ISSN 0126-0472 EISSN 2087-4634
Accredited by DGHE No: 36b/E/KPT/2016

Media Peternakan, December 2016, 39(3):195-202
DOI: https://doi.org/10.5398/medpet.2016.39.3.195
Available online at http://medpet.journal.ipb.ac.id/

Nutrient Content, Protein Fractionation, and Utilization of Some Beans as Potential Alternatives to Soybean for Ruminant Feeding

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(Received 27-06-2016; Reviewed 05-08-2016; Accepted 06-10-2016)

ABSTRACT

This experiment aimed to determine nutrient content, protein fraction, and *in vitro* rumen fermentation of some alternative beans in comparison to soybean. Samples used were napier grass, soybean, redbean, groundnut, pigeonpea, cowpea, bambarabean, and mungbean. Samples were determined for their proximate composition, Van Soest's fiber fraction, and Cornell protein fraction. The samples were subsequently evaluated for their fermentation characteristics and digestibility by using a two-stage *in vitro* rumen fermentation technique, maintained at 39 °C for 2 × 48 h. The *in vitro* incubation was performed in three consecutive runs by following a randomized complete block design in which each sample per run was represented by four fermentation tubes. Results revealed that all experimental beans contained high crude protein (CP), i.e. above 200 g/kg dry matter (DM), but only soybean and groundnut had CP contents higher than 300 g/kg DM. Redbean had the lowest crude fiber and acid detergent fiber contents among the beans. Soybean contained high proportion of rapidly degraded CP fraction, but low in slowly degraded and unavailable CP fractions. High proportion of slowly degraded CP fraction was found in redbean and bambarabean. Redbean, pigeonpea, cowpea, and mungbean were better than soybean, groundnut, and bambarabean with regard to DM degradability and DM digestibility values ($P<0.05$). Concentration of total VFA was the highest in the incubation of redbean. It was concluded that groundnut, redbean, pigeonpea, cowpea, and mungbean have the potency to be used to substitute soybean for ruminant feeding.

Key words: bean, alternative feed, protein fraction, ruminant, rumen

ABSTRAK

Penelitian ini bertujuan untuk menentukan kandungan nutrien, fraksi protein, dan fermentasi rumen *in vitro* dari sejumlah kacang-kacangan alternatif kacang kedelai sebagai pakan ternak ruminansia. Bahan pakan yang digunakan dalam penelitian ini adalah rumput gajah, kacang kedelai, kacang merah, kacang tanah, kacang gude, kacang tunggak, kacang bogor, dan kacang hijau. Analisis komposisi proksimat, fraksi serat Van Soest, dan fraksi protein Cornell dilakukan pada bahan. Bahan kemudian dievaluasi secara *in vitro* dengan menggunakan teknik fermentasi rumen dua tahap pada suhu 39 °C selama 2 × 48 jam. Inkubasi *in vitro* dilakukan dalam tiga ulangan berdasarkan rancangan acak kelompok (masing-masing diwakili oleh empat tabung fermentasi). Hasil penelitian menunjukkan bahwa semua kacang-kacangan mengandung protein kasar (PK) yang tinggi, yakni lebih dari 200 g/kg bahan kering (BK), namun hanya kacang kedelai dan kacang tanah yang lebih tinggi dari 300 g/kg BK. Kacang merah memiliki kandungan serat kasar dan serat deterjen asam yang paling rendah di antara kacang-kacangan yang diuji. Kacang kedelai mengandung proporsi fraksi PK mudah terdegradasi yang tinggi, namun rendah fraksi yang lambat terdegradasi dan yang tidak tersedia. Fraksi PK lambat terdegradasi yang tinggi terdapat pada kacang merah dan kacang bogor. Kacang merah, kacang gude, kacang tunggak, dan kacang hijau memiliki degradasi dan kecernaan BK yang lebih tinggi dibandingkan dengan kacang kedelai, kacang tanah, dan kacang bogor ($P<0,05$). Konsentrasi total VFA paling tinggi terdapat pada inkubasi kacang merah. Disimpulkan bahwa kacang tanah, kacang merah, kacang gude, kacang tunggak, dan kacang hijau berpotensi untuk mensubstitusi kacang kedelai sebagai pakan ternak ruminansia.

Kata kunci: kacang-kacangan, pakan alternatif, fraksi protein, ruminansia, rumen

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INTRODUCTION

Adequate and balance nutrients are necessary to ensure optimal livestock production and health, including energy and protein supply. Typically, in Indonesia and elsewhere, energy demand of livestock is relatively easier to meet from feed rather than protein demand, causing unbalance supply between energy and protein. The use of protein supplements is a common approach to overcome such insufficient protein supply. Soybean, either fullfat or defatted soybean (soybean meal), has been used as a main protein supplement for monogastric and ruminant animals in many regions of the world (Campos *et al.*, 2014; Jolazadeh *et al.*, 2015; Liu *et al.*, 2016) including in Indonesia (Akhsan *et al.*, 2015; Faradillah *et al.*, 2015). Among protein supplements originated from plant sources, soybean is considered as superior with regard to its protein content and quality. Protein contents of soybean and soybean meal are around 35%-52% DM (Vollmann, 2016). Protein in soybean is highly digestible and rich in lysine, tryptophan, threonine, isoleucine, and valine, in which these amino acids are generally deficient in cereal grains (Yildiz & Todorov, 2014). However, with an increasing demand on soybean for animal feed and other purposes, and on the other hand, risks that may limit soybean production such as soil degradation, global warming (Hao *et al.*, 2010), etc., there is an urgent need to search for alternative protein sources other than soybean.

Beans are generally known for their high protein contents due to their symbiotic relationships with nitrogen fixing bacteria, i.e. *Rhizobium* sp. that are able to take up nitrogen from the air, thus have the capacity to accumulate more nitrogenous compounds in the tissue (Goh *et al.*, 2016). A number of beans available in Indonesia are redbean (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*), pigeonpea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), bambarabean (*Vigna subterranea*), and mungbean (*Phaseolus radiatus*). Although these beans have been traditionally used for human consumption in Indonesia (Haliza *et al.*, 2007; 2010), they are rarely used as animal feed. Furthermore, the informations about their nutritional contents, qualities, and utilizations for animals are very limited. Therefore this experiment aimed to determine nutrient content, protein fraction, and *in vitro* rumen fermentation of some alternative beans in comparison to soybean as a reference of commonly used protein supplement.

MATERIALS AND METHODS

Sample Collection and Preparation

Samples used in the present experiment were napier grass (*Pennisetum purpureum*), soybean (*Glycine max*), redbean (*Phaseolus vulgaris*), groundnut (*Arachis hypogaea*), pigeonpea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), bambarabean (*Vigna subterranea*), and mungbean (*Vigna radiata*). Napier grass was collected from experimental station of Faculty of Animal Science, Bogor Agricultural University, and the beans were purchased from a traditional market in Bekasi, Indonesia. All samples were immediately oven-dried at 60 ^{o}C for 24 h and then ground to pass a 1 mm sieve for further chemical composition analysis and *in vitro* incubation.

Chemical Composition Determination

Samples were determined for their dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and ether extract (EE) contents according to AOAC (2005). An oven at 105 ^{o}C and a furnace at 550 ^{o}C were employed to determine DM and OM contents of samples, respectively. Contents of CP and EE were determined by using micro-Kjeldahl and Soxhlet extraction apparatus, respectively. Sequential digestion with H_2SO_4 and NaOH solutions was performed to obtain CF. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by following the procedures of Van Soest *et al.* (1991). Analysis of NDF was performed without using α-amylase and sodium sulfite and expressed exclusive of residual ash. Determination of ADF was similar to NDF except that the solution used was acid detergent solution. Gross energy was determined by using a bomb calorimeter.

Determination of CP fraction followed the protocol of Licitra *et al.* (1996) that is based on the original Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen *et al.*, 1992). The CP was divided into three main fractions, i.e. fraction A (non-protein nitrogen, NPN), fraction B (true protein), and fraction C (unavailable protein). Fraction B is further divided into three fractions namely B1, B2, and B3 in which they have different degradation rates in the rumen. Fraction B1 is a rapidly degraded CP in the rumen, whereas B2 and B3 are intermediately and slowly degraded CP, respectively. Fraction A was obtained by precipitating true protein with trichloroacetic acid (TCA); it was calculated by the difference between CP and the precipitated true protein. Fraction C is regarded as acid detergent insoluble crude protein (ADICP) and was determined by measuring CP content of ADF. Similar to ADICP, neutral detergent insoluble crude protein (NDICP) was determined by measuring CP content of NDF. Procedures for ADICP and NDICP measurements were adopted according to Jayanegara *et al.* (2016). Determination of B1, B2, and B3 fractions require determination of soluble crude protein (SCP). The SCP is defined as true protein soluble in buffer at rumen pH. It was measured by mixing 0.5 g sample with 50 mL borate-phosphate buffer and 1 mL of 10% sodium azide solution. Subsequently the B fractions were calculated as follow:

B1 = SCP – NPN (fraction A)
B2 = CP – SCP – NDICP
B3 = NDICP – ADICP (fraction C)

All of the chemical composition analyses were performed in duplicate. Proximate composition and Van Soest's fiber fractions were expressed as g/kg DM whereas fiber fractions were expressed as proportions to their corresponding CP contents (g/kg CP).

In Vitro Rumen Fermentation

The ground samples were evaluated for their fermentation characteristics and digestibility by using a two-stage *in vitro* rumen fermentation technique (Tilley & Terry, 1963). An amount of 0.5 g sample was inserted into a fermentation glass tube and added with 40 mL McDougall's buffer. About 10 ml of rumen fluid was then added into the tube. Rumen fluid was obtained from two fistulated Ongole crossbred cattle, taken through the fistula before morning feeding. The cattle were cared for according to animal welfare standard of LIPI Cibinong Bogor. All tubes were continuously flushed with CO_2 for 30 s to ensure anaerobic condition and immediately closed with ventilated rubbers. The *in vitro* incubation was performed in three consecutive runs (replicates) at different weeks in which each sample per run was represented by four fermentation tubes; two tubes were completed after 48 h incubation with buffered-rumen fluid (first stage) and the remaining tubes were continued for another 48 h incubation with pepsin-HCl solution (second stage). After the first stage of incubation, the tubes were centrifuged at 4,000 rpm for 10 min. The supernatant was taken for subsequent VFA analysis and determination of ammonia concentration by using gas chromatography technique and Conway micro-diffusion method, respectively. The residue was analysed for DM, OM, and CP to obtain DM degradability (DMDe), OM degradability (OMDe), and CP degradability (CPDe) values, respectively. In the second stage of *in vitro* fermentation, the supernatants in the remaining tubes were discarded after centrifugation. Subsequently, an amount of 50 mL pepsin-HCl 0.2% solution was added into each tube and incubation was performed for another 48 h, but without closing with the ventilated rubbers. The residue was separated with supernatant through filtration using Whatman paper no. 41 and analysed for DM, OM, and CP to obtain DM digestibility (DMDi), OM digestibility (OMDi), and CP digestibility (CPDi) values.

Statistical Analysis

Chemical composition data were descriptively tabulated. *In vitro* incubation data were analysed by analysis of variance (ANOVA) following a randomized complete block design. Different batches of rumen fluid (taken at different weeks) served as the blocks. The following statistical model was employed:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} is the observed value for i^{th} treatment and j^{th} replicate, μ is the overall mean, τ_i is the treatment effect, β_j is the block effect (replicate) and ε_{ij} is the random residual error. The significancy was stated when the ANOVA result showed $P<0.05$ for a certain variable. Comparison among treatments was performed by using Duncan's multiple range test. Prior to ANOVA, the data were checked for outlier values; any values ≤ –2 or ≥ 2 of their standardized residuals were categorized as outliers. Pearson correlation test was applied to the data to observe relationship among chemical composition and *in vitro* rumen fermentation parameters. All statistical analyses were performed by employing SPSS software version 20.

RESULTS

Chemical Composition

All experimental beans contained high CP, i.e., above 200 g/kg DM. Soybean and groundnut had CP contents higher than 300 g/kg DM (Table 1). Napier grass contained much higher CF, NDF, and ADF than those of the beans. Among all beans, redbean had the lowest CF and ADF contents. Other beans that had lower ADF in comparison to soybean were cowpea and mungbean. The content of EE was particularly high in groundnut and soybean. The two beans were also high in GE contents as compared to the other beans. Fraction A was high in napier grass but low in soybean (Table 2). Soybean contained high proportion of fraction B1 and B2, but low fraction B3 and C. High proportion of fraction B3 as well as NDICP was found in napier grass, redbean, and bambarabean. Fraction C was particularly very high in napier grass. Although bambarabean and mungbean were also high in fraction C, their contents were approximately one-third than that of napier grass.

In Vitro Rumen Fermentation

Napier grass had the lowest DMDe and DMDi in comparison to other feeds ($P<0.05$; Table 3). All beans generally had high DMDe and DMDi. Among the beans,

Table 1. Chemical composition (g/kg DM) and gross energy content of some feed materials (kcal/kg DM)

Feedstuff	OM	CP	CF	NDF	ADF	EE	GE
Napier grass	902	113	371	666	489	39.0	4252
Soybean	952	377	93.8	235	139	219	5691
Redbean	961	260	55.8	323	93.1	17.6	4474
Groundnut	975	339	128	200	174	476	6997
Pigeonpea	958	242	108	313	168	13.2	4257
Cowpea	963	273	69.8	417	117	18.0	4684
Bambarabean	950	237	76.0	366	161	68.9	4594
Mungbean	965	266	58.0	222	119	14.7	4420

Note: DM= dry matter; OM= organic matter; CP= crude protein; CF= crude fiber; NDF= neutral detergent fiber; ADF= acid detergent fiber; EE= ether extract; GE= gross energy.

Table 2. Protein fraction of some feed materials (g/kg CP)

Feedstuff	A	B1	B2	NDICP	B3	C
Napier grass	315	49.0	142	494	168	326
Soybean	45.6	571	296	86.9	24.5	62.4
Redbean	182	543	54.6	221	161	59.8
Groundnut	146	577	169	109	28.0	80.8
Pigeonpea	220	322	352	106	24.4	81.2
Cowpea	143	515	256	86.9	27.7	59.3
Bambarabean	258	150	383	210	106	103
Mungbean	180	449	243	128	11.4	116

Note: CP= crude protein; A= non-protein nitrogen; B1= rapidly degraded protein; B2= intermediately degraded protein; NDICP= neutral detergent insoluble crude protein; B3= slowly degraded protein; C= unavailable protein.

redbean, pigeonpea, cowpea, and mungbean were better than soybean, groundnut, and bambarabean with regard to DMDe and DMDi values (P<0.05). Patterns of OMDe and OMDi values were similar to those of DMDe and DMDi, respectively. With regard to CPDe and CPDi, soybean and groundnut were superior in comparison to other beans (P<0.05). The lowest CPDe and CPDi were found in napier grass and followed by bambarabean. Proportions of CPDe to CPDi for napier grass, soybean, redbean, groundnut, pigeonpea, cowpea, bambarabean, and mungbean were 58%, 92%, 81%, 92%, 70%, 80%, 64%, and 82%, respectively.

The highest concentration of total VFA was found in the incubation of redbean and the lowest was found in napier grass (Table 4). Groundnut produced the lowest total VFA among all experimental beans (P<0.05). Incubation of pigeonpea, cowpea, bambarabean, and mungbean resulted in similar total VFA concentrations. Proportion of C_2 was the highest for napier grass (P<0.05) whereas proportion of C_3 was the highest for bambarabean and redbean (P<0.05). The lowest proportion of C_4 was found in napier grass incubation (P<0.05). All beans produced ammonia at concentrations above 20 mM in which soybean was superior in generating ammonia as compared to other beans (P<0.05). The lowest ammonia concentration among the beans was found in the incubation of bambarabean.

Correlation between Chemical Composition and i*In Vitro* Rumen Fermentation Parameters

The concentration of CP was positively correlated with CPDe, CPDi, and ammonia concentration (P<0.01; Table 5). Fiber components, especially CF and ADF were negatively correlated with DMDe, DMDi, OMDe, and OMDi (P<0.05) but positively correlated with C_2 and C_4 proportions (P<0.05). The contents of EE and GE did not have any significant correlation with *in vitro* rumen fermentation parameters. Protein fraction B1 was positively correlated with CPDe, CPDi, and ammonia concentration (P<0.01) whereas, on the contrary, NDICP was inversely related with the *in vitro* rumen fermentation parameters (P<0.01). Fraction B3 had no significant correlation with CPDe and CPDi but it negatively correlated with ammonia concentration (P<0.05). Fraction C was negatively correlated with CPDe, CPDi, and ammonia (P<0.05).

DISCUSSION

Although all alternative beans had relatively high CP contents, none of them had equal CP in comparison to soybean. Typical CP contents in redbean, groundnut, pigeonpea, cowpea, bambarabean, and mungbean are (mean±sd) 248±15, 297±31, 232±90, 252±22, 198±31, and 258±28 g/kg DM, respectively (FAO, 2016), in which data on CP contents of the beans in the present experiment were within the range reported by FAO. Protein in soybean is known to be easily degraded in the rumen and therefore it is high in the proportion of

Table 3. *In vitro* degradability and digestibility of some feed materials (g/kg) (n= 3 replicates)

Feedstuff	DMDe	DMDi	OMDe	OMDi	CPDe	CPDi
Napier grass	266^{a}	475^{a}	260^{a}	429^{a}	244^{a}	422^{a}
Soybean	548^{b}	755^{c}	415^{bc}	740^{c}	796^{e}	861^{d}
Redbean	672^{c}	888^{d}	567^{d}	882^{d}	636^{d}	787^{c}
Groundnut	564^{b}	698^{b}	454^{c}	683^{b}	787^{e}	854^{d}
Pigeonpea	698^{c}	893^{d}	613^{d}	889^{d}	531^{c}	763^{c}
Cowpea	676^{c}	878^{d}	588^{d}	874^{d}	625^{d}	783^{c}
Bambarabean	526^{b}	774^{c}	384^{b}	758^{c}	444^{b}	697^{b}
Mungbean	703^{c}	896^{d}	619^{d}	892^{d}	652^{d}	793^{c}
SEM	21.0	21.4	21.4	23.8	26.2	18.7
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means in the same column with different superscripts differ significantly (P<0.05).
DMDe= dry matter degradability; DMDi= dry matter digestibility; OMDe= organic matter degradability; OMDi= organic matter digestibility; CPDe= crude protein degradability; CPDi= crude protein digestibility; SEM= standard error of mean.

Table 4. *In vitro* ruminal volatile fatty acid (VFA) profile and ammonia concentrations of some feed materials (n= 3 replicates)

Feedstuff	Total VFA (mM)	C2 (%)	C3 (%)	C4 (%)	Ammonia (mM)
Napier grass	46.8^{a}	66.5^{c}	17.5^{a}	15.9^{a}	7.15^{a}
Soybean	59.5^{bc}	61.1^{ab}	18.8^{ab}	20.1^{bc}	45.6^{f}
Redbean	79.6^{d}	60.8^{ab}	20.6^{c}	19.0^{b}	31.6^{d}
Groundnut	48.6^{ab}	62.2^{b}	18.5^{ab}	19.4^{bc}	37.2^{e}
Pigeonpea	64.3^{c}	59.6^{a}	19.6^{bc}	20.8^{c}	27.6^{c}
Cowpea	65.8^{c}	60.6^{ab}	18.8^{ab}	19.2^{bc}	34.7^{e}
Bambarabean	61.6^{c}	60.4^{ab}	21.2^{c}	18.4^{b}	21.0^{b}
Mungbean	64.4^{c}	59.7^{a}	19.5^{bc}	20.8^{c}	35.4^{e}
SEM	2.13	0.503	0.311	0.640	1.73
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Means in the same column with different superscripts differ significantly (P<0.05).
C2= acetate; C3= propionate; C4= butyrate; SEM= standard error of mean.

Table 5. Correlation coefficient between feed chemical composition and *in vitro* ruminal fermentation parameters (n= 8)

Variables	CP	CF	NDF	ADF	EE	GE	A	B1	B2	NDICP	B3	C
DMDe	0.53	–0.89**	–0.72*	–0.90**	–0.15	–0.04	–0.49	0.66	0.18	–0.83*	–0.58	–0.85**
DMDi	0.45	–0.91**	–0.63	–0.90**	–0.31	–0.19	–0.42	0.55	0.25	–0.77*	–0.48	–0.83*
OMDe	0.35	–0.75*	–0.56	–0.76*	–0.28	–0.17	–0.36	0.58	0.07	–0.70	–0.53	–0.69
OMDi	0.46	–0.92**	–0.64	–0.91**	–0.29	–0.18	–0.43	0.56	0.25	–0.78**	–0.49	–0.84**
CPDe	0.96**	–0.69	–0.88**	–0.76*	0.56	0.68	–0.93**	0.95**	–0.03	–0.84**	–0.66	–0.80*
CPDi	0.93**	–0.87**	–0.93**	–0.91**	0.40	0.52	–0.84**	0.88**	0.17	–0.94**	–0.69	–0.94**
Total VFA	0.16	–0.71*	–0.29	–0.70	–0.55	–0.45	–0.22	0.37	–0.09	–0.36	0.08	–0.60
C2	–0.57	0.95**	0.75*	0.92**	0.15	0.05	0.46	–0.50	–0.48	0.85**	0.59	0.87**
C3	0.13	–0.69	–0.37	–0.62	–0.31	–0.29	0.05	0.02	0.28	–0.28	0.13	–0.53
C4	0.69	–0.80*	–0.86**	–0.81*	0.07	0.16	–0.63	0.65	0.38	–0.90**	–0.80*	–0.79*
Ammonia	0.96**	–0.74*	–0.86**	–0.80*	0.41	0.54	–0.96**	0.93**	0.09	–0.88**	–0.71*	–0.83*

Note: *= P<0.05; **= P<0.01.
CP= crude protein; CF= crude fiber; NDF= neutral detergent fiber; ADF= acid detergent fiber; EE= ether extract; GE= gross energy; A= non-protein nitrogen; B1= rapidly degraded protein; B2= intermediately degraded protein; NDICP= neutral detergent insoluble crude protein; B3= slowly degraded protein; C= unavailable protein; DMDe= dry matter degradability; DMDi= dry matter digestibility; OMDe= organic matter degradability; OMDi= organic matter digestibility; CPDe= crude protein degradability; CPDi= crude protein digestibility; VFA= volatile fatty acid; C2= acetate; C3= propionate; C4= butyrate.

rumen degradable protein (Maxin *et al.*, 2013; Akbarian *et al.*, 2014). The present study confirmed such finding as shown by the high proportions of protein fraction B1 and B2 in soybean as well as by the high value of CPDe and ammonia concentration in the *in vitro* incubation of soybean. To our knowledge, this is the first study in Indonesia that attempted to determine various protein fractions in feedstuffs by using CNCPS method. Sniffen *et al.* (1992) stated that protein fraction B1 is rapidly degraded in the rumen and easily converted to peptides, amino acids, and ammonia by rumen microbes, whereas some fraction B2 is fermented in the rumen and some escapes to the next gastro-intestinal tract; this depends on the relative rates between digestion and passage. Further, the authors reported that the digestion rate constants for B1 and B2 fractions of proteinaceous feeds were 50-400%/h and 2-14%/h, respectively (Sniffen *et al.*, 1992). Some beans such as groundnut, pigeonpea, and cowpea showed high proportions of B1 and B2 like soybean, and hence, indicated high proportion of rumen degradable protein of the beans.

Such high proportion of degradable protein is not always good. When protein is rapidly degraded in the rumen, it may not synchron with the rate of energy and carbon provision for microbial protein synthesis, thus decreasing its conversion efficiency (Yang *et al.*, 2010; Seo *et al.*, 2013). This condition may lead to the accumulation of ammonia concentration in the rumen and blood stream, and when ammonia concentration in blood is above the threshold it may cause toxicity response (Bartley *et al.*, 1976; Holder *et al.*, 2013). Therefore a balance proportion of degradable and undegradable (but digestible) protein is important to avoid such inefficiency of microbial protein synthesis and ammonia toxicity. Wang *et al.* (2008) observed that high ratio of rumen degradable to rumen undegradable protein resulted in high urinary N and total N excretion. Further, a reduction of rumen degradable to rumen undegradable protein ratio improved the efficiency of N utilization in lactating dairy cows by decreasing N excretion in urine and faeces. Marghazani *et al.* (2012) fed different proportion of rumen undegradable protein to early lactating Sahiwal cows, i.e., 30, 40, 50, or 60% (iso-energetic and iso-proteic diets). It was observed that the cows fed with 40% rumen undegradable protein resulted in a maximum nitrogen balance and production performance. A number of treatments may be applied to shift the highly degradable protein in soybean towards more undegradable protein (by-pass protein) such as by using tannins (Jolazadeh *et al.*, 2015) and formaldehyde (De Campeneere *et al.*, 2010). These compounds have been known to be able to protect protein and resistant to rumen degradation by microbes (Jayanegara *et al.*, 2013, 2015; Mahima *et al.*, 2015). Saponins may also potentially be used for protecting protein degradation in the rumen due to their chemical interaction and inhibition on growth and activity of proteolytic microbes such as *Streptococcus bovis*, *Butyrivibrio fibrisolvens*, and *Prevotella bryantii* (Jayanegara *et al.*, 2014).

Redbean and bambarabean apparently good sources of rumen undegradable protein as shown by their high proportions of fraction B3. Protein fraction B3 is insoluble in neutral detergent solution but it is soluble in acid detergent solution (Higgs *et al.*, 2012). This fraction is slowly degraded in the rumen and the high percentage of B3 fraction escapes degradation. Digestion rate constants of protein B3 for grains, proteinaceous feeds, and forages were 0.06-0.55, 0.05-0.30, and 0.08-2.0%/h, respectively (Sniffen *et al.*, 1992). However, it has to be noted that high proportion of undegradable protein is meaningless if it can not be digested and utilized in the lower gastro-intestinal tract. In the case of bambarabean, the high proportion of protein fraction C may limit its protein utilization. Protein fraction C is also known as acid detergent insoluble CP (ADICP). It represents the protein linked to lignin, tannin-protein complexes, heat-damaged protein, and Maillard products (Licitra *et al.*, 1996). Further, it is highly resistant to microbial enzymes, does not provide amino acids post-ruminally (Sniffen *et al.*, 1992), and generally considered unavailable for ruminants (Pelletier *et al.*, 2010). The negative correlations between protein fraction C with CPDe, CPDi, and ammonia concentration in the present study confirmed such concept. Our previous study also observed a negative relationship between ADICP proportion in feedstuffs and their protein digestibility (Jayanegara *et al.*, 2016).

Apart from the good quality of protein found in redbean, low CF and ADF contents in the bean show its potency as animal feed. Such low fiber content leads to high DMDe, DMDi, OMDe, and OMDi values since lignocellulose is known to be hardly degraded and fermented by microbes under anaerobic environment as present in the rumen (Laconi & Jayanegara, 2015; Rouches *et al.*, 2016). High digestibility of redbean is confirmed by the high total VFA production as an end product of microbial metabolism in the rumen, particularly from carbohydrate (both structural and non-structural) fermentation (Scharen *et al.*, 2016). Confirming this result, a main factor determining total VFA production rate is rumen fermentable organic matter intake; there is a strong linear positive relationship between both variables (Noziere *et al.*, 2011). In the case of groundnut that produced low total VFA, it is apparently due to the high EE or fat content. Fat in the form of triglyceride undergoes lipolysis in which fatty acids are separated from glycerol (Buccioni *et al.*, 2012). Glycerol is further metabolized to result VFA but fatty acids are not metabolized by rumen microbes. Rather, fatty acids undergo saturation process of the double bonds known as biohydrogenation (Jayanegara *et al.*, 2012). Therefore the contribution of fat to VFA is only from glycerol fermentation and it is considered as small amount, taking into consideration that glycerol is a three carbon molecule whereas fatty acids are medium to long chain (>12 carbon molecule), depend on the origin of the fat. Additionally, one molecule of triglyceride is consisted of one molecule of glycerol and three molecules of fatty acids. The VFA is later used as energy source by the host animals and may contribute to about 70% of their total energy requirement (Bergman, 1990).

Each individual VFA has its own fate after absorption in which acetate is a precursor of milk fat and propionate is a precursor of glucose and milk sugar or lactose synthesis (Aluwong *et al.*, 2010; Fievez *et al.*, 2012). In the present study, higher percentage of acetate is due to higher proportion of fiber, either CF, NDF or ADF. This result is supported by a meta-analysis study of Noziere *et al.* (2011) that observe an increase in molar percentage of acetate with increasing proportion of digested NDF in the digested organic matter, in which the relationship is curvilinear. Further in that study, higher proportion of digested NDF in the digested organic matter results in a decrease in molar percentage of propionate. Although the correlation coefficient between fiber and propionate in this experiment was negative, it did not show any significant relationship. Bannink *et al.* (2006) outlined that more cellulose (fiber) fermentation will increase acetate production whereas more starch fermentation will increase propionate production. This is because of different metabolic fate between fibrolytic bacteria that produce more acetate and amylolytic bacteria that produce more propionate in the rumen (Alemu *et al.*, 2011).

CONCLUSION

Groundnut, redbean, pigeonpea, cowpea, and mungbean have the potency as alternatives to soybean, at least partially, for ruminant feeding. All experimental beans except redbean and bambarabean are similar to soybean in which they are high with degradable protein proportion, whereas the other two beans contain substantially higher proportion of undegradable protein. Redbean in particular may strategically be used as a source of protein by-pass in ration. Although CP content of redbean is not as superior as soybean, it has a comparative advantage due to its low CF and ADF contents. In the case of bambarabean, its utilization may be limited since it contains considerable proportion of undigested protein (fraction C).

ACKNOWLEDGEMENT

This research was funded by Indonesian Ministry of Research, Technology, and Higher Education through "Penelitian Unggulan Perguruan Tinggi – Penelitian Unggulan Divisi" research grant. All authors are grateful to Mr. Sofyan, Mrs. Eneh, and Mrs. Dian Anggraeni for excellent technical helps during the experimental period.

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