In vitro evaluation of ruminal digestibility and fermentation characteristics of local feedstuff-based beef cattle ration [version 2; peer review: 1 approved with reservations]

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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 dry matter digestibility (DMD), organic matter digestibility (OMD), ammonia (NH₃) 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: T₁ (Leersia hexandra Swartz 100 %); T₂ (Neptunia plena L. Benth 100%); T₃ (Leersia hexandra Swartz 15% + (Neptunia plena L. Benth 15% + 70 % Other Feedstuffs); T₄ (Leersia hexandra Swartz 20% + (Neptunia plena L. Benth 20% + 60% Other Feedstuffs); T₅ (Leersia hexandra Swartz 25% + (Neptunia plena L. Benth 25% + 50% Other Feedstuffs). In vitro approaches were used to determine the DMD, OMD, NH₃ production, and VFA in beef cattle. The data were analyzed using ANOVA at a significance level of 95%, and a Duncan Multiple Range Test.

Results: The results showed that the highest DMD (P<0.05) was derived from T₅ (56.47%), followed by T₄ (56.45%) and T₃ (55.90%). T₅=62.40% significantly (P<0.05) generated the highest OMD followed by T₄=61.95% and T₃=60.82%. This treatment had the highest NH₃ value, namely 5.02 mM, compared with T₃=4.55 mM, T₄=4.50 mM, T₂=4.22 mM, and T₁=3.99 mM. Furthermore, T₅ had the highest VFA (P<0.05) compared with T₄, T₃, T₂, and T₁ with the value of 150.5, 133.0, 130.5, 130.0, and 123.5 mM, respectively.

Conclusions: The local feedstuff-based ration contributed to beef cattle production.
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-Mari 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, Leersia hexandra 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 (Forejová 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 (Na₂CO₃), 15% sulfuric acid (H₂SO₄), 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, CO₂, and ice for stopping the fermentation process.

Preparation of feed sample
Feedstuff sample materials were prepared through physical treatment consisting of cutting, drying, 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 T₁ (*Leersia hexandra* Swartz 100%); T₂ (*Neptunia plena* L. Benth. 100%); T₃ (*Leersia hexandra* Swartz 15% + (*Neptunia plena* L. Benth 15% + 70 % Other Feedstuffs); T₄ (*Leersia hexandra* Swartz 20% + (*Neptunia plena* L. Benth 20% + 60% Other Feedstuffs); T₅ (*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, NH₃ production, and VFA in a laboratory setting (Gosselink et al. 2004;

| Feedstuffs               | Nutritional content (%) |
|-------------------------|-------------------------|
|                         | 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 |
| *Leersia hexandra* 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.
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 NH₃ was carried out by the Conway microdiffusion technique. Measurement of NH₃ 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 CO₂ 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 (Na₂CO₃) 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 NH₃ can be bound with boric acid, after 24 hours the titration is carried out with H₂SO₄ 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% H₂SO₄ 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, NH₃ fermentation level, and VFA fermentation level were calculated by using the following equations (Hristov et al. 2019; Daru and Mayulu 2020).

DMD equation:

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

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

Remarks:

\(M\) sample = sample weight \times \% DM

DM residue = weight after oven-CP-filter paper

OM sample = weight of DM sample \times \% OM

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

OM residue = weight after oven-tanur-filter paper

Blank = weight after oven-CP-filter paper

\(\text{NH}_3\) production equation:

\[
\text{NH}_3\,\text{production (mM)} = (\text{mL titrant} \times \text{NH}_2\text{SO}_4 \times 1000) \tag{3}
\]

Remarks: \(N=\text{H}_2\text{SO}_4\) solution normality

VFA production equation:

\[
\text{VFA production (mM)} = (a - b) \times \text{NHCl} \times 1000/5 \tag{4}
\]

Remarks:

\(a\) = Titrant volume of the blank (mL)

\(b\) = Titrant volume of the sample (mL)

The \textit{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.

\textbf{Results and discussion}

\textbf{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 \textit{et al.} 2017; Mayulu \textit{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 \textit{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 \textit{et al.} 2018). It is also an important indicator in measuring the nutritional quality of feed (Al-\textit{Arif} \textit{et al.} 2017). Low quality of feed or rations is caused by high crude fiber content, including ADF and NDF (Güls¸en \textit{et al.} 2004). Dry matter consists of all nutrients, while organic matter comprises all nutrients excluding ash. DM digestibility in beef cattle plays an important role in evaluating feed nutrients absorbed by the digestive tract (Al-\textit{Arif} \textit{et al.} 2017). A decrease in this parameter is affected by the ratio of stems and forage leaves (Kamal \textit{et al.} 2020). Table 3 shows the \textit{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, \(T_3\) produced the highest DMD but was not significantly different
smallest value were T5 = 62.40%, T4 = 61.95%, T3 = 60.82%, T1 = 52.89%, and T2 = 49.31%. The DMRT results showed that the highest OMD was derived from T5, but it wasn’t significantly different from T3 and T4. T5 treatment had a significantly higher OMD (p < 0.05) compared to T1 and T2. Organic matter digestibility derived from T1, T3, T4, and T5 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 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.

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 quality, 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).

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

| Parameter | Treatment | T1 | T2 | T3 | T4 | T5 |
|-----------|-----------|----|----|----|----|----|
| DMD       | (%)       | 41.30^b±3.96 | 42.94^a±1.51 | 55.90^a±0.73 | 56.45^a±1.88 | 56.47^a±0.31 |
| OMD       | (%)       | 52.89^b±4.22 | 49.31^a±1.17 | 60.82^a±1.02 | 61.95^a±1.40 | 62.40^a±0.28 |

Remarks: Different superscripts show a significant difference (P < 0.05), where T1 = 100% Neptunia plena L. Benth, T2 = Ration of 15% Neptunia plena L. Benth + 15% Leersia hexandra Swartz + 70% other feedstuffs, T3 = Ration 20% Neptunia plena L. Benth + 20% Leersia hexandra Swartz + 60% other feedstuffs, T4 = Ration 25% Neptunia plena L. Benth + 25% Leersia hexandra Swartz + 50% other feedstuffs.

Table 4. Means of in vitro N-NH3 and volatile fatty acid (VFA) of beef cattle ration based on local feedstuffs.

| Parameter | Treatment | T1 | T2 | T3 | T4 | T5 |
|-----------|-----------|----|----|----|----|----|
| N-NH3     | (%)       | 3.99^c±0.20 | 4.22^bc±0.34 | 4.55^b±0.25 | 4.50^b±0.28 | 5.02^a±0.17 |
| VFA       | (%)       | 123.5^c±4.18 | 130.0^bc±0.00 | 130.5^bc±7.58 | 133.0^p±8.37 | 150.5^p±7.58 |

Remarks: Different superscripts show significant difference (P < 0.05), where T1 = 100% Neptunia plena L. Benth, T2 = Ration 15% Neptunia plena L. Benth + 15% Leersia hexandra Swartz + 70% other feedstuffs, T3 = Ration 20% Neptunia plena L. Benth + 20% Leersia hexandra Swartz + 60% other feedstuffs, T4 = Ration 25% Neptunia plena L. Benth + 25% Leersia hexandra Swartz + 50% other feedstuffs.

from T1 and T3, T5 treatment resulted in a significantly higher DMD (p < 0.05) than T1 and T2. Local feedstuff usage in the ration with percentages of 15, 20, and 25 produced T4 = 56.47%, T5 = 56.45%, and T3 = 55.90%. These values were higher compared with single feedstuff T1 (100% Neptunia plena L. Benth) and T2 (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 T1 and T2 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.
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 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 derived from T$_5$ which contained 11.68% CP and 59.39% TDN. A higher ammonia value was obtained 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; Kongphithee 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$_5$ 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 contribute to beef cattle production 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

Data is available under the terms of the Creative Commons Zero “No Rights Reserved” Data Waiver (CC0 1.0 Public Domain Dedication).

Acknowledgments

The author is grateful to the Head and Staff of Feed Nutrient Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University who provided facilities to support this research.

References

Abbasi IH, Raja F, Abbasi MEAE, et al.: Post-ruminal effects of rumen-protected methionine supplementation with low protein diet using long-term simulation and in vitro digestibility technique. AMB Express. 2018;8(1): 1-8. Publisher Full Text

Acland j: Association of official analytical chemist AOAC-1990, official method of analysis. East Africa Crops. 1985; 33–38. Washington D.C. Adesogan AT, Arniola KG, Jiang Y, et al.: Symposium review: technologies for improving fiber utilization. J. Dairy Sci. 2019; 102(6): 5726–5755. Publisher Abstract | Publisher Full Text

Al-Arif A, Anam M, Suvandi LT, et al.: The nutrients contents, dry matter digestibility, organic matter digestibility, total digestible nutrient, and N, rumen production of three kinds of cattle feeding models. KnE Life Sci. 2017; 3: 338-343. Publisher Full Text

Al-Masri MR: In vitro rumen fermentation kinetics and nutritional evaluation of kochia indica as affected by harvest time and cutting regimen. Anim. Feed Sci. Technol. 2010; 157: 55-63. Publisher Full Text

Andriarimalala, J, Herilalao CC, Kpmasse P, et al.: Nutritional potential of bamboo leaves for feeding dairy cattle. Pesqui. Agropecu. Trop. 2019; 49. Publisher Full Text

Baranak PS, Ally K, Lokesh E, et al.: In vitro assessment of nutritive value of unconventional feed resource as livestock feed. Int. J. Livest. Res. 2017; 7(6): 159-169. Publisher Full Text

Baran, Sedat M, Altaji S, et al.: The determination of nutrient value, digestibility and energy levels of compound feeds used for ruminant nutrition by in vitro methods. Turkish Journal of Agriculture-Food Science and Technology. 2017; 5(7): 832-835. Publisher Full Text

Beigh Y, Afzal AM, Ganai, et al.: Prospects of complete feed system in ruminant feeding: a review. Vet. World. 2017; 10(4): 424-437. Publisher Abstract | Publisher Full Text

Chathurika S, Sujari S, Manawadu A, et al.: Enhance in vitro rumen fermentation of panicum maximum with biological supplements. Jurnal Ilmu Ternak dan Veteriner. 2019; 24(2): 68-72. Publisher Full Text

Churches S: Invasive plant risk assessment: Calotrope (Calotropis Procerca), Biosecurity Queensland. 2016: 1–18. Publisher Full Text

Daru TP, Mayulu H: Optimization of land resources through forages development. Eur. J. Mol. Clin. Med. 2020; 7(7): 5000–5013.

Deutschnann K, Phatsara C, Sarachakula C, et al.: In vitro gas production and in vivo nutrient digestibility and growth performance of that indigenous cattle fed fresh and preserved pangola grass. Ital. J. Anim. Sci. 2017;16(3): 521-529. Publisher Full Text

Dijkstraa, Kebreab E, Bannink A, et al.: Application of the gas production technique to feed evaluation systems for ruminants. Anim. Feed Sci. Technol. 2005; 123-124: 561-578. Publisher Full Text

Evan T, Marcos CN, Ranilla MB, et al.: In vitro and in situ evaluation of broccoli wastes as potential feed for ruminants. Animals. 2020; 10(11): 1-14. PubMed Abstract | Publisher Full Text

Fondevila M, Espés BP: A new in vitro system to study the effect of liquid phase turnover and ph on microbial fermentation of concentrate diets for ruminants. Anim. Feed Sci. Technol. 2008; 144: 196-211. Publisher Full Text

Foretová J, Lád F, Tínáctý J, et al.: Comparison of organic matter digestibility determined by in vivo and in vitro methods. Czech J. Anim. Sci. 2005; 50(2): 47-53.

Gosselink (MJ), Dulphy JP, Poncet C, et al.: A comparison of in situ and in vitro methods to estimate in vivo fermentable organic matter of forages in ruminants. NJAS-Wagen. J. Life Sci. 2004; 52(1): 25-45. Publisher Full Text

Gülseren N, Çoşkun B, Urucalılar HD, et al.: Prediction of nutritive value of a native forage, prangos uechritzii, using in situ and in vitro measurements. J. And Environ. 2004; 56(1): 167-179. Publisher Full Text

Halmeimy BFA, Rinne M, Lammiminen M, et al.: Alternative and novel feeds for ruminants: nutritive value, product quality and environmental aspects. Animal. 2018; 12: s295-s309. Publisher Abstract | Publisher Full Text

Hasan S, Mupvisa A, Khaeren PI, et al.: Potential of complete feed formulated from local raw materials on beef cattle performance. European J. BioSci. 2020; 14(1): 1-6.

Hristov AN, Bannink A, Crompton LA, et al.: Invited review: nitrogen in ruminant nutrition: a review of measurement techniques. J. Dairy Sci. 2019; 102(7): 5811-5852. Publisher Full Text

Imysa A, Laconi EB, Wirayawan KG, et al.: In vitro digestibility of ration containing different level of palm oil frond fermented with phanerochaete chrysosporium. Media Peternakan. 2013; 36(2): 131-136. Publisher Full Text

Kamal MT, Hashem MA, Al Mamun M, et al.: Investigating the quality of commercial beef cattle feeds and feed ingredients used in Bangladesh. SAARC J. Agric. 2020; 18(1): 197-208. Publisher Full Text

Kongphitee K, Sommart K, Phonbumrung T, et al.: Feed intake, digestibility and energy partitioning in beef cattle fed diets with cassava pulp instead of rice straw. Asian Australas. J. Anim. Sci. 2018; 31(9): 1431-1440. PubMed Abstract | Publisher Full Text

Krisan S, Nyholm L, Nousiainen J, et al.: Comparison of in vitro and in situ methods in evaluation of forage digestibility in ruminants. J. Anim. Sci. 2012; 90(9): 3162-3173. PubMed Abstract | Publisher Full Text

Lin H, Hong Zhang X, Chen J, et al.: Phytohemagglutinin potential of Leersia Hexandra Swartz of copper contaminated soil and its

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Version 2

Reviewer Report 17 March 2023

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

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? Yosra Ahmed Soltan
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.

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**Version 1**

Reviewer Report 27 September 2022

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

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**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

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). 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.

References
1. Soltan Y, Abdalla Filho A, Abdalla A, Berenchtein B, et al.: Replacing maize with low tannin sorghum grains: lamb growth performance, microbial protein synthesis and enteric methane production. Animal Production Science. 2021; 61 (13). Publisher Full Text

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.
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