International Journal of Agriculture and Biosciences 2024 13(3): 488-493.
https://doi.org/10.47278/journal.ijab/2024.158
This is an open-access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

In Vitro Rumen Fermentability and Physical Properties of Bali Cattle Ration Based on Agro-Industrial by-Product with Different Ratio of Rumen Degradable Protein and Non-Fiber Carbohydrate

Jasmal A Syamsu [1,5], Asmuddin Natsir [1], Abdul Alim Yamin [1], Mardiati Zain [2], Ujang Hidayat Tanuwiria [3], Yunilas [4] and Ichlasul Amal [6]

[1]Department of Animal Nutrition, Faculty of Animal Science, Universitas Hasanuddin, Makassar, Indonesia
[2]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia
[3]Department of Animal Nutrition, Faculty of Animal Science, Universitas Padjadjaran, Bandung-Sumedang, Indonesia
[4]Department of Animal Science, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia
[5]Center for Research and Development of Livestock Resources and Tropical Animal, Universitas Hasanuddin, Makassar, Indonesia
[6]Faculty of Vocational, Universitas Hasanuddin, Makassar, Indonesia
*Corresponding author: jasmal.syamsu@unhas.ac.id

ABSTRACT

The research aimed to evaluate in vitro rumen fermentability and physical properties of Bali cattle with different ratios of rumen degradable protein (RDP) and non-fiber carbohydrate (NFC). The effect of the ratio of non-fiber carbohydrates to *in vitro* rumen degradable protein of the agro-industrial by-product based on ration for Bali cattle. The experiment was arranged in a 3x3 factorial completely randomized design with four replicates. The first factor was RDP level 55, 60, and 65%, respectively; the second was NFC 35, 40, and 45%. Feed was formulated based on nine combination ratios, i.e., R1=55:35, R2=55:40, R3=55:45, R4=60:35, R5=60:40, R6=60:45, R7=65:35, R865:40, R9=65:45. Parameters measured were physical properties included angle of repose, bulk density, and specific gravity, *in vitro* parameters were dry matter (DM), organic matter (OM), crude fiber digestibility (CFD), volatile fatty acid (VFA), and ammonia (NH3). Results showed that the formulated ratio based on RDP and NFC interaction RDP and NFC did not affect the bulk density and specific gravity. However, the RDP level significantly affected the angle of repose. *In vitro* tests showed no interaction between RDP and NFC on dry matter digestibility (IVDMD) and *in vitro* crude fiber digestibility (IVCFD). However, *in vitro*, the interaction between RDP and NFC influenced organic matter digestibility (IVOMD). RDP and NFC levels significantly influenced IVDMD and IVOMD. The interaction of RDP and NH3 did not significantly influence NH3 concentration and total *in vitro* VFA, but RDP level significantly influenced total VFA; meanwhile, NFC did not influence both NH3 concentration and total VFA. The combination of 65:35 (RDP and NFC) in beef cattle ration resulted in good digestion products, VFA, and NH3 to supply protein and energy.

Keywords: Rumen degradable protein, Non-fiber carbohydrate, Agro-industrial by-products, Bali cattle

Article History
Article # 24-613
Received: 09-May-24
Revised: 30-May-24
Accepted: 14-Jun-24
Online First: 23-Sep-24

INTRODUCTION

Population growth and public awareness of the importance of animal protein have influenced beef demand (Rosmalia et al., 2023). Population growth continues to increase but is not accompanied by optimal beef production. The availability of national beef production is still needing more. Therefore, the government must import cattle and meat from abroad.

Bali cattle contribute significantly to the national meat supply. Bali cattle have quite good abilities in utilizing feed. It can still survive well in conditions where feed is less available (Heraini et al., 2023). On the other hand, when feed is available in sufficient quantities and is of good

Cite this Article as: Syamsu JA, Natsir A, Yamin AA, Zain M, Tanuwiria UH, Yunilas and Amal I, 2024. In Vitro rumen fermentability and physical properties of Bali cattle ration based on agro-industrial by-product with different ratio of rumen degradable protein and non-fiber carbohydrate. International Journal of Agriculture and Biosciences 13(3): 488-493. https://doi.org/10.47278/journal.ijab/2024.158

A Publication of Unique Scientific Publishers

quality, the live weight increases (compensatory growth). Therefore, the provision of quality feed is of prime importance to maintaining the productivity level of Bali cattle.

Nutrition improves performance and meat production (Pinottia et al., 2023). The nutritional adequacy of the feed consumed by livestock must contain balanced nutrition to achieve optimal productivity (Amanah et al., 2024). One of the most important factors determining a feed's nutritional value is its digestibility. Digestibility measurement of feed ingredients *in vitro* is an effort to determine the amount of nutrients contained in feed ingredients that will be degraded and digested in the digestive tract. Rations formulated based on protein requirements are not the main focus in ruminants because microbes can be a source of protein for the host. Therefore, the current ration formulation must be based on the RUP content of the diet (Zain et al., 2024) with a balanced formulation of carbohydrates and protein according to the needs of livestock (Okon et al., 2023). Consuming sufficient amounts of energy shows a better response to protein utilization. (Yunilas, et al., 2023).

Ruminants' protein requirements consist of rumen degradable protein (RDP) and rumen undegradable protein (RUP). RDP is needed as a nitrogen source in microbial protein synthesis. Rumen microbes need nitrogen and carbon to synthesize rumen microbial proteins. Meanwhile, carbon must be available as non-fiber carbohydrates (Rosmalia et al., 2022). Therefore, there is a dire need to research feed ingredients from agro-industry waste for better utilization for the ruminant feed and determine their appropriate ratio.

MATERIALS & METHODS

Feed Preparation

Five sources of agro-industrial by-products (copra meal, groundnut meal, corn meal, rice bran, and full-fat soy) were collected from different locations, and elephant grass was used as a fiber source. After collection, each sample was dried and ground through a 1mm sieve using a hammer mill and mixed using a horizontal mixer. Feed composition and nutrient contents are presented in Table 1. Nutrient contents were analyzed using the AOAC method (AOAC, 2005), and fiber fraction was analyzed using the method of Goering and Van Soest (1970). Feed composition is presented in Table 1. Nutrient contents (moisture, crude protein, fat, crude fiber, Non-Free Extract, and insoluble ash) were according to the AOAC method (AOAC 2005). Fiber fractions (i.e., Acid Detergent Fiber, Neutral Detergent Fiber, cellulose, hemicellulose, lignin, and insoluble ash) were as per Goering and Van Soest (1970) method.

Experimental Design

The experiment was arranged in a 3 x 3 factorial completely randomized design with four replicates. The first factor was RDP level, i.e., 55, 60, and 65%, respectively. The second factor was NFC 35, 40, and 45%, respectively. Feed was formulated based on nine combination ratios: R1=55:35, R2=55:40, R3=55:45, R4=60:35, R5=60:40, R6=60:45, R7=65:35, R865:40, and R9=65:45.

Table 1: Feed composition

Feed Ingredients	Feed Composition (%)								
	R1	R2	R3	R4	R5	R6	R7	R8	R9
Elephant Grass	43	40	30	43	35	28	29.5	25	17
Groundnut	6	3	6	3	3	3	18	15	20
Copra Meal	5	8	10	10	16	21	5	20	19
Corn	8	10	13	13	12	11	29.5	21	31
Rice Bran	25	28	30	14	17	20	2	3	4.5
Full-Fat Soy	12	10	10	16	16	16	15	15	7.5
Mineral	1	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100	100
RDP	55	55	55	60	60	60	65	65	65
NFC	35	40	45	35	40	45	35	40	45

R1=55:35, R2=55:40, R3=55:45, R4=60:35, R5=60:40, R6=60:45, R7=65:35, R865:40, R9=65:45; RDP: Rumen Degradable Protein; NFC: Non-Fiber Carbohydrate.

Parameter Measured

Parameters measured in this research were physical properties, which consist of angle of repose, bulk density, and specific density. In vitro, rumen fermentability included volatile fatty acid (VFA), ammonia (NH_3), dry matter digestibility (IVDMD), organic matter (IVOMD) and crude fiber digestibility (IVCFD). The procedure of each parameter, like i*n vitro* rumen fermentation (VFA, NH3, IVDMD, IVOMD, and IVCFD), is described below:

Angle of Repose

The angle of repose measurement was carried out by dropping the material at a height of 15cm through the same funnel against a flat plane with yellow cardboard measuring 40cm long and 40cm wide. The height of the stack of materials should always be below the funnel. The ingredients were weighed 100g and then poured on slowly. The funnel walls were made to prevent the material from falling constantly. The height and diameter formed were measured using a ruler.

The angle of repose (*tg a*) was determined by measuring the base diameter (*d*) and height (*t*) of the pile when the material bounced after being dropped (Syamsu et al., 2015).

Bulk Density

The bulk density measurement method is done by pouring 20g of the material into a cup (100mL). Each observation should avoid shaking during measurement. The bulk density was calculated by dividing the weight of the material by the volume of the space occupied (g/mL). The highest room volume was added to measure the volume, and the lowest volume was divided by two. Volume was measured by adding the highest value to the lowest volume and dividing it by 2. The bulk density (g/cm^3) was calculated as the weight of the sample (g) divided by volume (cm^3) (Khalil, 1999a).

Specific Density

Specific gravity is a comparison between weight and material volume. A 10g sample was mixed with 50 mL of distilled water. The material residue was mixed with water and allowed to settle at the bottom. The final volume reading was performed after the volume did not move. The difference between the initial and final volumes was material volume (Khalil, 1999b).

In Vitro Fermentation

The second experiment evaluated the RDP ratio to NFC rations based on local feed ingredients on *in vitro* fermentation and digestibility of Bali cattle rations. The treatment diet was carried out as performed by Tilley and Terry (1963). The rumen fluid was obtained from the slaughterhouse in a thermos (39ºC) and filtered. The McDougall solution was used as a buffer (9.8g Na-HCO3, 4.65g Na2HPO4.2H2O, 0.57 KCl, 0.47g NaCl, 0.12g MgSO4.7H2O, and 0.04g CaCl2 into 1L distilled water). A 0.5g sample was placed into a fermenter tube. Samples were fermented by incubating 10mL rumen fluid and 40mL McDougall buffer solution for 4 hours at 39°C in a shaker water bath under anaerobic conditions. A pH meter measured the pH value (Hanna Instrument, HI98191). For the molar proportion of individual volatile fatty acids (VFA), 5mL of the sample was collected, one drop of H2SO4 98% was added, and the sample was stored at -20°C for further analysis. At the end of the fermentation process, two drops of $HgCl_2$ solution were added. The supernatant was collected by centrifugation at 3500rpm for 15min and then stored at -20°C till the NH3 concentration and total VFA concentration were analyzed.

VFA and NH_3 Concentration

The steam distillation method was used in the VFA analysis. Briefly, 5mL supernatant was taken in a distillation tube, and 15% H_2SO_4 was added. Then, it was immediately covered with a rubber cap and connected to a cooling flask. After that, the distillation tube was inserted into a flask containing boiling water (heated continuously during distillation). The hot water vapor would press against the VFA and condense in the cooler. The water formed was collected in an Erlenmeyer flask containing 5mL of 0.5N NaOH until it reached 300mL. PP (Phenolphthalein) indicator was added 2 – 3 drops and titrated with HCl 0.5N until the color of the titrate changed from pink to colorless.

NH_3 concentration was measured by smearing the cup's rim with Vaseline (Conway, 1958). Briefly, 1mL of the supernatant of the sample was taken and then placed at one end of the Conway cup groove. After that, the saturated Na_2CO_3 solution was placed at one end of the Conway cup next to the supernatant (must not be mixed). Then, 1mL of boric acid solution with a red indicator was placed in a Conway cup. The cup was smeared with Vaseline and closed until it was airtight. The Na_2CO_3 solution was mixed with the supernatant until evenly distributed by shaking and tilting the cup. After that, it was left for 24 hours at room temperature, then the cup lid was opened, and the boric acid indicator was titrated with 0.005N H_2SO_4 until the color changed from red to blue.

In Vitro Digestibility

For IVDMD and IVOMD, a fermenter tube was filled with 0.5g of sample and 40mL of McDougall's solution. The tube was put into a shaker bath at 39°C and then filled with 10mL rumen fluid; the tube was shaken with CO_2 flowing for 30 seconds until the pH was 6.5 to 6.9 and then closed with a ventilated rubber, and fermented for 48 hours. After 48 hours, the rubber cap of the fermenter tube was opened, and 2-3 drops of $HgCl_2$ were added to kill microbes. The fermenter tube was centrifuged at 5,000rpm for 15min. The substrate was separated into a sediment at the bottom and a clear supernatant at the top. The supernatant was discarded, and the sediment obtained from centrifugation (5,000rpm for 15min) was added to 50 mL of 0.2% pepsin-HCl solution. This mixture was then incubated again for 48 hours without a rubber cap. The remaining digestion was filtered using Whatman filter paper # 41 with the help of a vacuum pump. The residue on the filter paper was put into a porcelain cup and then placed in a 105°C oven for 24 hours. After 24 hours, the porcelain cup, filter paper, and residue were removed, put into a desiccator and weighed to determine the dry matter content (Table 2). The material in the cup was ignited or ashed in an electric furnace for 6 hours at a temperature of 450-600°C, then weighed to determine the level of organic material. As a blank, residue from fermentation without feed ingredients was used.

Statistical Analysis

The data were obtained and statistically analyzed by One-Way Analysis of Variance, followed by Duncan's New Multiple Range Test using SPSS (SPSS for Windows, 16.0, SPSS Inc.).

RESULTS & DISCUSSION

Physical Properties

The physical properties of the ration are shown in Table 3. The results showed that RDP and NFC treatments influenced significantly ($P<0.05$) the angle of repose. Meanwhile, the interaction of RDP and NFC did influence. Feed ingredients used varied according to the formulation. Results showed that a 65:35 ratio (RDP*NFC) generated a larger angle of repose (48.86º) compared to other ratios.

The angle of repose is the maximum angle at which the body can be positioned without sliding or slumping to the horizontal surface (Pekel et al., 2020). The high course particles and proportions of elephant grass used in formulations change the mobility of materials to perform larger angles. Feed ingredient types and composition or formulation are also determining factors affecting the angle of repose. Moreover, particle size and shape also influence the angle of repose. Nwankwojike & Nduk (2012) reported that the average angle of repose of digested palm oil mash was 48.86º. Fu et al. (2020) reported that the angle of repose is influenced by angular ballast, friction coefficient, and lower sphericity or roundness. On the other hand, moisture also affects the friction coefficient; the increased friction coefficient is due to the increase in moisture content of ingredients (Ulyanov et al., 2020).

The results of specific gravity measurements showed that RDP and NFC ratio did not significantly influence specific gravity. Specific gravity is crucial in various processing, handling, and storage processes. Specific gravity is a determining factor of bulk density, specific density is the ratio of the material mass to volume, and bulk density is a comparison between the mass of the material with the volume of space occupied through the pouring process (Retnani et al., 2011).

Table 2: Dietary nutrient contents of ration based on dry matter

RDP	NFC	Nutrient Composition (%)					
		Moisture	Crude Protein	Fat	Crude Fiber	NFE	Insoluble Ash
55	35	8.50	16.48	9.10	19.05	44.43	10.94
	40	8.85	17.06	8.78	16.49	47.87	9.80
	45	9.03	17.05	10.57	13.50	49.16	9.71
60	35	9.23	16.97	8.07	16.42	48.62	9,93
	40	9.28	18.64	8.81	14.54	48.12	9.90
	45	9.42	20.89	9.16	13.85	46.73	9.37
65	35	9.31	17.96	11.05	15.33	44.41	11.25
	40	9.28	20.74	9.75	13.55	44.51	11.45
	45	9.76	18.50	10.12	10.86	49.33	11.19
Fiber Fraction (%)							
		ADF	PDF	Cellulose	Hemicellulose	Lignin	Insoluble Ash
55	35	26.02	46.22	19.61	20.19	5.07	1.34
	40	25.16	42.98	18.47	17.82	5.51	1.19
	45	21.68	38.61	16.12	16.92	4.78	0.78
60	35	27.46	46.79	20.40	19.33	5.93	1.13
	40	24.41	43.04	18.27	18.63	5.14	1.00
	45	23.16	43.81	17.78	20.64	5.02	0.36
65	35	22.08	41.36	15.83	19.27	4.53	1.73
	40	24.30	41.87	17.26	17.57	5.24	1.80
	45	19.41	36.45	13.45	17.04	4.99	0.97

RDP: Rumen Degradable Protein; NFC: Non-Fiber Carbohydrate; NFE: Nitrogen Free Extract; ADF: Acid Detergent Fiber; PDF: Neutral Detergent Fiber

Table 3: Physical properties of RDP and NFC ratios

Physical Properties	RDP	NFC			Mean	P-value		
		35	40	45		RDP	NFC	RDP*NFC
Angle of response (°)	55	48.30	47.41	48.49	48.07a	0.010	0.019	0.764
	60	49.30	48.42	48.79	48.84b			
	65	49.40	48.37	48.82	48.86b			
	Mean	49.00a	48.07b	48.70a				
Specific density (kg/m^3)	55	251.98	267.26	272.82	264.02	0.176	0.897	0.881
	60	245.04	236.11	236.11	239.09			
	65	250.00	260.91	251.98	254.30			
	Mean	249.01	254.76	253.64				
Bulk Density (kg/m^3)	55	463.33	465.75	465.75	464.94	0.339	0.889	0.52
	60	452.72	468.39	465.23	462.11			
	65	498.42	471.29	466.28	478.66			
	Mean	471.49	468.47	465.75				

RDP: Rumen Degradable Protein; NFC: Non-Fiber Carbohydrate

In the present study, bulk density measurement was not statistically (P>0.05) influenced by RDP and NFC ratio. However, bulk density was greatly influenced (P<0.05) by the type of feed ingredients, such as fibrous feedstuff. Fibrous feedstuff such as elephant grass, rice bran, and coconut meal were used in this study. More voluminous ingredients were used, resulting in higher bulk density values for the feed. According to Jaelani et al. (2016), particle size and fineness of material affect bulk density. Feed with a high NFC ratio used a higher proportion of ground grain and seed (corn and full-fat soya) in formulation change bulk density. Lisowski et al. (2020) reported that the specific and bulk densities of pellets produced from the blend of hay and straw were the highest. Yang et al. (2020) reported that the digestible fiber-to-starch ratio of pelleted had linear decreasing effects on bulk density.

The physical properties of feed ingredients play an important role in feed processing and handling (Syamsu et al., 2015; Macho et al., 2020). Generally, physical properties measurement is needed to evaluate the characteristics of feed ingredients before feed processing. On the other hand, the physical properties of feed ingredients are directly correlated to nutritional content. Ridla et al. (2023) reported that the physical properties and nutrient content of rice bran are closely correlated, and bulk density and compacted bulk density of rice bran positively correlate with crude protein content. Conversely, it has a negative correlation to crude fiber.

***In Vitro* Fermentation and Digestibility**

IVDMD and IVOMD are presented in Table 4. The interaction between RDP and NFC did not influence IVDMD but influenced IVOMD. The level of RDP significantly influenced IVDMD and IVOMD. The 60% of RDP generated the highest IVDMD, but the highest IVDMD was 35% of NFC level. However, 65% of RDP generated the highest IVOMD. Furthermore, there was no interaction between the RDP and NFC ratio RDP and NFC levels.

The feed quality was not only determined by chemical composition but also influenced by fiber fraction and digestibility (Pratama et al., 2018). Digestibility is closely related to chemical composition, particularly crude fiber content. Hartono et al. (2015) stated the crude fiber-containing ingredient has a thicker cell wall, so it causes lower feed digestibility. Generally, the low crude fiber content of ingredients will be more easily digested due to the thin cell walls of the material; thus, digestive juices are easily penetrated during fermentation, and rumen microbes could break down crude fiber (cellulose and hemicellulose) as well.

Rumen fermentation characteristics (NH3 and VFA) are shown in Table 4. The interaction of two factors (RDP and NH3) did not significantly influence NH3 concentration

Table 4: RDP and RAC ratio of rations based on local feed ingredients on in vitro fermentation and digestibility of Bali cattle rations

Parameters	RDP	NFC			Mean	P-value		
		35	40	45		RDP	NFC	RDP*NFC
In vitro dry matter digestibility (%)	55	61.75	59.92	56.71	59.46a	0.001	0.529	0.935
	60	74.47	75.46	74.04	74.65b			
	65	72.43	68.44	68.22	69.70b			
	Mean	69.55	67.94	66.32				
In vitro organic matter digestibility (%)	55	74.38	80.38	65.56	73.44a	0.001	0.582	0.023
	60	77.46	69.41	97.61	81.49b			
	65	95.66	95.59	93.35	94.87c			
	Mean	82.50	81.79	85.51				
In vitro crude fiber digestibility (%)	55	8.88	11.06	12.19	10.71	0.080	0.856	0.32
	60	12.69	10.24	8.55	10.49			
	65	6.07	8.82	7.63	7.50			
	Mean	9.21	10.04	9.45				
NH3 concentration (mM)	55	20.86	16.95	17.57	18.46a	0.039	0.081	0.273
	60	22.06	18.53	26.55	22.38b			
	65	21.20	21.87	26.26	23.11b			
	Mean	21.37	19.12	23.46				
Total volatile fatty acid (VFA) production (mM)	55	150.39	72.28	81.73	101.47a	0.002	0.028	0.063
	60	100.44	40.77	130.59	90.60a			
	65	156.66	152.19	147.86	152.24b			
	Mean	135.83b	88.41a	120.06ab				

Mean values bearing different alphabets in a column under specific parameters differ significantly (P<0.05). RDP: Rumen Degradable Protein; NFC: Non-Fiber Carbohydrate; VFA: Volatile Fatty Acid.

and total VFA, but RDP level significantly influenced total VFA; meanwhile, NFC did not influence either NH3 concentration and total VFA. NH3 concentration increased linearly from 18.46 to 23.11mM by increasing the level of RDP, but the VFA value showed inconsistent results. The VFA values were 101.47(55% RDP), 90.60 (60% RDP), and 152.24mM (65% RDP). The feed contained 55% of RDP fell, then increased when the RDP level was increased to 65%. NFC also showed similar results.

RDP of formulated feed was influenced by nitrogen availability for microbial synthesis. Supplying nitrogen and energy in appropriate time and adequate amounts could optimize rumen microbial synthesis (Afzalzadeh et al., 2010). Furthermore, microbial activities would increase protein degradation by producing NH3 (Putri et al., 2021). The increase in NH3 is due to the increasing RDP level. It indicates that a higher number of degradable proteins were formed during incubation. It is necessary to achieve a higher RDP ratio using more dietary protein sources. Ruminants require nitrogen resources in two forms, i.e., protein and NPN. All dietary protein is degraded in the rumen to produce approximately 60% of NH3 (Wulandari et al., 2017). Ammonia is the primary precursor of protein microbial synthesis through deamination because microbes cannot indirectly utilize protein sources (Jayanegara et al., 2017).

Feed digestion is necessary to produce VFA as an energy source for ruminants (Tampoebolon et al., 2019). VFA is a fermentation product produced by microbial activity (Yang et al., 2020). A 65% RDP to 35% NFC combination could result in a higher VFA (156.66mM) than other ratios. Dijkstra (1994) stated that VFA is one of the main fermentation products produced from the fermentation of organic matter in the rumen; relative amounts of VFA that are formed depend on several factors, such as the type and availability of substrate, as well as the presence of certain microbiological species. Sun et al. (2020) reported that corn contains a high NFC, which would increase total VFA.

Conclusion

Agricultural by-products are potentially utilized as beef cattle feed based on physical and nutritional quality. The effect of the ratio with RDP, NFC, and its interaction on physical properties showed different results. It showed that interaction between RDP and NFC did not affect the angle of repose, bulk density, and specific gravity, but RDP level significantly affected the angle of repose. The research results also show that the best combination of RDP and NFC is 65% and 35% in several aspects. It positively impacts the digestibility of OM, DM, and CF. RDP level significantly influenced total VFA but did not influence NH3 concentration.

Authors Contribution

JAS, AN, MZ, UHT, and YNL designed the research; JAS, AN, AAY, and IA collected and analyzed data and compiled and reviewed the manuscript. All the authors read and approved the final version of the manuscript.

Acknowledgment

We appreciate the Institute for Research and Community Service of Hasanuddin University for the funding through The Indonesian Collaborative Research Program by contract number 01825/UN4.22/PT.01.03/ 2023.

REFERENCES

Afzalzadeh, A., Rafiee, H., Khadem, A.A. and Asadi, A. (2010). Effects of ratios of non-fibre carbohydrates to rumen degradable protein in diets of Holstein cows: 2. Rumen and plasma parameters and nitrogen metabolism. *South African Journal of Animal Science*, *40*(3), 213-220.

AOAC, (2005). Official Methods of Analysis 18th ed., Association of Official Analytical Chemists, Washington, DC, USA.

Amanah, U., Zain, M., Elihasridas, Putri, E. M., and Pazla, R. (2024). Comparison of nutritive value and RDP-RUP contents of tropical legumes from two different areas with in-vitro methods. *International Journal of Veterinary Science, 13*(1), 66-73.

Conway, E. J. (1958). Microdiffusion Analysis and Volumetric Error. Macmillan Publishing Co.

Dijkstra, P. (1994). Production and absorption of volatile fatty acids in the rumen. *Livestock Production Science*, *39*(1), 61-69.

Fu, J.J., C. Chen., J. F. Ferellec., and J. Yang. (2020). Effect of particle shape on repose angle based on hopper flow test and discrete element method. *Advances in Civil Engineering*, 2020, 8811063.

Goering, H. K. and Van Soest, P. J., (1970). Forage Fiber Analysis (Apparatus Reagents, Procedures and Some Applications). Agriculture Handbook. United States Department of Agriculture, Washington DC.

Hartono, R., Fenita, Y., and Sulistyowati, E. (2015), Uji in vitro kecernaan bahan kering, bahan organik dan produksi N-NH3 pada kulit buah durian (durio zibethinus) yang difermentasi jamur tiram putih (pleurotus ostreatus) dengan perbedaan waktu inkubasi. *Jurnal Sain Peternakan Indonesia*, *10*(2), 87-94.

Heraini, D., Fatmila, D.T., Pardede, B.P., Yudi, Y., and Purwantara, B. (2023). Age and individual variation affect semen quality and field fertility of Bali bulls (*Bos sondaicus*): A preliminary study. *Developing Modern Livestock Production in Tropical Countries*. www.taylorfrancis.com

Jaelani, A., Dharmawati, S., and Wacahyono, (2016). Pengaruh tumpukan dan lama masa simpan pakan pelet terhadap kualitas fisik. *Ziraa'ah*, *41*(2), 261-268.

Jayanegara, A., Novandri, B., Yantina, N., and Ridla, M. (2017). Use of black soldier fly larvae (*Hermetia illucens*) to substitute soybean meal in ruminant diet: An *in vitro* rumen fermentation study. *Veterinary World*, *10*(12), 1439–1446.

Khalil, (1999a). Pengaruh kandungan air dan ukuran partikel terhadap perubahan perilaku fisik bahan pakan lokal: kerapatan tumpukan, kerapatan pemadatan tumpukan, dan berat jenis. *Media Peternakan*, *22*(1), 1-11.

Khalil, (1999b). Pengaruh Kandungan Air dan Ukuran Partikel terhadap Sifat Fisik Pakan Lokal: Sudut Tumpukan dan Faktor Higroskopis. *Media Peternakan, 22*(1), 33-42.

Lisowski, A., Matkowski, P., Dąbrowsk, M., Piątek, M., Świętochowski, A., Klonowski, J., Mieszkalski, L., and Reshetiuk, V. (2020). Particle size distribution and physicochemical properties of pellets made of straw, hay, and their blends. *Waste and Biomass Valorization,* 11, 6-75.

Macho, O., Demková, K., Gabrišová, L., Cierny, M., Mužíková, J., Galbavá, P., Nižnanská, Ž., Blaško, J., Peciar, P., Fekete, R., and Peciar, M. (2020). Analysis of static angle of repose with respect to powder material properties. *Acta Polytechnica, 60*(1), 73-80.

Nwankwojike and Nduka, B. (2012). Angle of repose of digested oil palm fruit mash and pulp on steel. *International Journal of Innovative Technology & Creative Engineering*, *2*(2), 12-17.

Okon, P., Bachmann, M., Wensch-Dorendorf, M., Titze, N., Rodehutscord, M., and Rupp C., (2023). Feed clusters according to *in situ* and *in vitro* ruminal crude protein degradation. *Animals, 13*, 1–18.

Pinottia, L., Mazzolenia, S., Moradeia, A., Lina, P., and Luciano, A. (2023). Effects of alternative feed ingredients on red meat quality: a review of algae, insects, agro-industrial by-products and former food products. *Italian Journal of Animal Science*, *22*(1), 695710.

Pekel, A. Y., A. Çalık., E. Kuter., M. S. Alataş., S. B. Öklen., A. Kızıl., M. Bulat., and Ö. Cengiz., (2020). Impact of chemical and physical properties on flowability characteristics of corn distillers dried grains with solubles. *International Agrophysics, 34*, 195-202.

Pratama, S. M., Wajizah, S., Jayanegara, A., and Samadi, (2018). Evaluation of agro-industrial by-products as potential local feed for ruminant animals: chemical composition, fiber fractions and *in vitro* rumen fermentation. *Animal Production, 20*(3), 155-164.

Putri, E. M., Zain, M., Warly, L., and Hermon, H. (2021). Effect of rumen-degradable-to-undegradable protein ratio in ruminant diet on *in vitro* digestibility, rumen fermentation, and microbial protein synthesis. *Veterinary Word, 14*(3), 640-648.

Retnani, Y., Herawati, L., and Khusniatiuji, S., (2011). Sifat fisik ransum broiler starter bentuk crumble berperekat tepung tapioka, bentonit dan onggok. *Jurnal Ilmu dan Teknologi Peternakan,* 1 (2), 88-97.

Ridla, M., Adjie, R. H. N., Ansor, S., Jayanegara, A., and Martin, R. S. H. (2023). Correlation of Physical Characteristics and Nutrient Content of Rice Bran. *Jurnal Peternakan*, *20*(1), 1-8.

Rosmalia, A., Permana, I. G., and Despal, D. (2022). Synchronization of rumen degradable protein with non-fiber carbohydrate on microbial protein synthesis and dairy ration digestibility. *Veterinary World, 15*(2), 252-261.

Rosmalia, A., Permana, I. G., Despal, D., Toharmat, T., Pambudi, F. R., and Arif, S. I. Z. (2023). Effect of dietary non-fiber carbohydrate sources and sulfur supplementation on *in vitro* ruminal fermentation and digestibility of the dairy ration. *Iranian Journal of Applied Animal Science*, *13*(2), 231-240.

Sun, L., Lee, M., Jeon, S., and Seo, S. (2020). Evaluation of the associative effects of rice straw with timothy hay and corn grain using an in vitro ruminal gas production technique. *Animals*, *10*(325), 1-12.

Syamsu, J. A., Yusuf, M., and Abdullah, A. (2015). Evaluation of Physical Properties of Feedstuffs in Supporting the Development of Feed Mill at Farmers Group Scale. *Journal of Advanced Agricultural Technologies, 2*(2), 147-150.

Tampoebolon, B. I. M., Prasetiyono, B. W. H. E., and Mukodiningsih, S., (2019). The effect of fermentation with different times of corn husk which has obtained ammoniation treatment in the production of VFA-NH3 by in vitro digestibility. *IOP Conf. Ser.: Earth Environment Science, 247,* 012073.

Ulyanov, V., Utolin, V., Luzgin, N., Krygin, S., and Parshina, M. (2020). Studying physical and mechanical characteristics of corn feed. *BIO Web of Conferences, 17*, 00209.

Yang, G., Zhao, F., Tian, H., Li, J., and Guo, D. (2020). Effects of the dietary digestible fiber-to-starch ratio on pellet quality, growth and cecal microbiota of Angora rabbits. *Asian-Australas Journal Animal Science, 33*(4), 623-633.

Yunilas, Y., Zain, M., Tanuwiria U.H., and Syamsu, J.A., (2023). The balance of rumen degradable protein and readily available carbohydrate in sheep rations on *in vitro* fermentability. *Journal Advance Veterinary and Animal Resource*, *10*(4), 738–743.

Wulandari, Widyobroto, B. P., Noviandi, C. T., and Agus, A. (2017). In vitro degradation and rumen fermentation characteristics of soybean meal protected with different levels of formaldehyde. The 7th International Seminar of Tropical Animal Production (ISTAP).

Zain, M., Tanuwiria, U. H., Syamsu, J. A., Yunilas, Y., Pazla, R., Putri E. M, Makmur, M., Amanah, U., Shafura, P. O., and Bagaskara, B. (2024). Nutrient digestibility, characteristics of rumen fermentation, and microbial protein synthesis from Pesisir cattle diet containing non-fiber carbohydrate to rumen degradable protein ratio and sulfur supplement. *Veterinary World, 17*(3), 672–681.