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/2023.073

Comparison of Nutritive Value and RDP-RUP Contents of Tropical Legumes from Two Different Areas with In-Vitro Methods

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Article History: 23-216 Received: 24-May-23 Revised: 27-Jun-23 Accepted: 07-Jul-23

ABSTRACT

This study aims to obtain the chemical composition, RDP-RUP content, and digestibility of tropical legumes from two different regions using the in-vitro method. The legumes used included: *A. hypogea*, *A. pintoi*, *C. calothyrsus*, *C. mucunoides, G. sepium, I. zollingeriana, L. leucocephala, M. oleifera* and *S. grandiflora*. The chemical composition of feed ingredients was determined using proximate and van soest analysis. Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) were measured using the in vitro Tiley and Terry method. The research design used was a randomized block design with areas (Luak and dramaga) as treatment then repeated three times. Research data were analyzed using Analysis of Variance (ANOVA) with SPSS software version 25. The results obtained from this study were not significant differences (P>0.05) between treatments for each parameter observed. *I. zoliingeriana* was the legume with the highest CP (30.40%) content among all the legumes observed. DMD ranged from 58.03% (*A. pintoi*) – 72.68% (*I. zollingeriana*). The highest RDP content was found in *I. zollingeiana* (71.28%). The highest RUP content was found in *C. mucunoides* (46.94%). The conclusion from this study is that tropical legumes of the same type but grown in areas with different environmental conditions tend to have almost the same quality. The database of feed nutrient content is very useful in formulating livestock rations. A good ration formulation is based on the ratio of RDP and RUP so that the needs of rumen microbes and host livestock are met. This research is highly useful to help formulate ruminant livestock rations based on the ratio of RDP and RUP, due to limited information from previous studies.

Key words: Tropical legumes, RDP, RUP, nutrients, digestibility.

INTRODUCTION

Legumes are forages that are high in protein. Protein in ruminants is divided into two, namely RDP protein (Rumen Degradable Protein) or protein that is degraded in the rumen and RUP (Rumen Undegradable Protein) or protein that escapes rumen degradation. The RDP and RUP values of legumes vary greatly by species. Legumes that are degraded (RDP) in the rumen will provide NH3 for the needs of rumen microbial protein synthesis. Meanwhile, legumes that are not degraded (RUP) in the rumen will be a source of amino acids for host livestock along with microbial protein that lyses to the post rumen (Putri et al. 2019).

Ruminant livestock must be given feed that has a balanced RDP-RUP content. Feed containing high RUP will decrease microbial protein production while feed containing high RDP will produce high NH3 in the rumen. Therefore, it is necessary to pay attention to a balanced ratio of RDP and RUP for ruminants in order to optimize their productivity (Putri et al. 2019).

The productivity of ruminants depends on the adequacy of the nutrients they consume from their feed. The feed consumed must contain balanced nutrients to support optimal productivity. One of the unique nutrient requirements for ruminants is protein. Ruminants need protein for themselves and microbes in the rumen that help the digestive process through fermentation (Zain et al. 2020). Feed proteins that is degraded in the rumen (RDP = Rumen Degradable Protein) will be broken down into ammonia and used for microbial protein synthesis.

Cite This Article as: Amanah U, Zain M, Elihasridas, Putri EM 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. https://doi.org/10.47278/journal.ijvs/2023.073

Meanwhile, those that are not degraded in the rumen (RUP=Rumen Undegradable Protein) or protein by-pass will be broken down into amino acids and used by the host livestock together with microbial protein which lyses to the post rumen (Putri et al. 2019). Both microbial protein and protein by-pass are equally important, so a balance between the two needs to be considered. So far, the development of ration formulations is still based on protein requirements, due to the lack of information regarding the RDP-RUP content in feed ingredients.

Utomo (2012) reported that legumes are proteinaceous roughages, namely a high protein fiber feed source. Leguminosae is a forage animal feed that is rich in protein. Different types of legumes vary in nutritional contents and degradability in the rumen (Rahmat et al. 2021). The level of protein degradation in tropical legumes in the rumen that is the difference between RDP and RUP is the chemical structure and level of protein solubility, microbial population, rumen pH, and rate of passage (Kaufman 2016) as well as the environmental conditions where it grows. Environmental conditions such as temperature, rainfall, and light intensity are external factors that greatly affect the nutrient quality of tropical legumes (Hasan 2015).

There are differences in the quality of plant nutrients based on the environment in which they grow and the importance of paying attention to the RDP-RUP ratio in the preparation of ruminant livestock rations, it is necessary to conduct a study to know the degradable and undegradable proteins present in tropical legumes. Among the tropical legume species available are *Gliricidia sepium, Leucaena leucocephala, Calliandra calothyrsus, Indigofera zollingeriana, Moringa oleifera, Calopogonium mucunoides, Arachis hypogaea, Sesbania grandiflora,* and *Arachis pintoi*. Each of these legumes comes from the regions of West Sumatra (Luak District, Limapuluh Kota District) and West Java (Dramaga District, Bogor Regency). These two areas were chosen because they have contrasting ambient temperature, rainfall, altitude, light intensity and soil type.

MATERIALS AND METHODS

Ethical Approval

This research did not use any live animals so, ethical approval was not needed.

Study Period and Experimental Site

This research was conducted at the Ruminant Nutrition Laboratory, Faculty of Animal Husbandry, Andalas University from September to November 2022.

Sampling

The legumes used came from two regions, namely Luak, Limapuluh Kota Regency, West Sumatra and Dramaga, Bogor Regency, West Java. Nine types of legumes used were *A. Hypogea, A. Pintoi, C. calothyrsus, C. mucunoides, G. sepium, I. zollingeriana, L. leucocephala, M. oleifera* and *S. Grandiflora.* The geographical location of the Luak, Limapuluh Kota Regency, West Sumatra, is 0º25’28,71"S and 100º50’47,80"E and at elevation 110-2,261 meters above sea level. The average temperature in the area is 22-25°C with a rainfall of 2700mm/year. Meanwhile, the geographical location of the Dramaga, Bogor Regency, West Java, is 6°18'0"S and 107°13'30"E and is 15-2500 meters above sea level. 23-25°C is the average temperature in the area with a rainfall of 4198mm/year.

Samples that had been collected from each region (Luak and Dramaga) were dried for 48h using an oven at 60°C then chopped and grinded (1mm). After that, each sample was stored in airtight plastic until analysis. This experiment used goat rumen liquor taken from abattoirs. The rumen fluid was put into a thermos with a temperature of 39°C by filtering it using 5 layers of gauze (100µm) while flowing CO2 gas to keep it anaerobic.

Chemical Analysis of Samples

Nutrient contents that includes of moisture, ash, crude protein, crude fiber and ether extract were analyzed by proximate analysis (AOAC 2005). Analysis of the moisture is carried out by weighing the sample as needed (2g or 3g) then in the oven for 8h at 105°C, then to get the ash content value, the sample is put into the furnace at 600°C for 4-5h. Analysis of crude protein goes through several stages, namely destruction, distillation and titration. Crude fiber was analyzed by heating the sample with 0.3N H_2SO_4 solution, then filtered and fired. Crude fat is analyzed by soxhletation process. Van soest analysis is used to determine the content of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), cellulose, and Acid Detergent Lignin (ADL) (Goering and Van Soest 1970). NDF analysis was carried out by heating the sample with NDS (Neutral Detergent Soluble) solution, then filtering it and placing it in an oven. ADF analysis was carried out by heating the sample with ADS (Acid Detergent Soluble) solution, then filtering it and baking it in the oven, then soaking it with 72% H2SO4 to get the cellulose content, and then heating it to get the ADL content.

In-vitro Method

The digestibility of each feed ingredient, RDP and RUP levels, as well as rumen fluid characteristics in vitro were measured in vitro using the Tilley and Terry method (1963). The in-vitro process begins with taking rumen fluid at the abattoir. Then the rumen fluid is filtered so that it is separated from the dregs. Furthermore, rumen liquor mixed with buffer in a ratio of 1:4 (McDougall 1947). 2.5g of each sample was then put into a 250mL Erlenmeyer tube then added a mixture of rumen fluid and buffer (250mL). Then the Erlenmeyer tube was tightly closed while flowing CO2 gas so that it remains anaerobic. The sample is then placed in a shaking incubator at 39°C at 90rpm for 48h. When the incubation period ends, erlenmeyer tube was immersed in ice cubes to stop microbial activity, followed by measuring the pH. Then centrifugation was carried out to separate the supernatant and residue at 3000rpm at 40°C. The supernatant was used for the analysis of total NH3 and VFA. The method of Conway and O'Malley (1942) was used to determine the NH3 concentration, and the total VFA concentration was determined using the steam distillation method (Prosedure 1996). The residue that has been filtered with Whatman No. filter paper. 41 was used to determine the digestibility and content of RDP-RUP following the proximate analysis method.

Statistical Analysis

The data that has been obtained was tested statistically using analysis of variance with SPSS V.25 software. The mean of each parameter that has a significant or highly significant effect is further tested using the Tukey HSD test.

RESULTS

Chemical Composition of Tropical Legume

Based on the observations that have been made, 9 types of legumes from different areas indicate non-significant different results (P>0.05). Data in Table 1 which includes DM, ash, CP, CF and EE indicate non-significant different (P>0.05) among treatments (Luak and Dramaga). The same thing was also found in Table 2. namely NDF, ADF, cellulose and lignin. *I. zollingeriana* from the Dramaga area contained the highest CP among all the legumes studied (30.40%) while the lowest was found in *A. pintoi* from the Luak area (18.64%). The NDF content ranges from 27.28- 45.83%. While the ADF ranged from 16.39-33.94%.

In-vitro Digestibility of Dry Matter and Organic Matter, RDP, RUP

Presents Data on the Digestibility of Dry Matter (DMD), Digestibility of Organic Matter (OMD), RDP and RUP. DMD, OMD, RDP and RUP indicate non-significant (Table 3) difference (P>0.05) between treatments (Luak and Dramaga areas). DMD ranged from 58.03% (*A. pintoi* from Luak) – 72.68% (*I. zollingeriana* from Dramaga). OMD ranged from 58.43% (*A. pintoi* from Luak) - 74.43% (*I. zollingeriana* from Dramaga). The highest RDP content was found in *I. zollingeiana* (71.28%). The highest RUP content was found in *C. mucunoides* (46.94%).

Rumen Fluid Characteristics

pH, VFA, and NH3 are characteristics of rumen fluid. The results indicated that the treatment (Luak and Dramaga) had no significant effect (P>0.05) on the characteristics of rumen fluid. as presented in Table 4. 6.79-6.97 is the range for rumen pH. VFA concentrations ranged from 61.83-96.67mM and NH3 concentrations ranged from 14.21-33.21mg/100mL.

DISCUSSION

Chemical Composition of Tropical Legume

Legumes are highly nutritious, providing essential amino acids, complex carbohydrates, fibre, unsaturated fatty acids, vitamins and minerals essential (Bouchenak and Myriem 2013; Rebello et al. 2014). The nutrient composition (DM, ash, CP, CF and EE) of each type of legume varies greatly (Zain et al. 2023). Legume DM content ranged from 18.83% (*A. pintoi*) – 29.06% (*C. calothyrsus*). ash ranged from 6.46% (*C. calothyrsus*) – 10.42% (*A. hypogea*). CP content was in the range of 18.64% (*A. pintoi*) – 30.40% (*I. zollingeriana*). CF content ranged from 10.58% (*I. zollingeriana*) – 22.25% (*C. mucunoides*). The content of EE ranged from 2.09% (*A. hypogea*) – 5.41% (*S. grandiflora*). This range is optimal for rumen microbial growth. This is because rations containing crude fat above 5% will interfere with rumen microbial activity during the digestion process (Indah et al. 2020). The NDF content ranged from 27.28% (*I. zollingeriana*) – 39.39% (*A. hypogea*). The ADF content ranged from 16.39 (*I. zollingeriana*) – 33.94% (*C. mucunoides*). The cellulose content ranges from 12.21% (*C. calothyrsus*) – 27.51% (*G. sepium*). ADL content ranged from 1.62% (*I. zollingeriana*) – 7.23% (*A. pintoi*).

The nutrient content of *L. lecocephala*, *I. zollingeriana* and *G. sepium* showed results that were in line with research by Putri et al. (2019) where *I. zollingeriana* contained the highest CP (31.22%) followed by *L. leucocephala* (25.47%) and *G. sepium* (23.84%). *C. mucunoides* contains higher CP than previous studies (Evitayani et al. 2004) but is similar in terms of EE, ADF and ADL content and lower in NDF content. The CP, CF and EE content of *S. grandiflora* in this study was higher than previous studies (Rahmat et al. 2021) which reported that the CP, CF and EE contents were 19.69% respectively; 10.50%; 2.54% but in line with the DM and ash content. The CP and EE content of *C. calothyrsus* in this study were lower than previous studies (Makmur et al. 2020) but contained higher CF. A previous study reported that *M. oleifera* contained CP 30.29% higher than the results of this study while the content of fiber fractions such as NDF, ADF Cellulose and ADL showed lower results (Moyo et al. 2011). Zain et al. (2023) reported that *A. pintoi* and *A. hypogea* contain nutrient compositions that are in line with this study.

Nutritional requirements in terms of protein for ruminants are very important. The ration given must contain sufficient protein for rumen microbial activity and livestock protein needs in tissues. Proteins that are degraded in the rumen into NH3 are needed for body protein synthesis by rumen microbes, while the protein that escapes degradation will enter the post-rumen along with microbial protein and then become a source of amino acids for host livestock (Zain et al. 2020). The provision of CP with a high level of degradation in the rumen will increase NH3 production so that it is inefficient for livestock, increases feed costs, and damages the environment (Savari et al. 2018). So that the ration must also be balanced for the RDP and RUP.

Tropical legumes contain higher CP compared to grasses (Kumar et al. 2015). Legumes are suitable for used as ruminant animal feed because they contain high CP and CF (Permana et al. 2022). Feed protein in ruminants must be able to meet the needs of rumen microbial synthesis and be able to be digested post-rumen for the needs of the host (Savari et al. 2018; Zain et al. 2020). Microbial protein (MP), RUP and endogenous protein will contribute together to enter the post rumen and become a source of amino acids for host livestock (ruminants) (White at al. 2017). High-producing livestock require a source of amino acids from proteins that escape rumen degradation so protein sources from microbes are not sufficient to meet their amino acid needs (Bahrami-Yekdangi et al. 2014). Pazla et al. (2018) reported that high protein feed will produce more N so it is good for rumen microbial activity. Rumen microbes need N to synthesize proteins in their bodies.

The fiber fraction is a constituent of plant cell walls. Legumes contain fiber fractions such as NDF, ADF, and cellulose which are lower than grass. Rumen microbes will

Table 1: Chemical composition of proximate analysis for tropical legumes

Legumes	Parameters									
	DM		Ash		CP		CF		EE	
	L	D	L	D	L	D	L	D	L	D
A.hypogea	21.40±0.24	20.65±0.23	9.65±0.21	10.42±0.22	24.54±0.23	25.40±0.24	19.50±0.24	20.38±0.23	3.01±0.61	2.09±0.59
A.pintoi	19.17±0.18	18.83±0.19	7.77±0.15	8.33±0.17	18.64±0.20	19.35±0.21	20.62±0.33	21.85±0.30	3.68±0.67	2.68±0.68
C.calothyrsus	29.06±0.40	27.62±0.41	5.58±0.24	6.46±0.23	26.37±0.23	25.51±0.22	13.37±0.51	15.28±0.49	3.59±0.39	2.55±0.37
C.mucunoides	24.38±0.21	23.59±0.21	8.58±0.22	9.36±0.21	19.14±0.13	19.57±0.13	21.53±0.20	22.25±0.19	3.51±0.38	2.73±0.37
G.sepium	21.47±0.26	20.56±0.25	8.63±0.23	9.49±0.23	25.85±0.66	28.22±0.67	14.04±0.33	15.28±0.34	4.01±0.77	3.21±0.75
I.zollingeriana	26.44±0.31	25.59±0.30	8.62±0.21	9.36±0.25	29.53±0.23	30.40±0.24	11.50±0.26	10.56±0.25	3.54±0.25	2.83±0.21
L.leucocephala	26.92±0.44	26.49±0.42	7.62±0.24	8.48±0.24	23.56±0.23	24.41±0.22	15.66±0.18	16.32±0.16	4.01±0.72	2.99±0.65
M.oleifera	27.54±0.52	26.60±0.50	9.26±0,10	9.57±0.11	27.12±0.14	27.63±0.13	14.92±0.39	16.35±0.38	4.51±0.36	3.88±0.23
S.grandiflora	23.39±0.68	22.60±0.68	6.65±0.21	744±0.22	24.42±0.26	25.33±0.25	13.95±0.38	15.35±0.39	5.41±0.59	4.49±0.48

DM=dry matter; CP=crude protein; CF=crude fiber; EE=ether extract; L=Luak (West Sumatera); D=Dramaga (West Java)

Table 2: Chemical composition of van soest analysis for tropical legumes

Legumes	Parameters							
	ADF		NDF		Selulosa		ADL	
	L	D	L	D	L	D	L	D
A.hypogea	24.04±0.51	22.19±0.53	39.64±0.30	39.39±0.30	18.03±0.51	16.14±0.49	5.27±0.37	5.02±0.37
A.pintoi	26.30±0.50	28.07±0.51	42.19±0.55	43.59±0.55	18.29±0.46	19.94±0.47	6.72±0.49	7.23±0.49
C.calothyrsus	18.70±012	18.55±0.14	36.82±0.57	34.86±0.56	13.17±0.18	12.51±0.19	4.86±0.13	5.31±0.12
C.mucunoides	32.72±0.36	33.94±0.37	44.34±0.42	45.83±0.43	25.44±0.25	26.28±0.24	6.87±0.11	7.15±0.11
G.sepium	31.02±0.42	30.18±0.41	38.09±0.16	37.47±0.18	27.21±0.41	25.70±0.40	3.31±0.26	3.92±0.24
I.zollingeriana	16.39±0.41	17.78±0.40	28.44±0.48	27.28±0.45	14.34±0.20	14.89±0.20	1.62±0.07	2.36±0.08
L.leucocephala	27.29±0.37	25.96±0.38	37.44±0.28	36.42±0.28	21.21±0.12	20.80±0.12	5.38±0.24	4.44±0.21
M.oleifera	25.70±0.34	26.81±0.35	35.30±0.35	34.02±0.34	22.71±0.23	23.44±0.24	2.63±0.16	3.14±0.17
S.grandiflora	28.03±0.53	26.89±0.50	34.37±0.21	34.87±0.21	23.71±0.55	21.85±0.52	3.91±0.23	4.89±0.24

NDF=neutral detergent fiber; ADF= acid detergent fiber; ADL=acid detergent lignin

Table 3: In- vitro Dry Matter and Organic Matter Digestibility, RDP, RUP tropical legumes

Legumes	Parameters							
	IVDMD		IVOMD		RDP		RUP	
	L	D	L	D	L	D	L	D
A.hypogea	62.73±0.15	63.49±0.20	61.39±0.22	62.62±0.21	56.84±0.51	57.56±0.49	43.16±0.51	42.44±0.48
A.pintoi	58.03±0.75	59.61±0.75	58.33±0.53	61.07±0.53	54.53±0.36	55.07±0.36	45.47±0.36	44.93±0.33
C.calothyrsus	67.68±0.29	66.18±0.29	69.05±0.32	67.81±0.30	56.73±0.44	54.46±0.42	43.27±0.44	45.54±0.47
C.mucunoides	58.07±0.73	58.88±0.72	59.22±0.62	60.04±0.63	53.06±0.25	53.78±0.22	46.94±0.25	46.22±0.27
G.sepium	69.41±0.46	70.04±0.48	70.44±0.38	71.09±0.38	67.23±0.46	69.52±0.40	32.77±0.46	30.48±0.46
I.zollingeriana	71.63±0.63	72.68±0.64	72.44±0.52	74.43±0.56	71.28±0.39	73.05±0.31	28.72±0.39	26.95±0.41
L.leucocephala	61.30±0.22	62.32±0.23	60.65±0.27	61.33±0.27	56.96±0.53	59.54±0.53	43.04±0.53	40.46±0.54
M.oleifera	68.38±0.42	68.92±0.41	69.28±0.28	69.83±0.28	68.17±0.56	68.98±0.54	31.83±0.56	31.02±0.56
S.grandiflora	64.69±0.23	65.92±0.24	66.12±0.37	67.59±0.36	54.71±0.75	55.54±0.79	45.29±0.75	44.46±0.71

IVDMD=in-vitro dry matter digestibility; IVOMD= in-vitro organic matter digestibility; RDP=rumen degradable protein; RUP=rumen undegradable protein.

Table 4: Rumen Fluid Characteristic tropical legumes

Legumes	Parameters					
	pH		VFA		NH3	
	L	D	L	D	L	D
A. hypogea	6.80±0.47	6.81±0.43	67.83±0.51	69.17±0.52	23.57±0.90	26.42±0.10
A. pintoi	6.82±0.34	6.79±0.33	64.50±0.51	65.83±0.56	14.54±0.94	18.69±0.97
C.calothyrsus	6.97±0.35	6.85±0.34	80.00±1.18	78.33±1.12	16.46±0.60	14.21±0.61
C.mucunoides	6.95±0.07	6.85±0.09	61.83±0.66	62.50±0.65	21.68±0.44	23.59±0.44
G.sepium	6.82±0.23	6.81±0.21	81.67±1.18	83.33±1.20	26.78±0.17	27.63±0.17
I.zollingeriana	6.86±0.46	6.90±0.44	95.00±1.18	96.67±1.19	32.30±0.38	33.21±0.35
L.leucocephala	6.93±0.64	6.90±0.65	71.83±0.66	72.50±0.69	28.96±0.84	33.21±0.88
M.oleifera	6.95±0.51	6.83±0.51	80.00±1.18	81.67±1.17	19.41±0.89	23.32±0.82
S.grandiflora	6.91±0.32	6.84±0.34	75.00±1.18	76.67±1.18	23.62±0.26	24.70±0.27

VFA=volatil fatty acid

easily enter into the cell wall so that they digest feed more quickly if given a ration with a lower crude fiber content (Yanti et al. 2021). The cell wall content which included ADF, cellulose NDF and ADL from each type of tropical legume was also indicates not-significant different (P>0.05). *C. mucunoides* had the highest cell wall content among all the tropical legumes studied. The content of NDF and ADF of *C. Mucunoides* reached 45.83% and 33.94%, respectively. The content of the cell wall is a part that becomes a benchmark for the level of digestibility. This is due to the presence of ADL which is difficult to digest which always binds to the fiber fraction to form lignocellulose and lignohemicellulose bonds (Ajayi et al. 2021). Furthermore, Zain et al. (2023) said that in general the lignin content in legumes is higher than grass.

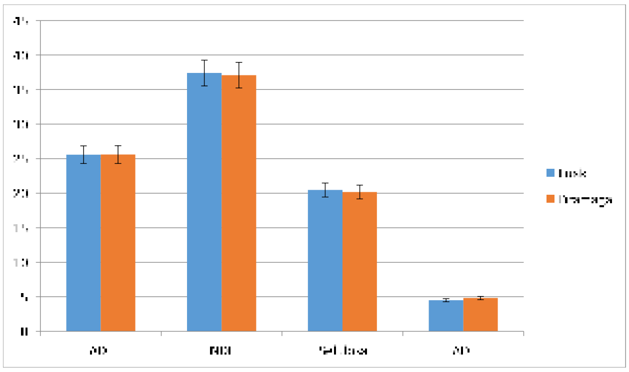

Fig. 1: Van Soest analysis tropical legumes

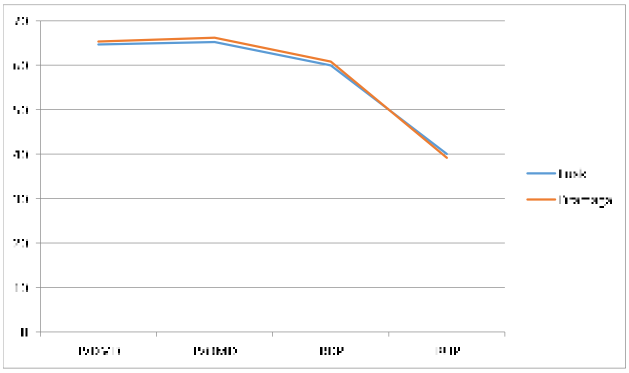

Fig. 2: In- vitro Dry Matter and Organic Matter Digestibility, RDP, RUP tropical legumes

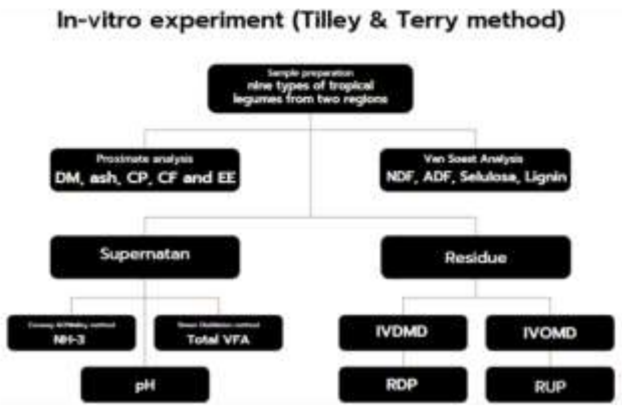

Fig. 1: Flow diagram of experimental methodology

Dry Matter and Organic Matter Digestibility

In-vitro of Dry Matter Digestibility (IVDMD) ranged from 58.03% (*A. pintoi*) – 72.68% (*I. zollingeriana*), meanwhile In-vitro of Organic Matter Digestibility (IVOMD) ranged from 58.43% (*A. pintoi*) – 74.43% (*I. zollingeriana*). The digestibility of tropical legume dry matter was showed in Table 3. The Luak and Dramaga areas showed statistically similar digestibility (P>0.05). Several factors contributed to this non-significant result, including the environmental conditions of the two areas. The environmental temperature of the Luak area averages 22-23°C while the Dramaga area has an average temperature of 23-25°C. Sampling was carried out using the same method and the same harvesting age of 45 days. Post-harvest treatment was also carried out using the same procedure; such as drying in an oven at 60°C for 48h. Avoid drying in the sun because the light intensity of the two areas will certainly be different so that the dryness level of the samples will also be different. This is done to minimize heterogeneous environmental factors in the two areas so that the results obtained are more precise.

IVDMD and IVOMD *C. mucunoides* and *L. leucocephala* were reported by (Evitayani et al. 2004) respectively 59.1%; 65.2% and 71.8%; 70.5% is not much different from the results of this study. A previous study by (Rahmat et al. 2021) said that IVDMD of several types of legumes such as *G. sepium*, *C. calothyrsus*, *I. zollingeriana* and *S. grandiflora* were incubated in-sacco for 48h in a row 76.32%; 38.90%; 82.97% and 81.94% and IVOMD respectively 75%; 37.82%; 81.99% and 81.06%. The digestibility was higher because it used a different method of measuring digestibility than this study. The higher the fiber content in the feed, the more difficult it is for rumen microbes to digest, thereby reducing digestibility. Jamarun et al. (2017) said that the nutrient content of feed and rumen microbes affected feed fermentation in the rumen. In addition to fiber, protein and energy also affect the digestibility of feed. Increasing protein, energy and RDP further increases nutrient digestibility (Putri et al. 2021). IVOMD follows the IVDMD pattern, because most DM contains OM.

High digestibility of dry matter and organic matter indicates good rumen fermentation conditions (Putri et al. 2021). The higher the digestibility of dry matter and organic matter, the better the fermentation process in the rumen feed. Good rumen fermentation conditions indicate optimal microbial activity thereby increasing enzyme production by rumen microbes to digest feed, which has a positive impact on increasing dry matter degradation and reducing the risk of adverse rumen conditions. High digestibility can increase the productivity of ruminants, because they can use nutrients optimally (Sharif et al. 2019). This result is in line with previous studies (Hao et al. 2018) which states that proper and sufficient synchronization between nutrient supply and substrate metabolism for microbes can increase microbial growth and nutrient digestibility of feed.

If the NDF and ADF content of the feed increases, the digestibility of forages such as legumes will decrease because they are plant cell wall components that are difficult for rumen microbes to digest (Stergiadis et al. 2015). Wilson and Hatfield (1997) said that plant cell wall lignification affects the level of feed degradation in the rumen. Rumen microbes will find it increasingly difficult to degrade feed if the lignification of the cell wall is higher. One of the main factors affecting the digestibility of tropical legumes is the content of their cell walls (Hadi et al. 2011). However, it is possible that other nutrients can also affect digestibility. Another factor that can increase digestibility is protein degradation in the rumen by microbes during the fermentation process (Tarigan and Ginting 2011).

Rumen Degradable and Undegradable Protein

The RDP and RUP content in this study were shown in Table 3. The results showed non-significant different among treatments (P>0.05). The RDP value ranged from 53.06% (*C. mucunoides*) – 73.05% (*I. zollingeriana*) while the RUP value ranged from 26.95 (*I. zollingeriana*) – 45.47% (*C. mucunoides*). RDP and RUP content in this study were consistent with previous studies (Putri et al. 2019; Zain et al. 2023). The high and low RDP and RUP values were also caused by the tannin content. Feeds with higher tannin content further reduce protein digestibility in the rumen, due to the presence of tannin complex bonds with proteins (Putri et al. 2019).

Rumen degradable protein (RDP) refers to the fraction of protein in feed that can be degraded by rumen microbes. Feed that contains a good source of protein for ruminants must be able to provide sufficient nitrogen for rumen microbial growth and can be digested post-rumen for the benefit of the host and has high biological value (Tedeschi et al. 2015). Rumen undegradable protein (RUP) or also called bypass protein is a protein fraction in the feed that enters the post rumen because it is able to go through the rumen degradation process. This indicates that the high productivity of ruminants is supported by the provision of balanced rations between degradable protein (RDP) and protein by-pass (RUP) (Savari et al. 2018).

Provision of balanced RDP and RUP in livestock ruminant feed is very important to meet animal protein needs. RDP provides a source of amino acids needed by rumen microbes for microbial protein synthesis, while RUP provides amino acids that can be directly used for animal body protein synthesis. The correct combination of RDP and RUP in livestock rations will ensure adequate availability of amino acids for growth, milk production, reproduction and other bodily functions. Analysis of feed composition and determination of RDP and RUP content in feed can help optimize livestock performance and feed efficiency. The balance of feed RDP and RUP must be considered so that excess N in the rumen does not occur as a fulfillment of MP needs (White et al. 2017).

Feed ingredients containing RDP and RUP vary depending on the degree of degradation in the rumen and the utilization of nitrogen by rumen microbes (Tacoma et al. 2017). Rumen microbes utilize RDP as a nitrogen source in their body's protein synthesis. Meanwhile, RUP was needed especially for livestock that are in a period of growth or are currently producing high. High-producing ruminants usually require more complete amino acids so a protein supply only from microbial protein is not sufficient. Therefore, large amounts of feed protein must avoid rumen degradation to meet ruminant protein needs (Bahrami-Yekdangi et al. 2014). The provision of rations that pay attention to the RDP-RUP ratio has a very positive impact on livestock. Bonchenari et al. (2020) reported that the growth rate of dairy calves was higher when given rations containing the RDP and RUP ratio.

Rumen Fluid Characteristic

The good or bad conditions of fermented feed in the rumen are described by the pH value (Rosmalia et al. 2022). This research has a pH ranging from 6.79-6.97. The pH value is still within the normal range of 5-7 (Puniya et al. 2015). In previous studies, increasing feed protein did not affect pH (Yang et al. 2016). Similar rumen fluid pH values from in-vitro tropical legumes were also reported by (Rahmat et al. 2021; Zain et al. 2023). Rumen microbial growth can be disrupted by fluctuations in rumen pH. This is because rumen microbes are sensitive to changes in pH, especially protozoa. Rumen pH is generally neutral, but when rumen pH decreases, energy sources that are normally used for microbial protein synthesis are used to maintain the pH to remain neutral (Uddin et al. 2015) thereby disrupting microbial protein synthesis.

Mutsvangwa et al. (2016) stated that in the rumen fermentation pattern, changes in N-NH3 in the rumen reflect the CP and RDP levels of the feed. The concentration of N-NH3 indicates how much feed protein can be digested in the rumen. Its value was determined by the ability of rumen microbes to degrade feed protein and how quickly feed ingredients can be digested in the rumen. Table 4 shows the variations in N-NH3 values. I.zollingeriana produced the highest N-NH3 production. The level of N-NH3 is influenced by the nutritional content of feed ingredients, especially crude protein (Table 1).

VFA is the result of fermentation of organic matter in the rumen. VFA is used by ruminants as an energy source. A high VFA concentration in the rumen indicates a high level of digested organic matter. Previous research by Putri et al. (2021) reported that the high total VFA concentration is associated with increased nutrient digestibility. This is because VFA is a product of nutrient degradation so that an increase in total VFA will increase nutrient digestibility. Table 4 shows that the highest VFA concentration is found in I.zollingeriana with a value of 96.67mM. The higher the organic matter digested in the rumen, the higher the VFA concentration (Table 2). Rumen microbes use VFA as a source of energy for their body's protein synthesis, while ATP, as a product of other feed degradation, will be used by host livestock (Brooks et al. 2012; Hackmann and Firkins 2015).

Conclusion

The conclusion from this study is that tropical legumes of the same type but grown in areas with different environmental conditions tend to have almost the same quality. The database of feed nutrient content is very useful in formulating livestock rations. A good ration formulation is based on the ratio of RDP and RUP so that the needs of rumen microbes and host livestock are met. This research is highly useful to help formulate ruminant livestock rations based on the ratio of RDP and RUP, due to limited information from previous studies.

Acknowledgment

Funding for this research grant from the Faculty of Animal Science Andalas University No. 038/UN16.06.D/KPT/XI/2023.

Author's contribution

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

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