P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article https://doi.org/10.47278/journal.ijvs/2022.218

Evaluation of Legumes, Roughages, and Concentrates Based on Chemical Composition, Rumen Degradable and Undegradable Proteins By *In Vitro* Method

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Article History: 22-741 Received: 06-Nov-22 Revised: 26-Nov-22 Accepted: 29-Nov-22

ABSTRACT

The database of rumen-degradable and undegradable proteins (RDP-RUP) of feed in Indonesia still needs to be improved. RDP-RUP-based diet has high urgency because it contains metabolizable proteins, an accurate material utilized by ruminants for maintenance, growth, and production. The present research aims to discover the grade of rumen-degradable proteins (RDP) and undegradable proteins (RUP) and the chemical contents in legumes, roughages and concentrates used as cattle feed by farmers from Indonesia. Proximate and Van Soest analyses were used to specify chemical contents. Dry matter and organic matter digestibility, RDP-RUP, and rumen fermentation characteristics were set by in vitro Tilley and Terry Method. The inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) tool was used to determine mineral contents, and the fatty acid profile was determined following the AOCS method. The highest protein contents in legumes were in *Calliandra calothyrsus* (26.08%) and the lowest in *Arachis pintoi* (14.36%). In roughages, the highest protein contents were in corn straw (11.04%) and the lowest in rice straw (6.44%). Among concentrates, the highest protein content in soybean meal (40.97%) and the lowest in onggok (4.33%). In legumes, roughages, and concentrates, the highest RDP was in *Sesbania grandiflora* (79.97%), corn husk (46.83%), and soybean meal (80.67%) while the lowest RDP was in *Calopogonium mucunoides* (58.19%), rice straw (5.07%) and onggok (37.88%), respectively. In conclusion, knowing the nutrient composition of feed ingredients is helpful for diet formulation. Additionally, we must use RDP and RUP in the composition of ruminant cattle feeds to meet the needs of the animals and rumen microorganisms. Since there is still a need for knowledge on the RDP and RUP of feeds, this study helps create a ruminant livestock diet based on RDP and RUP levels.

Key words: RDP, RUP, Chemical Compositions, Ruminant.

INTRODUCTION

Feed ingredients' nutritional value has prime importance in ration formulation. The quality of feed nutrients directly impacts the performance and production of ruminants. The composition, pace, and digestibility of the ruminant animals' feed determine its nutritional value. The primary goal of feed ingredient chemical analysis is to foretell how livestock will respond to being fed in the form of rations. Therefore, knowledge of the chemical components of feed materials is crucial for creating livestock rations (Kumar et al. 2015).

The proteins in ruminants' ration formulation play an important role as proteins are utilized by both ruminants and rumen microbes. Rumen microbes require NH_3 and RDP products to synthesize microbial proteins. At the same time, host animals use RUP and microbial proteins synthesized by rumen microbes (Tedeschi et al. 2015). RDP is a fraction of proteins degraded in the rumen-by-rumen microbes for microbial protein synthesis.

Cite This Article as: Zain M, Despal, Tanuwiria UH, Pazla R, Putri EM and Amanah U, 2023. Evaluation of legumes, roughages, and concentrates based on chemical composition, rumen degradable and undegradable proteins by *in vitro* method. International Journal of Veterinary Science 12(4): 528-538. https://doi.org/10.47278/journal.ijvs/2022.218

Proteolytic enzymes of rumen microbes rapidly deaminate these protein fractions to produce ammonia and carbon (Haryanto 2014; Tedeschi et al. 2015). The results of protein synchronization with feed energy maximize the use of nutrients by ruminants and microbial protein synthesis (Moradi and Zadeh 2013). Microbial proteins are used by livestock as amino acids post-hydrolysis in the intestine (Haryanto 2014; Pazla et al. 2018a).

Besides that, RUP, a protein fraction, does not degrade in the rumen, so it bypasses the post-rumen and digests in the intestines. RUP increases amino acid passage in the duodenum. Absorption of RUP depends on digestibility in the post-rumen. However, the feed can bypass the rumen due to low digestibility (Akhtar et al. 2016).

Adequate nitrogen for rumen microbes in the form of RDP and a direct supply of proteins (RUP) for the host animals are required in the feed. RDP and RUP ratios appropriate in the ration are required to maximize livestock production more effectively. Giving RUP over RDP decreases rumen microbial proteins production, volatile fatty acids (VFA), and the ability of rumen microbes in carbohydrate fermentation. In contrast, excess RDP causes the rumen to produce excessive NH_3. The liver must use energy to convert the extra NH_3 to urea. As Indonesia lacks information on aspects, there is dire need to generate such information on the levels of RDP and RUP of feed formulation for ruminants; this is why this study was planned and executed.

MATERIALS AND METHODS

Study Period and Experimental Site

This research was carried out at Andalas University's Ruminant Laboratory of Animal Science Faculty from July to September 2022.

Sampling

We analyzed for RDP, RUP, dry matter, and organic matter digestibility of *Calliandra calothyrsus, Arachis pintoi, Sesbania grandiflora, Calopogonium mucunoides, Arachis hypogea,* native grass, corn straw, corn husk, rice straw, tofu waste, coconut cake, soybean meal, and onggok. Samples were collected around the town of Payakumbuh, West Sumatera, Indonesia. Each sample was dried using sunlight in the Greenhouse, Faculty of Animal Husbandry, Andalas University. Afterward, drying was carried out at 60ºC (in the oven) for 48 hours until the sample could be chopped and mashed (1mm) using a grinder machine. After that, each sample was stored in an air-tight plastic pack until they were analyzed.

Chemical Analysis of Samples

Each sample was determined to contain crude protein, ash, dry matter, and crude fat by proximate analysis (AOAC 2005). Van Soest's analysis was carried out to know neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose, and acid detergent lignin (ADL) (Goering and Van Soest 1970).

In Vitro Method

The Tilley and Terry method was applied to determine each feed ingredient's digestibility, RDP and RUP levels, and rumen characteristics *in vitro* for 48 hours for roughages and 24 hours for concentrates, and the incubation period ended by immersing the Erlenmeyer tube in ice water to stop the microbial activity, followed by a pH measurement. The supernatant was separated from the residue and centrifuged for 30min at 3000rpm at 40˚C. The supernatant was frozen for NH_3 and total VFA analysis. NH_3 concentration was determined using the Conway method, and steam distillation was used to determine the total concentration of VFA. After filtration with Whatman No. 41 filter paper, the residues were dried at 60˚C in an oven, then analyzed to determine nutrient digestibility following the proximate analysis method.

Determination of mineral contents

Samples of concentrates, roughages, and legumes were dried at 60˚C in an oven, meshed, and made powder. Then in 1g powder, added 2mL of distilled water dried 150˚C for 15min, then the sample was cooled at room temperature, and after adding 25mL of distilled water, the sample was filtered through 45 grit filter paper. ICP-OES, an inductively coupled plasma optical emission spectroscopy instrument, was used to assess the mineral contents (Mg, P, Ca, S, Co, and Zn).

Determination of Fatty Acid Composition

A standard method was followed to analyze the VFA before foraging samples. AOCS (1993) solvent-based extraction of lipids and the creation of methyl esters of fatty acids (FAME) was followed by utilizing gas to transmethylate FAME. The produced FAME was then subjected to a gas analysis. Chromatography (Agilent model 7890B, USA-based Technologies, Inc.), outfitted with Supelco SPTM 100 m x 0.25 mm x 0.2 m capillary column to separate the methyl ester and an ionized sensor) was used to detect it (AOAC 2000). The temperature increased by 3 running ramps at 30°C each minute. Both the injectors' indicators were placed at 225°C and 240°C, respectively. As a carrier gas, high-purity nitrogen (N_2) with a split of 1:100 was used at a flow rate of 18cm/sec. By comparing the retention durations with FAME standards, it was possible to identify the fatty acids present in the sample. The amount of fatty acids present was expressed as a percentage.

Statistical Analysis

All data were statistically analyzed using analysis of variances with SPSS software version 21.0. The means of each parameter that had a significant effect with the probability level of P<0.05 were further analyzed using the post-hoc Duncan's multiple range test.

RESULTS

Chemical Composition of Legumes, Roughages, and Concentrates

We observed that chemical composition was significantly (P<0.05) different among feed ingredients (Table 1). The highest crude protein contents and crude fat percentage were in *Calliandra calothyrsus* (26.08%), *Sesbania grandiflora* (5.95%), and the lowest in *Arachis pintoi* (14.36%), and *Arachis hypogea* (1.84%), respectively. NDF and ADF were high in *Calopogonium mucunoides* (56.13%) and *Arachis hypogea* (41.00%) while low in *Arachis pintoi* (44.07%) and *Sesbania grandiflora* (36.33%), respectively. The highest contents of cellulose, hemicellulose, and acid detergent lignin were found in *Arachis hypogea* (29.27%), *Sesbania grandiflora* (16.34%), and *Calliandra calothyrsus* (21.33%), respectively. *Arachis pintoi* contained hemicellulose; the lowest ADLs were 7.10 and 9.64%. The lowest cellulose value was found in *C. calothyrsus* (16.84%).

Chemical contents among the roughages were significantly (P<0.05) different (Table 2). Corn straw contained the highest crude protein (11.04%), followed by crude protein in native grass, corn husk, and rice straw (6.44%). Field grass was found to have the most significant crude fat (2.49%), while the lowest was in a corn husk (1.15%). The highest NDF and ADF contents were found in a corn husk (82.67%) and rice straw (57.99%), while the lowest was found in corn straw (69.30%) and corn husk (46.06%), respectively. Corn husk had the highest cellulose (36.43%) and hemicellulose (36.61%), while the lowest was in corn straw (29.43 and 19.83%), respectively. The highest ADL was found in corn straw (12.61%), whereas the lowest was in a corn husk (4.89%).

Table 3 presents the significant (P<0.05) difference in chemical compositions of various types of concentrates. Dry matter was higher (P<0.05) in coconut cake, onggok, and Soybean meal compared with tofu waste. Organic matter was higher in onggok and tofu waste compared with coconut cake and Soybean meal (Table 3). Soybean meal contained the highest crude protein (40.97%) and the lowest in onggok (4.33%). The highest crude fat was in coconut cake (15.90%), and the lowest was in onggok (0.10%). The highest crude fiber was found in tofu waste (19.00%), and the lowest was in onggok (4.18%). The highest nitrogen-free extract (NFE) contents (89.59%) were in onggok, while the lowest was in soybean meal (44.06%).

RDP, RUP, Dry Matter, and Organic Matter Digestibility

Dry matter digestibility (DMD), organic matter digestibility (OMD), RDP, and RUP of legumes, roughages, and concentrates were significantly (P<0.05) different (Table 4). In legumes, DMD was significantly (P<0.05) higher in *Sesbania grandiflora*, *Arachis hypogea*, and *Arachis hypogea* compared to *Calliandra calothyrsus*. The statistically highest OMD was found in *Arachis pintoi*, *Arachis hypogea*, and *Sesbania grandiflora* compared with *Calopogonium mucunoides* and *Calliandra calothyrsus*. *Sesbania grandiflora* and *Sesbania grandiflora* contained the highest RDP and RUP compared with other legumes (Table 4).

Table 1: Chemical composition of legumes

Parameters	*Calliandra calothyrsus*	*Arachis pintoi*	*Sesbania glandiflora*	*Calopogonium mucunoides*	*Arachis hypogea*
Dry matter	92.88±0.82d	91.59±0.26b	92.91±0.05cd	92.16±0.27c	90.87±0.10a
Organic matter	93.40±0.68d	92.08±0.05c	90.62±1.52b	92.67±0.76cd	86.71±3.78a
Crude protein	26.08±3.69c	14.36±2.76a	25.18±11.46c	17.68±2.26ab	20.80±11.87b
Crude fat	3.50±0.62b	1.88±0.17a	5.95±0.05c	3.08±0.94b	1.84±0.31a
Neutral detergent fiber	49.98±1.1a	44.07±5.36a	52.67±2.47b	56.13±1.31c	50.43±0.43b
Acid detergent fiber	39.38±0.12b	36.97±1.17a	36.33±0.25a	40.05±1.12b	41.00±1.55b
Cellulose	16.84±2.23a	26.98±3.25c	22.56±4.60b	25.98±1.84c	29.27±0.13c
Hemicellulose	8.60±0.99a	7.10±4.19a	16.34±2.72b	16.07±0.19b	9.43±1.97a
Acid detergent lignin	21.33±1.99c	9.64±2.26a	13.88±4.86b	13.61±0.89b	11.28±0.89b

Values (mean±SD) bearing different alphabets within the same row are significantly different at P<0.05.

Table 2: Chemical composition (%) of roughages

Parameters	Native grass	Corn straw	Corn husk	Rice straw
Dry matter	93.77±0.38b	91.94±0.88a	92.57±0.99a	95.33±1.80c
Organic matter	83.94±7.05a	91.33±0.19b	91.95±0.87b	82.10±0.19a
Crude protein	8.61±0.39b	11.04±0.97b	6.77±1.61a	6.44±0.97a
Crude fat	2.49±0.05	1.78±0.07	1.15±0.06	2.12±0.07
Neutral detergent fiber	72.26±3.10a	69.30±2.38a	82.67±4.71b	78.21±1.72b
Acid detergent fiber	46.73±2.22b	49.48±2.33b	46.06±2.79a	57.99±2.03c
Cellulose	30.94±0.63a	29.43±1.27a	36.43±1.95b	32.69±1.97a
Hemicellulose	25.53±5.33a	19.83±4.88a	36.61±7.50b	20.22±3.47a
Acid detergent lignin	11.96±0.10b	12.61±0.55b	4.8±0.01a	9.00±0.58b

Values (mean±SD) bearing different alphabets within the same row are significantly different at P<0.05.

Table 3: Chemical composition of concentrates

Parameters	Tofu waste	Coconut cake	Soybean meal	Onggok
Dry matter	76.82±0.51a	92.50±0.83b	89.52±0.08b	90.76±2.42b
Organic matter	97.53±0.14b	94.67±3.62a	92.66±0.15a	98.20±0.33b
Crude protein	25.86±0.95c	8.47±0.04b	40.97±1.49d	4.33±2.58a
Crude fat	2.32±0.67a	15.90±0.18b	0.62±0.06a	0.10±0.008a
Crude fiber	19.00±0.98b	13.10±6.23b	7.01±0.98a	4.18±0.22a
Nitrogen free extract	50.35±0.17b	57.20±0.13b	44.06±0.21a	89.59±0.11c

Values (mean±SD) bearing different alphabets within the same row are significantly different at P<0.05.

In roughages, the highest percentage of DMD, OMD, and RDP were found in corn husks, while rice straw produced the lowest DMD, OMD, and RDP. Rice straw produced the highest RUP, while the lowest was found in a corn husk. In concentrates, Soybean meal produced the highest DMD, OMD, and RDP, whereas soybean meal produced the lowest RUP. The lowest DMD and OMD were found in coconut cake. Onggok produced the lowest RDP and the highest RUP (Table 4).

Characteristics of Rumen Fermentation

The pH, NH_3, and VFA of the rumen fluid of legumes, roughages, and concentrates are presented in Table 5. The pH of legumes, roughages, and concentrates in the present study was significantly ($P>0.05$) different. The concentrations of NH_3 and VFA were significantly different ($P<0.05$). The highest NH_3 concentration in the legumes was found in *Arachis hypogea* (13.50mM) and the lowest in *Arachis pintoi* (8.00mM). Corn straw produced the highest NH_3 in the roughage species, while rice straw produced the lowest NH_3. In the concentrate type, the highest NH_3 was produced by soybean meal (29.22mM), and onggok had the lowest (2.42mM). The maximum level of VFA in legumes, roughages, and concentrate was produced by *Calopogonium mucunoides*, native grass, and onggok, with values of 107.50, 58.33, and 103.33mM, respectively. The lowest VFA was found in *Sesbania grandiflora*, corn husk, and tofu waste, with values of 77.50, 48.67 and 83.67 mM, respectively (Table 5).

Feed Minerals

The mineral contents of legumes, roughages, and concentrates are presented in Table 6. From the group of legumes, the mineral composition had a significant effect ($P<0.05$) except for Ca, Mg, and S ($P>0.05$). *Calopogonium mucunoides* contained the highest Ca, Mg, and Zn. *Sesbania grandiflora* contained the highest Co and P minerals, while the highest S mineral was found in *Arachis hypogea*. *Calliandra calothyrsus* contained the lowest Ca, Co, and S. *Arachis pintoi* contains the lowest Mg and P. The lowest Zn was found in *Arachis hypogea*.

In roughages, the mineral composition had a significant effect ($P<0.05$) except Mg ($P>0.05$). The highest Ca, Co, and Zn were contained in rice straw. Mg did not differ among roughages. The lowest P was found in rice straw compared to other roughages. Corn straw contained the highest S, but Ca and Co were the least in this food ingredient.

Coconut cake concentrate contains the highest minerals Ca, Co, and Zn. Tofu waste contained the lowest minerals, Ca, Co, and S, but the highest mineral Mg. Soybean meal contained the highest mineral S compared to other types of concentrates.

Fatty Acid Composition

Fatty acid composition from legumes, roughages, and concentrates is presented in Tables 7, 8, and 9. The highest content of SFA in legumes (Table 7) was found in *Calliandra calothyrsus* (39.87%) and the lowest in *Arachis hypogea* (25.88%). The highest contents of PUFA were found in *Arachis hypogea* (74.12%) and the lowest in *Calliandra calothyrsus* (60.13%). In legume species, C16:0 (13.33%) and C18:2n-6 (9.9%) had lower average percentages than C18:3n-3 (21.86%). *Calliandra calothyrsus* had the highest percentage of C18:3n-3 (21.86%), and *Arachis pintoi* (2.42%) was the lowest.

In roughages, the composition of total MUFA and PUFA had a significant effect ($P<0.05$), except the composition of total SFA ($P>0.05$). MUFA and PUFA did not differ among corn straw and corn husk; however, PUFA and MUFA were higher in field grass and rice straw, respectively (Table 8).

In concentrates (Table 9), the highest contents of SFA were found in coconut cake (87.78%) and the lowest in soybean meal (30.40%). As opposed to this, the highest contents of MUFA and PUFA were found in onggok (45.85%) and tofu waste (37.89%), and the lowest was coconut cake (7.05 and 5.17%), respectively.

Table 4: *In vitro* Nutrient Digestibility and RDP-RUP level of feeds

Feed Ingredients	*In vitro* digestibility			
	Dry matter digestibility (%)	Organic matter digestibility (%)	Rumen degradable protein (% CP)	Rumen undegradable protein (% CP)
Legumes				
Calliandra calothyrsus	52.15±2.25a	52.88±2.22a	61.07±1.84a	38.93±1.84b
Arachis pintoi	59.36±3.36b	60.82±3.24b	61.40±3.19a	38.60±3.19b
Sesbania grandiflora	62.08±5.98b	60.46±6.25b	79.97±3.16c	20.03±3.16a
Calopogonium mucunoides	51.46±0.32ab	51.71±0.32a	58.19±0.27a	41.81±0.27c
Arachis hypogea	60.19±0.59b	60.73±0.59b	72.79±0.41b	27.21±0.41a
Roughages				
Native grass	48.95±4.78b	45.23±5.13a	10.16±8.41a	89.84±8.41b
Corn straw	42.22±4.54a	42.65±5.26a	43.13±5.09b	56.87±5.09a
Corn husk	62.32±1.35c	61.39±1.39b	46.83±1.91b	53.17±1.91a
Rice straw	35.57±2.58a	34.77±2.61a	5.07±3.92a	94.93±3.92b
Concentrates				
Tofu waste	75.29±4.67b	76.37±4.47b	67.95±6.06b	32.05±6.06b
Coconut cake	33.01±6.67a	32.14±6.75a	72.59±2.73b	27.41±2.73a
Soybean meal	78.12±4.79b	77.95±4.83b	80.67±4.23c	19.33±4.23a
Onggok	76.85±7.08b	77.41±6.90b	37.88±18.98a	62.12±18.98b

Values (mean±SD) having different alphabets within the same column and group are significantly different at $P<0.05$.

Table 5: *In vitro* characteristics of rumen fluid of feeds

Feed ingredients	Parameters		
	pH	NH_3 (mM)	VFA (mM)
Legumes			
Calliandra calothyrsus	6.92±0.08	10.75±0.60b	82.5±3.54a
Arachis pintoi	6.82±0.02	8.00±0.60a	82.00±4.24a
Sesbania grandiflora	6.91±0.05	11.00±6.01b	77.50±3.54a
Calopogonium mucunoides	6.95±0.12	12.75±6.61c	107.50±3.54b
Arachis hypogea	6.80±0.12	13.50±4.21c	92.50±3.54a
Roughages			
Native grass	6.96±0.13	10.16±4.91b	58.33±2.89b
Corn straw	6.85±0.01	12.25±0.72b	56.67±1.77b
Corn husk	6.71±0.09	9.75±3.87a	48.67±1.51a
Rice straw	6.91±0.33	8.59±1.30a	53.33±3.64a
Concentrate			
Tofu waste	6.70±0.06	12.80±1.98b	83.67±3.09a
Coconut cake	6.69±0.17	6.71±0.13a	99.67±4.39a
Soybean meal	6.90±0.13	29.22±2.60c	103.33±3.64b
Onggok	6.56±0.21	2.42±0.07a	112.5±6.06b

Values (mean±SD) having different alphabets within the same column and group are significantly different at P<0.05.

Table 6: Mineral composition of feed ingredients

Feed ingredients	Minerals					
	Ca (%)	Co (ppm)	Mg (%)	P (%)	S (%)	Zn (ppm)
Legumes						
Calliandra calothyrsus	0.454±0.013	0.129±0.009a	0.15±0.0017	0.128±0.004b	0.012±0.0013	13.60±0.83a
Arachis pintoi	0.558±0.017	0.511±0.0023b	0.13±0.0025	0.071±0.009a	0.023±0.0017	15.00±0.71a
Sesbania grandiflora	0.466±0.072	0.514±0.017b	0.15±0.0043	0.607±0.0011c	0.022±0.0021	14.49±0.17a
Calopogonim mucunoides	0.559±0.023	0.247±0.0013a	0.16±0.0071	0.16z±0.0017b	0.02±0.0031	20.69±0.53b
Arachis hypogea	0.457±0.035	0.252±0.0053a	0.14±0.0021	0.107±0.0013b	0.024±0.0017	11.90±0.13a
Roughages						
Native grass	0.375±0.021a	0.188±0.0061a	0.11±0.0013	0.164±0.0021b	0.021±0.0013b	16.36±0.09b
Corn straw	0.330±0.017a	0.166±0.0011a	0.14±0.0027	0.13b±0.0017b	0.023±0.0009b	12.63±0.17a
Corn husk	0.398±0.033a	0.182±0.0021a	0.18±0.0013	0.138±0.0057b	0.009±0.0067a	14.20±0.21a
Rice straw	0.517±0.027b	0.395±0.0017b	0.15±0.0009	0.084±0.0067a	0.021±0.0013b	21.98±0.51b
Concentrates	Ca (%)	Co (ppm)	Mg (%)	P (%)	S (%)	Zn (ppm)
Tofu waste	0.375±0.013a	0.076±0.0071a	0.16±0.0013	0.132±0.0023	0.004±0.0017a	11.57±0.91a
Coconut cake	0.513±0.037b	0.384±0.0009b	0.13±0.0019	0.161±0.0051	0.021±0.0009b	16.16±0.72b
Soybean meal	0.429±0.041a	0.318±0.0051b	0.10±0.0013	0.106±0.0013	0.023±0.0013b	11.19±0.27a
Onggok	0.426±0.033a	0.320±0.0071b	0.11±0.0057	0.104±0.0007	0.019±0.0071b	10.14±0.13a

Values (mean±SD) having different alphabets within the same column and group are significantly different at P<0.05.

Table 7: Fatty acid profile (%) of legumes

Profile fatty acid (% total feed fat)		Legumes				
		Sesbania grandiflora	*Calliandra calothyrsus*	*Calopogonim mucunoides*	*Arachis pintoi*	*Arachis hypogea*
C14:0	Myristate acid	0.491±0.023a	1.907±0.055b	0.413±0.067a	2.036±0.076b	0.501±0.012a
C16:0	Palmitate Acid	8.644±0.33a	13.331±0.61b	12.379±0.02b	3.523±0.12a	13.124±0.12b
C18:0	Stearate Acid	4.596±0.48b	10.313±0.11c	5.485±0.34b	0.911±0.054a	4.392±0.69b
C18:1n9	Oleate acid(ω9)	3.795±0.14b	13.143±0.23b	4.394±0.59a	11.151±0.14b	12.353±0.62b
C18:2n6	Linoleic Acid (ω6)	8.309±0.76b	9.900±0.19b	14.864±0.18c	4.079±0.57a	32.607±0.81c
C18:3n3	Linolenic acid (ω3)	14.814±0.16	Not detected	21.867±0.33	2.416±0.34	10.466±0.69
C20:0	Arachidonic acid	1.853±0.26	Not detected	2.272±0.54	6.867±0.71	0.711±0.029
Total saturated fatty acids		30.55±0.18a	39.87±0.28b	33.54±0.45b	33.89±0.19b	25.88±0.62a
Total monounsaturated fatty acids		21.72±0.44a	50.23±0.28b	21.02±0.45a	52.15±0.76b	20.72±0.34a
Total polyunsaturated fatty acids		47.74±0.88b	9.90±0.43a	45.55±0.12b	13.96±0.36a	53.40±0.46b

Values (mean±SD) with different alphabets within the same row differ significantly (P<0.05).

Table 8: Fatty acid profile (%) of roughage

Profile fatty acid (% total feed fat)		Roughages			
		Field grass	Corn straw	Corn husk	Rice straw
C14:0	Myristate acid	0.657±0.54a	0.340±0.67a	0.296±0.65a	1.444±0.14b
C16:0	Palmitate Acid	20.978±0.51b	7.501±0.35a	7.051±0.48a	3.814±0.39a
C18:0	Stearate Acid	6.517±0.37b	4.194±0.66b	2.403±0.21a	1.204±0.40a
C18:1n9	Oleate acid(ω9)	13.213±0.44b	8.819±0.59a	7.502±0.09a	12.086±0.10b
C18:2n6	Linoleic Acid (ω6)	38.176±0.15c	12.910±0.62b	15.853±0.25b	4.628±0.53a
C18:3n3	Linolenic acid (ω3)	Not detected	4.033±0.56	2.440±0.34	0.368±0.04
C20:0	Arachidonic acid	Not detected	5.397±0.91	5.689±0.54	8.286±0.73
Total saturated fatty acids		35.92±0.28	34.45±0.08	36.63±0.12	34.93±0.23
Total monounsaturated fatty acids		25.91±0.26a	37.18±0.58b	35.88±0.24b	48.90±0.59c
Total polyunsaturated fatty acids		38.18±0.22c	28.37±0.04b	27.49±0.52b	16.17±0.72a

Values (mean±SD) with different alphabets within the same row differ significantly (P<0.05).

Table 9: Fatty acid profile of concentrate (%)

Profile fatty acid (% total feed fat)		Concentrates			
		Tofu waste	Coconut cake	Soybean meal	Onggok
C14:0	Myristate acid	0.400±0.026a	7.060±0.77c	1.185±0.051b	0.247±0.057a
C16:0	Palmitate Acid	8.683±0.22c	6.793±0.31c	2.896±0.05a	4.561±0.08b
C18:0	Stearate Acid	2.553±0.26	2.283±0.44	1.326±0.056	2.637±0.21
C18:1n9	Oleate acid(ω9)	6.036±0.59a	5.129±0.55a	10.905±0.34b	11.660±0.54b
C18:2n6	Linoleic Acid (ω6)	30.818±0.76c	3.364±0.43a	12.672±0.34b	5.446±0.67a
C18:3n3	Linolenic acid (ω3)	1.680±0.021	0.112±0.023	1.671±0.05	Not detected
C20:0	Arachidonic acid	4.520±0.86b	0.265a±0.037a	7.280±0.66c	7.410±0.43c
Total saturated fatty acids		33.80±0.12a	87.78±0.45b	30.40±0.27a	31.63±0.07a
Total monounsaturated fatty acids		28.32±0.56b	7.05±0.03a	43.83±0.10c	45.85±0.17c
Total polyunsaturated fatty acids		37.89±0.49c	5.17±0.18a	25.78±0.24b	22.52±0.45b

Values (mean±SD) with different alphabets within the same row differ significantly (P<0.05).

DISCUSSION

Chemical Composition

Dry matter, organic matter, crude fat, crude protein, crude fiber, ADF, NDF, cellulose, hemicellulose, and ADL differed significantly (P<0.05) for each feed ingredient. The highest NDF in legumes and roughages were in *Calopogonium mucunoides* (56.13%) and corn husks (82.67%), respectively. The high value of NDF affects the high methane concentration in feedstuffs (Hammond et al. 2014). Onggok feed ingredients contained the most easily digestible carbohydrates (NFE), while the soybean meal contained the lowest NFE. The high carbohydrates tend to produce high VFA concentrations by rumen fermentation. The concentration of VFA is formed from the process of reshuffling crude fiber by microbes so that the crude fiber contents in the feed affect the VFA value (Pazla et al. 2021a).

The ADF contents of roughages (Table 2) were higher than that of legumes (Table 3). Roughages and legumes have different degradations in the digestive tract, depending on the fraction of fiber constituents and their attachment to lignin (Ajayi et al. 2021). In general, the percentage of NDF, ADF, and cellulose in roughages in the present study was higher than that of legumes, but the contents of ADL of legumes were higher than that of roughages. Salama and Nawar (2016) tested the comparison of NDF, ADF, cellulose, and ADL values between several types of legumes and roughages. The results showed similar results to the current study.

The crude proteins in legumes, roughages, and concentrates were found significantly (P<0.05) higher in *Calliandra calothyrsus* (26.08%), corn straw (11.04%), and soybean meal (40.97%). In comparison, the lowest were *Arachis pintoi* (14.36 %), rice straw (6.44%), and onggok (4.33%), respectively. Feed proteins play an essential role in increasing the production and quality of milk and meat (Abdullah et al. 2012). Microbial and crude protein synthesis are closely linked processes (Gosselink et al. 2003; Galyean and Tedeschi 2014). Feed ingredients with high crude protein content tend to produce high concentrations of $N\text{-}NH_3$. Jamarun et al. (2017a) reported that feed proteins also affect ammonia production in the rumen. $N\text{-}NH_3$ concentration is influenced by protein sources from feed ingredients, while ATP is from carbohydrate fermentation. The factors that affect the $N\text{-}NH_3$ concentration could be feed proteins, protein degradation, energy sources, and the proportion of dissolved carbohydrates into ATP energy (Prayitno et al. 2018).

Dry Matter and Organic Matter Digestibility, RDP, and RUP

The high digestibility of soybean meal could be due to the high components of the cell contents, which are soluble components such as crude protein, crude fat, NFE, and soluble minerals (Table 3). According to Montcho et al. (2017), easily soluble and degraded organic matter help increase rumen microbial activity so that feed can be degraded effectively. Jamarun et al. (2017b) added that rumen microbes and feed nutrition influence degradation in the rumen.

The chemical composition of the tested feed ingredients influences the high and low digestibility of organic matter and dry matter. High protein contents can promote the growth of rumen microorganisms, thereby increasing the rate of feed degradation (Pazla et al. 2018b). Digestibility is also strongly influenced by the composition of the cell wall. The structure of fiber components can inhibit the penetration of microbial enzymes so that nutrient degradation is inhibited. The high lignin in plant cell walls makes rumen microbes difficult for degraded (Pazla et al. 2020), thus affecting the digestibility of organic matter (Dvořáčková et al. 2013).

In the present study, *Sesbania grandiflora* produced significantly (P<0.05) the highest DMD and OMD in legumes and not much different from *Arachis hypogea*. Corn husk is a forage source of energy for ruminants. The results showed that corn husks produced the highest DMD and OMD in forage types (P<0.05). The high digestibility of corn husks is affected by high hemicellulose (Table 2). Hemicellulose is hydrolyzed by rumen microbes and hemicellulase enzyme, whose final fermentation product is VFA (Liu et al. 2020). The DMD and OMD values of feed ingredients in the present study have exceeded those of agricultural by-product feedstuffs already tested (Aung et al. 2015).

Soybean meal (Table 4) has significantly (P<0.05) the highest crude protein degradation value (80.67%). Due to the high crude protein contents in soybean meal, proteins can be digested well by rumen microbes. The level of digestibility of crude protein is also influenced by the amount of protein that enters the digestive tract, the protein content of feed ingredients, and the feed rate in the digestive tract. Milford and Minson (1965) reported a close correlation between the crude proteins in 218 feed ingredients from tropical grasses and legumes and crude protein digestibility. Pazla et al. (2021b) obtained the RDP value of *Tithonia diversifolia* (71.50%) with a crude protein content of 31.02%. Sugarcane shoots produced an

RDP of 46.54% with a crude protein of 8.10% (Pazla et al. 2021c). In the present study, the lowest RDP value was in rice straw (5.07%), while crude proteins in straw were low (Table 2). According to Thulin et al. (2014), one factor that affects the digestibility of feed ingredients is crude protein content. High crude protein feed ingredients produce high digestibility as well.

Several factors influence feed protein degradation, namely, the ability of rumen microbes to degrade feed ingredients, microbial growth, the length of time feed proteins are in the rumen, protein sources, the amount of consumption, and particle size of feed consumed by animals (Putri et al. 2019; Zain et al. 2019; Zain et al. 2020; Sari et al. 2022). Protein degradation in the rumen is influenced by pH and the dominant microbial species. Bach et al. (2005) reported that proteolytic activity was increased in the presence of high pH in the diet of the dairy cattle but not in the concentrated diet of the beef species. The degradation rate depends on the protein's origin, which shows physicochemical characteristics and rumen microbial activity. Whetton et al. (1997) reported differences in the rate of crude protein degradation in *Gliricidia sepium* and *Calliandra calothyrsus*. Pamungkas et al. (2009) added that rapidly degraded proteins in the rumen would undergo hydrolysis faster than those that are slowly degraded in the rumen.

RUP is the proportion of feed proteins that escape rumen breakdown (Putri et al. 2021). The balance of RDP and RUP is essential to increase microbial protein synthesis (Rosmalia et al. 2022). The results of the present study showed that the highest ($P<0.05$) RUP was rice straw, and the lowest was soybean meal. Polyorach and Wanapat (2014) stated that rice straw could increase livestock productivity if supplemented with urea so that RDP increases. Petit and Veira (1991) stated that low protein degradation in the rumen could be associated with increased crude protein levels reaching the duodenum and would improve feed digestibility. Soybean meal is a high-protein feed component with a high level of degradation in the rumen. Between 22 and 53% of the proteins in soybean meal are resistant to breakdown in the rumen, while 86 and 100% are digestible in the small intestine (Stern et al. 2006).

The protein degradation and deamination of amino acids in the rumen continue even though there has been a relatively high accumulation of ammonia. This degradation process cannot be viewed as a beneficial or detrimental process because, on the one hand, the degradation process is expected to meet the needs of ammonia and peptides for rumen microbial growth. Meanwhile, on the other hand, high-quality protein is expected to experience less degradation in the rumen. The breakdown of proteins into ammonia is faster than ammonia for microbial protein synthesis, resulting in excess ammonia being absorbed and converted in the liver into urea and then excreted via urine. In other words, soybean meal with a high level of degradation has a biological value that is less profitable for ruminants because it cannot be utilized by livestock. Therefore, soybean meal needs to be protected from rumen microbial degradation to increase the supply of protein into the intestines and increase the supply of amino acids in host livestock (Ramaiyulis et al. 2016).

Characteristics Rumen Fluid

The pH of the rumen fluid is one of the determinants of whether or not the rumen conditions are suitable for the fermentation process. Belanche et al. (2021) stated that rumen microbial activity requires certain pH conditions related to ongoing rumen environmental conditions. Van Soest (1994) stated that the activity of cellulolytic bacteria was inhibited if the pH of the rumen fluid was below 6.2, and the activity is usually optimal at 6.7±0.5 rumen pH.

The pH values (6.56-6.96) on incubation of all feed ingredients, including legumes, roughages, and concentrates, were non significantly ($P>0.05$) different in the present study. These values indicate that all feed ingredients can provide a pH balance in the rumen fluid to support the microorganism's ecosystem. This pH value that is not much different is thought to be due to the length of incubation time (pH is measured at 48 hours after incubation), resulting in less availability of feed ingredients to be fermented by microbes.

The concentration of N-NH3 also determines the amount of feed proteins that can be digested in the rumen. Its value is significantly impacted by the rumen bacteria's capacity to break down feed proteins and how quickly it is digested. Table 5 shows the varying values of $N\text{-}NH_3$ concentrations. Soybean meal produced the highest concentration of $N\text{-}NH_3$. The high and low concentrations of $N\text{-}NH_3$ are influenced by the nutrient contents of the feed ingredients, especially crude protein (Table 1). Rumen microbes influence $N\text{-}NH_3$ concentration to form microbial proteins (Petri et al. 1988).

Ammonia concentration in the present study resulted in a significantly ($P<0.05$) different effect. Rice straw produced the lowest concentration of $N\text{-}NH_3$ in the roughages. Onggok produced the lowest $N\text{-}NH_3$ concentration in the concentrates, while the lowest concentration of $N\text{-}NH_3$ in the legumes was produced in *Arachis pintoi*. The higher degradation of proteins in the rumen will increase $N\text{-}NH_3$ concentration; conversely, with the lower degradation of protein in the rumen, the concentration of rumen $N\text{-}NH_3$ decreases. Jamarun et al. (2021) stated that $N\text{-}NH_3$ concentration was influenced by protein contents level of protein degradation, and protein solubility of feed ingredients. The degree of hydrolysis of feed proteins depends on their solubility, which is closely related to the concentration of ammonia. Qin et al. (2015) found the highest protein solubility in soybean meal, reaching 84.19% compared to fish meal and corn gluten meal. The concentration of NH^3 in the present study (Table 5) supported the synthesis of microbial proteins in the rumen except for the onggok material, which produced the lowest concentration of 2.42mM. Rahmadi et al. (2010) reported that the optimum ammonia concentration in supporting microbial protein synthesis was between 3.57-7.14mM. The synthesis and activity of rumen microbes depend on available energy (ATP). Hindratiningrum et al. (2011) that if the concentration of $N\text{-}NH_3$ in the rumen fluid is low, then ammonia fixation requires ATP, while the concentration of $N\text{-}NH_3$ is high enough without requiring ATP, ammonia is directly incorporated into microbial amino acids.

Volatile Fatty Acids (VFA) is formed from the fermentation of carbohydrates in cellulose, hemicellulose, sugar, pectin, fructans, and starch (Bannink et al. 2010).

Santoso and Hariadi (2009) reported that the agricultural industry's NDF contents of feed ingredients by-products produced different VFAs. VFA concentration in the present study was significantly (P<0.05) different among legumes, roughages, and concentrates. The highest VFA in legumes was found in *Calopogonium mucunoides* (107.50mM), and the lowest was in *Sesbania grandiflora* (77.50mM). The highest VFA in forage was found in native grass (58.33%), and the lowest was corn husk (48.67%), while onggok concentrate produced the highest VFA (112.5mM) and tofu waste produced the lowest VFA (83.67mM). Yanti et al. (2021) stated that the fiber fraction is often found in binding with lignin, making it difficult to digest by rumen microbes. Baba et al. (2017) added that the high contents of lignin in feed ingredients as a fiber component would make it difficult for microbes to degrade in the rumen. The high VFA in onggok occurs because the contents of non-structural carbohydrates (NFE) are also high. Pamungkas et al. (2009) used onggok as a source of rapid degradation energy to optimize rumen degradation kinetics in the preparation of ruminant diets. The concentration of VFA results from fermented feed ingredients, which describes the solubility of carbohydrates and proteins during the fermentation process (Wahyuni et al. 2014).

Bata and Hidayat (2010) stated that the concentration of VFA describes whether carbohydrates are readily fermented. The higher the VFA, the higher the carbohydrates and proteins are fermented in the rumen. Concentrated feed ingredients containing readily fermentable carbohydrates tend to produce high propionic acid (Hilali et al. 2018), while forage directs the fermentation produce acetic acid. Acetic acid is a non-glucogenic compound, and almost all can be oxidized. According to Muchlas et al. (2013), oxidation results in a substantial heat increment, lowering the efficiency value. On the other hand, propionate is a sugar precursor compound that is the main glucogenic.

Feed Minerals

Calcium (Ca), Phosphorous (P), Magnesium (Mg), and Sulfur (S) are macrominerals needed by livestock to constructing body parts like bones and teeth (Jamarun and Pazla 2022). The rumen microorganisms' ability to proliferate and digest fiber depends on the P (Suyitman et al. 2021). Sulfur is a mineral that rumen microbes require to produce sulfur-containing amino acids (Bal and Ozturk 2022). P and S can increase feed digestibility by stimulating rumen microbial growth (Pazla et al. 2018b). Minerals P, S, and Mg can increase livestock's rumen fluid VFA concentration and nutrient digestibility (Febrina et al. 2017). Increased microbial activity in cellulose digestion is possible when the rumen has average amounts of the minerals Ca, P, and Mg (Grace et al. 1977).

In the present study, the mineral contents of legumes had a significant (P<0.05) effect except for Ca, Mg, and S (P>0.05). The mineral contents of roughages had a significant effect (P<0.05) except Mg (P>0.05). Meanwhile, the mineral contents of concentrates had a significant effect (P<0.05) except Mg and P (P>0.05). Ca and Mg in all research feed ingredients were in the normal range, but P was only in *Sesbania grandiflora*, which was at normal levels, while S was below the normal range in all feed ingredients. McDowell et al. (1983) stated that the standard values for the mineral contents of feed ingredients are Ca 0.17-1.53%, P 0.17-0.59%, Mg 0.05-0.25%, and S 0.08-0.15%.

Zinc and cobalt are minerals often deficient in forage fodders (Sprinkle et al. 2020). Zinc (Zn) is a micromineral often deficient for rumen microbial growth and can maximize feed degradation (Petrič et al. 2020). Insufficient Zn tends to limit microbial protein synthesis (Pathak 2008). Additionally, Zn activates a variety of enzymes (Ibrahim et al. 2016). The contents of Zn in ruminant feed in Indonesia have been reported to be 20-38mg/kg DM (Little, 1986). This value is still low compared to the needs of ruminants for Zn, which is 40-130 mg/kg DM (EFSA 2014). Zn deficiency can limit enzymic activity and affect rumen microbial metabolism. Therefore, Zn must be balanced and sufficient to increase feed degradation and rumen microbial growth. The Zn contents in all research feed ingredients were below the normal range in the present study.

The highest Co in legumes, roughage, and concentrates were found significantly (P<0.05) in *Sesbania grandiflora*, rice straw, and coconut cake, respectively. Supplementing Co and Zn in dairy cow rations can increase milk production, proteins, and rumen microbes (Zhao et al. 2021). Cobalt supplemented in rations containing elephant grass, *Moringa oleifera*, and concentrates tend to increase VFA levels and microbial protein synthesis (Suhartati et al. 2016).

Fatty Acid Composition

Saturated fatty acids (SFA) are solid at ambient temperature and lack double bonds in their carbon chains. Monounsaturated fatty acids (MUFA) are liquid at room temperature, having one bond and two double bonds in the carbon chain. The carbon chain of polyunsaturated fatty acids (PUFA) has two or more double bonds and is liquid at room temperature (Ginsberg and Karmally 2000).

The composition of fatty acid in legumes, roughages, and concentrates can be observed in Tables 7, 8, and 9. In the present study, fatty acid composition varied among the feed ingredients significantly (P<0.05). The findings of the current study are consistent with the findings of Dias et al. (2017), who reported that C18:3n-3 dominated the forage fatty acid profile, followed by C16:0 and C18:2n-6. Makmur et al. (2019) also found that essential fatty acids C18:3n-3 predominated in grasses (*Panicum maximum, Cynodon plectostachyus, Pennisetum purpophoides, Pennisetum purpureum, and Brachiaria decumbens*) and legumes (*Gliricidia sepium, Calliandra calothyrsus, Stylosanthes guianensis*). C18:3n-3 fatty acids can boost omega-3 biosynthesis. We observed that C18:3n-3 was higher in legumes and roughages compared to concentrates. It indicates that forages are essential to the source of fatty acid to increase omega-3 in ruminant products.

Plant species, growth stage conservation, and nitrogen fertilization are the main factors that influence the fatty acid composition of forages (Khan et al. 2012). However, the contents of SFA and UFA (Unsaturated Fatty Acid) change again during the fat digestion process in the rumen (the process of biohydrogenation), that is, a saturation of unsaturated fatty acids, so that the final product in ruminant meat consists mainly of saturated fatty acids. Following Palmquist's (1988) statement, the biohydrogenation rate of PUFA to SFA is estimated at 90%.

Conclusion

According to the findings of this study, the RDP, RUP, and chemical composition of legumes, roughages, and concentrates varied. When making animal rations, knowledge of the nutritional value of feeds is helpful. Additionally, to satisfy the requirements of host animals and rumen microorganisms, ruminant cattle feeds must be made utilizing RDP and RUP. It is hoped that this study will help to create ruminant cattle rations based on RDP and RUP ratios, as the information is still scarce.

Author's Contribution

Mardiati Zain, Despal, and Hidayat Tanuwirya designed the concept, searched for funding, and drafted and reviewed the paper. Roni Pazla supervised the field and laboratory work. Ezi Masdia Putri conducted field and laboratory work as well as data tabulation. Ummi Amanah collected and prepared samples and conducted data analysis.

Competing Interest

All of the authors declare that they have no competing interests.

Acknowledgements

The funding for this study came from Andalas University with the Indonesian Collaborative Research Scheme A (Host) research scheme with the number: T/9/UN.16.17/PT.01.03/Pangan-RKI-A (Host)/2022 for the 2022 fiscal year.

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