.

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Animal nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.