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PROTEIN FRACTIONATION AND UTILIZATION OF SOYBEAN AND REDBEAN AT DIFFERENT DRYING TEMPERATURES

FRAKSINASI DAN UTILISASI PROTEIN KACANG KEDELAI DAN KACANG MERAH PADA SUHU PENGERINGAN YANG BERBEDA

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ABSTRACT

The objective of this study was to investigate the effect of drying temperatures on chemical composition, rumen fermentation, and digestibility of soybean and redbean (*in vitro*). Soybean and redbean were dried in an oven set at four different drying temperatures: 50, 60, 70, and 80ºC for 24 h in three replicates. The dried samples were then grilled and used further for chemical composition determination (proximate analysis, Van Soest analysis, and protein fraction) and *in vitro* rumen fermentation assay. Parameters measured in the *in vitro* assay were gas production, digestibility, pH, ammonia, and volatile fatty acids (VFA). Data obtained were analyzed by using analysis of variance and a posthoc test namely Duncan's multiple range test. Results showed that neutral detergent insoluble crude protein (NDICP) content was increased at higher drying temperature (70 or 80ºC) for both soybean and redbean (P<0.05). Similar to NDICP, higher temperature led to a greater acid detergent insoluble crude protein (ADICP) of soybean as well as those of redbean (P<0.05). Higher temperature decreased gas production rate (GPR) of both beans (P<0.05). Drying of soybean at 70 or 80ºC decreased crude protein digestibility (CPD) of soybean more than those of dried at 50 or 60ºC (P<0.05). Higher drying temperature resulted in a lower NH_3 concentration of both beans (P<0.05). In conclusion, drying temperature at 50 or 60ºC was considered to be safe to maintain the nutritional quality of soybean and redbean.

(Keywords: Drying, *In vitro*, Protein, Redbean, Soybean)

INTISARI

Penelitian ini bertujuan untuk mengevaluasi pengaruh suhu pengeringan yang berbeda terhadap komposisi kimia, fermentasi rumen secara in vitro, dan kecernaan kacang kedelai dan kacang merah. Kacang kedelai dan kacang merah dikeringkan di dalam oven pada suhu 50, 60, 70 dan 80ºC selama 24 jam dalam tiga ulangan. Sampel yang telah kering kemudian digiling dan dianalisis komposisi kimianya (analisis proksimat, analisis Van Soest, dan fraksi protein) serta dilakukan pengujian fermentasi rumen secara in vitro. Peubah yang diamati pada uji in vitro meliputi produksi gas, kecernaan, pH, konsentrasi amonia, dan volatile fatty acids (VFA). Data dianalisis dengan menggunakan analisis ragam dan uji lanjut Duncan. Hasil menunjukkan bahwa kandungan neutral detergent insoluble crude protein (NDICP) meningkat pada suhu pengeringan yang lebih tinggi (70 dan 80ºC) pada kacang kedelai dan kacang merah (P<0.05). Suhu yang tinggi juga meningkatkan kandungan acid detergent insoluble crude protein (ADICP) kacang kedelai dan kacang merah (P<0.05). Suhu tinggi menurunkan laju produksi gas pada kedua kacang-kacangan tersebut (P<0.05). Pengeringan pada suhu 70 dan 80ºC menurunkan kecernaan protein kasar kacang kedelai dibandingkan dengan suhu 50 dan 60ºC (P<0.05). Semakin tinggi suhu pengeringan menghasilkan konsentrasi NH_3 yang semakin rendah pada kedua jenis kacang-kacangan (P<0.05). Dapat disimpulkan bahwa pengeringan dapat dilakukan pada suhu 50 dan 60ºC dengan tetap dapat mempertahankan kualitas nutrisi kacang kedelai dan kacang merah.

(Kata kunci: In vitro, Kacang kedelai, Kacang merah, Protein, Suhu)

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Introduction

Protein is a main nutrient required by livestock, of both monogastrics and ruminants, to fulfill their maintenance, production, and reproduction requirements. Protein supplements are often used for feeding ruminant livestock since the requirements generally cannot be met by only consuming forage sources such as grasses and agricultural residues for optimum animal production. Some protein supplements that have been used to enhance feed protein content of ruminant livestock in Indonesia are: concentrate mixture (Astuti *et al.*, 2009; Wulandari *et al.*, 2014), and some feed stuff, such as: *katu* (*Sauropus androgynous*) leaves (Marwah *et al.*, 2010), moringa leaves (Jayanegara *et al.*, 2010), cassava leaves (Sudarman *et al.*, 2016), rice bran (Endrawati *et al.*, 2010), and mungbean fodder (Zahera *et al.*, 2015). Some beans can also been used as protein supplements from plant origins. To date, soybean is the most common bean that used as protein supplement for various livestock species due to its high protein content and quality. Soybean may be used either in the form of intact (full-fat) soybean (Zhang *et al.*, 2015) or defatted soybean (after oil removal by means of mechanical or chemical extraction) that commonly known as soybean meal (Weiss *et al.*, 2015). Other beans have been attempted as alternatives to soybean such as redbean, groundnut, pigeonpea, cowpea, bambarabean, and mungbean (Jayanegara *et al.*, 2016a). Although these beans are generally considered of lower quality in comparison to soybean, they are very useful when there is a shortage of soybean supply or when the price is seasonally high.

In ruminant ration, beans are typically used in meal form to facilitate homogenous mixture with other feed ingredients in concentrate. Therefore, beans should be dried prior of ground in a hammer mill to pass a certain screen size. Drying can be performed in many ways, such as: sun-drying, oven-drying, or freeze-drying. Sun-drying is the simplest way but it is not easy to be done in areas with high rainfall intensity, such as in Bogor. Further, it causes high variation of nutritional quality due to its dependence on nature (Sagar and Kumar, 2010). Freeze-drying probably is the best method to retain the nutritional value of materials (Kumar, 2012). However, it needs expensive equipment and may not be affordable for drying a big quantity of feed materials. Oven-drying is apparently a reasonable approach in term of simplicity, practicability, and cost. However, oven-drying should be performed at an optimum temperature, not too low or too high, so that it provides sufficient moisture removal on one hand and retains nutritional quality of the feed materials on the other hand. A study of Ramsumair *et al.* (2014) have shown that the increase of oven-drying temperature from 60 to 70^{o}C elevated neutral detergent fiber (NDF) contents of *Gliricidia sepium* and *Leucaena leucocephala* leaves, which indicated in the reduction of their nutritional values.

Drying feed ingredients is also of relevant with feed analysis as feed samples are usually dried before the chemical composition are determined. Drying feed stuffs for sample preparation can be performed by freeze-drying (Tassone *et al.*, 2014) or oven-drying at 40^{o}C (Purcell *et al.*, 2011), 50^{o}C (Jayanegara *et al.*, 2012), or 55 – 60^{o}C (Pagan *et al.*, 2009; Pelletier *et al.*, 2010). The last temperature is the range that used on common procedure for feed analysis. Information on optimum drying temperature for various beans in Indonesia still remains limited. Therefore, objective of this study was to investigate the influence of different drying temperatures, i.e. 50, 60, 70 and 80^{o}C on chemical composition, *in vitro* rumen fermentation, and digestibility of soybean and redbean. Soybean and redbean were selected since they have different characteristics; soybean is highly degraded in the rumen whereas redbean contains high proportion of undegradable (by-pass) protein (Jayanegara *et al.*, 2016a). Therefore they might elicit divergent pattern at different drying temperature.

Materials and Methods

Sample preparation

Soybean and redbean were obtained from a traditional markets in Bogor, approximately 12 kg fresh weight each. For each bean species, samples were divided into 12 portions (1 kg fresh each) and dried in an oven (Vacutherm^{TM} VT 6060 M, Thermo Electron LED GmbH, Langenselbold, Germany) set at four different drying temperatures, i.e. 50, 60, 70, and 80^{o}C for 24 h. Each drying temperature was performed in three replicates per bean species. Dried

samples were then milled in a hammer mill to pass through a 1 mm screen and used further for chemical composition determination and *in vitro* rumen fermentation assay.

Chemical composition determination

Each sample was determined for its proximate components, i.e. crude protein (CP) and eter extract (EE) according to AOAC (2005), Van Soest fiber fractions, i.e. NDF and acid detergent fiber (ADF) according to Van Soest *et al.* (1991), and protein fractions, i.e. neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) according to Licitra *et al.* (1996). These analyses were conducted in three replicates and each replicate was repeated twice.

Micro-Kjeldahl and Soxhlet extraction apparatus were employed for determining CP and EE contents, respectively. For NDF and ADF analyses, an amount of 1 g sample was inserted into a beaker glass and added with 100 ml of either neutral detergent solution or acid detergent solution. Samples were then boiled for 60 min and reflux with water stream. Residue was filtered under vacuum, washed with hot water and acetone, dried at 105ºC and ashed at 500ºC in a furnace. Neither α-amylase nor sodium sulfite was used for NDF determination. Residue of NDF and ADF were continued with CP determination to obtain NDICP and ADICP values, respectively. Proximate composition and Van Soest's fiber fractions were expressed as percent dry matter (DM) whereas NDICP and ADICP values were expressed as percent CP.

***In vitro* rumen fermentation**

In vitro incubation assay was conducted following the method of Theodorou *et al.* (1994). The incubation was performed in three runs (replicates), each at a different week, and each treatment per run was represented by two incubation units. An amount of 0.75 g sample was inserted into a 100 ml serum bottle, and then added 75 ml rumen fluid:buffer mixture (1:2 v/v). The incubation medium was continuously flushed with CO_2 to maintain anaerobic condition. Rumen fluid was obtained from three fistulated Ongole crossbred cattle before morning feeding. Buffer solution was prepared by mixing 9.8 g $NaHCO_3$, 3.71 g $Na_2HPO_4.7H_2O$, 0.57 g KCl, 0.47 g NaCl, 0.12 g $MgSO_4.7H_2O$, and 0.04 g $CaCl_2$ in 1000 ml distilled water. Serum bottles were sealed with butyl rubber stoppers and aluminum crimp seals shortly before starting the incubation. The bottles were placed in a water bath set at 39ºC, and the *in vitro* incubation was performed for 72 h. Gas production was vented and recorded at 1, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, 48, 60, and 72 h. Manual shaking was performed in each time of gas recording.

Samples were with drawn for pH, volatile fatty acid (VFA), and ammonia (NH_3) measurements at 24 h, and for dry matter digestibility (DMD) and crude protein digestibility (CPD) determinations at 48 h. Determination of pH was conducted using a pH meter. Concentration of NH_3 was measured by employing Conway micro-diffusion technique (Conway and Byrne, 1933). Measurement of VFA concentration (acetate, propionate, and butyrate) was performed using gas chromatograph (GC 8A, Shimadzu Corp., Kyoto, Japan) with a column containing 10% SP-1200, 1% H_3PO_4 on 80/100 Cromosorb WAW. Chromatogram of VFA sample was compared with known concentration of VFA standard. Total VFA was obtained through the summation of each partial VFA. Serum bottles used for digestibility determination were opened and added 2 drops of $HgCl_2$ to stop the microbial fermentation activity. The contents were transferred into tubes and centrifuged at 4,000 rpm for 10 min. Supernatant was discarded and residue was added with 50 ml 0.2% pepsin-HCl solution for subsequent incubation performed for 48 h. Afterwards, contents were filtered by Whatman paper no. 41 and analyzed for DM and CP. Values of DMD and CPD were obtained by difference between initial values and residues (corrected for blanks).

Statistical data analysis

Data on proximate analysis, Van Soest's fiber fraction, protein fraction, *in vitro* fermentation characteristics and digestibility were analyzed by following a 2×4 factorial experimental design. The first factor was different beans (soybean or redbean) and the second factor was different drying temperatures (50, 60, 70, or 80ºC). Block used for *in vitro* data was the different batch of rumen fluid sampling. Data in which their standardized residuals lower than –2 or higher than 2 were categorized as outliers and therefore were removed from the dataset. When ANOVA result showed significantly different at $P<0.05$ for a particular parameter,

data were further analyzed with a posthoc test using Duncan's multiple range test to compare among different treatments. All the statistical analyses were performed by using SPSS software version 20.0.

Results and Discussion

Content of CP in soybean was approximately twice than that of redbean (P<0.05; Table 1). Increasing temperature levels caused only minor change on CP contents of both soybean and redbean. The EE content of redbean was much lower in comparison to soybean (P<0.05). There was a significant interaction between different beans and drying temperatures on NDF (P<0.05), but it was not the case for ADF. Higher drying temperature did not significantly change NDF content of soybean. In the case of redbean, at temperature 60°C or above, NDF content of the bean increased by approximately 25%. The ADF contents of both soybean and redbean were greater when oven-dried at 70 or 80°C than those at 50 or 60°C (P<0.05). Interaction between drying temperature and bean was significant for NDICP (P<0.05). The content of NDICP increased at higher drying temperature (70 or 80°C) for both soybean and redbean (P<0.05) but at different magnitude. Content of ADICP was greater in redbean in comparison to soybean (P<0.05). As with NDICP, higher temperature led to a greater ADICP both in soybean and redbean.

Part of protein in plant is located in the cell wall (Tan *et al.*, 2013) and this is generally limitedly utilized by rumen microbes in comparison to protein located in the cytoplasm. In feed evaluation system, such protein is known as NDICP and ADICP (Licitra *et al.*, 1996; Jayanegara *et al.*, 2016b). The NDICP and ADICP is a protein fraction that insoluble in neutral detergent and acid detergent, respectively. While NDICP is slowly degraded or undegraded in the rumen, ADICP is thought to be completely indigestible and does not provide amino acids in the lower gastro-intestinal tract (Pelletier *et al.*, 2010) and therefore contributes to low quality of protein in feed. It is consisted of lignin-associated protein, tannin-protein complexes and heat-damaged protein (Licitra *et al.*, 1996). Our previous study revealed a negative relationship between NDICP or ADICP proportion to total CP and *in vitro* crude protein digestibility (Jayanegara *et al.*, 2016b). Apparently drying temperature at 50 and 60°C is able to maintain the quality of protein in soybean and redbean.

Table 1. Chemical composition of soybean and redbean which dried at different temperatures

Item	Bean	Temperature (°C)				Average
		50	60	70	80	
CP (%DM)	Soybean	43.8±0.49	44.1±0.48	44.7±0.19	44.8±0.13	44.3±0.54^{b}
	Redbean	21.8±0.13	22.4±0.17	22.7±0.19	23.2±0.58	22.5±0.57^{a}
	Average	32.8±12.7^{a}	33.2±12.5^{ab}	33.7±12.7^{bc}	34.0±12.5^{c}	
EE (%DM)	Soybean	19.9±0.33	19.2±0.44	20.4±0.89	20.2±1.12	19.9±0.78^{b}
	Redbean	3.21±1.28	2.76±1.46	2.50±0.13	2.25±0.70	2.68±0.83^{a}
	Average	11.5±9.65	11.0±9.51	11.5±10.4	11.2±10.4	
NDF (%DM)	Soybean	20.7±0.64^{a}	22.1±1.05^{a}	22.1±0.19^{a}	22.3±0.43^{a}	21.8±0.84
	Redbean	27.9±0.40^{b}	35.0±0.08^{c}	35.6±1.47^{c}	35.4±0.02^{c}	33.5±3.48
	Average	24.3±4.20	28.5±7.47	28.8±7.82	28.8±7.59	
ADF (%DM)	Soybean	15.8±0.27	15.4±0.67	16.6±0.13	17.2±1.24	16.2±0.92^{b}
	Redbean	10.5±0.34	10.8±0.11	11.6±0.28	12.2±0.16	11.3±0.73^{a}
	Average	13.2±3.07^{a}	13.1±2.68^{a}	14.1±2.85^{b}	14.7±2.98^{b}	
NDICP (%CP)	Soybean	6.58±0.29^{a}	6.41±0.22^{a}	11.7±0.03^{c}	11.1±0.17^{c}	8.95±2.64
	Redbean	10.1±0.01^{b}	9.42±0.01^{b}	12.5±0.96^{d}	14.0±0.08^{e}	11.5±1.99
	Average	8.35±2.05	7.91±1.74	12.1±0.74	12.5±1.65	
ADICP (%CP)	Soybean	6.37±0.61	6.30±0.09	7.36±0.06	7.32±0.98	6.84±0.69^{a}
	Redbean	8.07±0.10	9.29±0.27	9.68±0.21	10.1±0.34	9.29±0.84^{b}
	Average	7.22±1.04^{a}	7.80±1.74^{ab}	8.52±1.34^{bc}	8.73±1.73^{c}	

^{a,b,c} Different superscripts within the same parameter are significantly different at P<0.05.
CP = crude protein; DM = dry matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein.

Protein quality starts to decline at a drying temperature of 70°C or above as shown by the greater NDICP and ADICP values. At high temperature, carbohydrate degradation products react with protein to form dark-colored and insoluble polymers known as Maillard or browning reaction (Pelletier *et al.*, 2010; Khan *et al.*, 2015).

In vitro incubation of redbean resulted in a greater gas production potential (GPP) than that of soybean (P<0.05), but conversely, gas production rate (GPR) was lower in redbean (P<0.05; Table 2). Higher temperature caused minor change on GPP but it decreased GPR of both beans (P<0.05). Gas resulted in an *in vitro* system is produced by the action of rumen microbes during degradation and fermentation of feed or substrate; it is an end product of microbial metabolism. Additionally, gas is also produced from the buffering process of VFA generation by bicarbonate buffer present in artificial saliva (Getachew *et al.*, 1998). The gas itself is consisted of mainly CO_2 and CH_4, and small amounts of H_2, O_2, and N_2 (McDonald *et al.*, 2011). From organic compounds present in feed, carbohydrate is the main component contributing to gas production. Protein fermentation contributes also to gas production but the amount is much smaller in comparison to carbohydrate, whereas lipid has negligible contribution on gas production (Getachew *et al.*, 1998). Greater GPP in the incubation of redbean was due to its greater carbohydrate content (both non-structural and structural carbohydrate) than that of soybean. Further, redbean contained much lower fat than the other bean. Fat does not contribute to gas production because rumen microbes do not ferment long-chain fatty acids. Triglyceride, the main form of fat, undergoes lipolysis in the rumen to result glycerol and fatty acids by the action of *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* that release lipolytic enzymes (Lourenco *et al.*, 2010). Unsaturated fatty acids are biohydrogenated to produce various fatty acid isomers with higher saturation degree, but their carbon chains are not degraded and metabolized by rumen microbes (Buccioni *et al.*, 2012). Glycerol may be fermented to VFA particularly propionate but such conversion does not significantly produce gas (Avila-Stagno *et al.*, 2014).

Although GPP is reflected more by carbohydrate fermentation, it is apparent that GPR is reflected more by protein fermentation. Greater GPR observed in soybean incubation was due to its greater protein content as compared to redbean. Moreover, type of protein present in redbean and soybean is different. Jayanegara *et al.* (2016a) observed that, by using Cornell protein fractionation system (Sniffen *et al.*, 1992; Licitra *et al.*, 1996), soybean contained high proportion of soluble and rapidly degraded protein whereas redbean was dominated by intermediately and slowly degraded protein. Such different properties of protein present in soybean and redbean therefore explain the differences of GPR between the beans. Apparently the presence of Maillard products (indicated by NDICP and ADICP contents) affected GPR as shown by

Table 2. *In vitro* gas production and digestibility of soybean and redbean which dried at different temperatures

Item	Bean	Temperature (°C)				Average
		50	60	70	80	
GPP (ml)	Soybean	158±3.0	161±5.3	158±3.9	156±2.4	158±4.2[a]
	Redbean	232±5.1	243±5.8	241±13	242±6.7	240±8.9[b]
	Average	195±39[a]	202±43[b]	200±45[ab]	199±45[ab]	
GPR (ml/h)	Soybean	0.111±0.01	0.110±0.01	0.106±0.01	0.101±0.01	0.107±0.01[b]
	Redbean	0.074±0.01	0.068±0.01	0.063±0.01	0.061±0.01	0.067±0.01[a]
	Average	0.092±0.02[c]	0.089±0.02[bc]	0.085±0.02[ab]	0.081±0.02[a]	
DMD (%)	Soybean	81.1±5.7	81.6±5.4	82.3±5.8	82.3±5.9	81.8±5.3[a]
	Redbean	87.0±3.6	89.7±5.1	84.8±2.9	87.5±1.6	87.3±3.7[b]
	Average	84.1±5.5	85.7±6.5	83.6±4.6	84.9±5.0	
CPD (%)	Soybean	91.0±1.4[e]	90.4±0.6[e]	86.9±1.7[d]	86.0±2.2[d]	88.6±2.7
	Redbean	76.2±1.3[b]	77.4±1.5[b]	80.8±4.5[c]	68.4±2.0[a]	75.7±5.3
	Average	83.6±7.8	83.9±6.9	83.8±4.6	77.2±9.4	

[a,b,c,d] Different superscripts within the same parameter are significantly different at P<0.05.
GPP = gas production potential; GPR = gas production rate; DMD = dry matter digestibility; CPD = crude protein digestibility.

lower GPR with increasing levels of drying temperatures. Since Maillard products are consisted of carbohydrate-protein insoluble polymers, both components cannot be fermented to result gas production after they are linked together at high temperature. In other studies, heat treatment had been shown to increase Maillard reaction products (Wellner *et al.*, 2011), and their concentrations were elevated at higher drying temperatures (Michalska *et al.*, 2016) and period (Lin *et al.*, 2016) as also observed in the present study.

Although *in vitro* dry matter digestibility (DMD) of redbean was greater as compared to soybean ($P<0.05$), it was reversed in the case of crude protein digestibility (CPD). Drying soybean at 70 or 80^{o}C resulted in lower CPD than that dried at 50 or 60^{o}C ($P<0.05$). Drying redbean at 70^{o}C improved CPD than that dried at lower temperature, but when the temperature was above 70^{o}C then the digestibility was dropped. Lower DMD of soybean in comparison to that of redbean is apparently because of its greater ADF content. Supporting the result, Jayanegara *et al.* (2009) observed a strong negative correlation between ADF content and digestibility. Similarly, Laconi and Jayanegara (2015) also found a negative relationship between DMD or OMD and ADF as shown by loading plot of principal component analysis. The ADF is consisted of primarily lignocellulose structure and resistant to microbial degradation in the rumen. De Boever *et al.* (2005) stated that the structural components of feed ingredients originated from plants such as cell wall, NDF, ADF, cellulose and lignin reduce nutrient digestibility of ration, while soluble carbohydrate, starch and protein improve the nutrient digestibility. With regard to protein digestibility, greater CPD found in soybean than that of redbean is apparently due to the different nature of protein in both beans. Soybean protein has been characterized by its high proportion of degradable protein in the rumen (Maxin *et al.*, 2013; Akbarian *et al.*, 2014) whereas redbean protein is rich in slowly degraded fraction (Jayanegara *et al.*, 2016a). These facts explain the difference of CPD between soybean and redbean. Drying temperatures at 50 and 60°C are apparently safe for processing of feed ingredients as shown by the insignificance CPD of soybean and redbean. At higher temperatures, especially at 80^{o}C, it seems that formation of Maillard products is intensified and thus decreases the CPD of the beans. This was confirmed by the negative relationship between CPD and ADICP, in which Maillard products are recovered within the parameter (Figure 1).

In vitro incubation of redbean revealed a lower pH than that of soybean ($P<0.05$; Table 3). Different drying temperature did not change pH of soybean and redbean incubations. The pH range in the present study is within normal pH for rumen microbes to proliferate and degrade macromolecules entering the rumen, i.e. between 6.0 to 7.0. Rumen pH below 5.6 or 5.0 is considered as an indication for sub-acute or acute acidosis, respectively, and such condition may be more prevalent in animals consuming a high proportion of grains like in feedlot (Gonzalez *et al.*, 2012). Lower pH found in the incubation of redbean is due to greater carbohydrate content (particularly non-fiber carbohydrate) in the bean as compared to soybean which is rich in CP and EE. While CP and EE contribute little on ruminal pH dynamics, amount and type of carbohydrate is a main determining factor for rumen pH. Non-fiber carbohydrates such as starch, sugar, and pectin are rapidly degraded and fermented in the rumen and therefore decreases rumen pH whereas, on the contrary, structural carbohydrate generally possesses a slow degradation rate (Li *et al.*, 2014). Concentration of NH_3 was found to be greater in the *in vitro* incubation of soybean than that of redbean ($P<0.05$). Higher drying temperature resulted in a lower NH_3 concentration ($P<0.05$). The origin of ruminal NH_3 is from degradation of feed protein, deamination of amino acid, and rumen microbial lysis (Bach *et al.*, 2005). Ammonia is an important precursor for the synthesis of amino acids and microbial protein since rumen microbes generally are lack of capability to directly transporting amino acids into their cells (Pengpeng and Tan, 2013). Ammonia concentration in rumen fluid is a balance between rate of protein degradation and rate of NH_3 utilization by rumen microbes. Range of NH_3 concentration was considerably greater in comparison to literatures, i.e. 6 – 21 mmol/l (McDonald *et al.*, 2011). It is most probably that NH_3 is accumulated since no absorption process was took place in the *in vitro* system which is different with *in vivo* condition. Further, the samples used were

Table 3. *In vitro* rumen fermentation characteristics of soybean and redbean which dried at different temperatures

Item	Bean	Temperature (°C)				Average
		50	60	70	80	
pH	Soybean	6.87±0.10	6.87±0.10	6.83±0.05	6.83±0.05	6.85±0.08[b]
	Redbean	6.62±0.16	6.57±0.14	6.62±0.16	6.63±0.19	6.61±0.15[a]
	Average	6.74±0.18	6.72±0.19	6.73±0.16	6.73±0.17	
NH_3 (mmol/l)	Soybean	46.9±2.1	40.9±2.2	30.4±1.6	27.8±2.0	36.5±8.1[b]
	Redbean	30.4±1.0	25.1±3.9	16.8±2.6	13.1±1.2	21.3±7.3[a]
	Average	38.6±8.7[d]	33.0±8.8[c]	23.6±7.4[b]	20.4±7.8[a]	
Total VFA (mmol/l)	Soybean	31.8±15.7	55.3±13.1	37.5±14.3	37.6±10.9	40.5±14.8
	Redbean	44.0±30.4	50.8±24.8	47.0±10.1	52.6±2.6	48.6±17.7
	Average	37.9±22.7	53.0±17.9	42.2±12.2	45.1±10.9	
C_2 (%VFA)	Soybean	64.4±0.9	66.0±2.2	65.0±1.7	65.4±2.4	65.2±1.7
	Redbean	64.1±1.7	62.9±1.4	64.5±1.1	64.4±0.7	64.0±1.3
	Average	64.2±1.2	64.5±2.4	64.8±1.3	64.9±1.7	
C_3 (%VFA)	Soybean	24.3±0.4	23.3±1.1	23.9±0.8	24.2±1.4	23.9±1.0
	Redbean	23.8±1.6	24.3±1.0	23.5±0.7	23.6±0.5	23.8±0.9
	Average	24.1±1.1	23.8±1.1	23.7±0.7	23.9±1.0	
C_4 (%VFA)	Soybean	11.3±0.9	10.7±1.2	11.1±1.0	10.5±1.0	10.9±0.9[a]
	Redbean	12.0±1.3	12.9±0.7	12.0±0.6	12.1±0.7	12.2±0.8[b]
	Average	11.7±1.1	11.8±1.5	11.6±0.9	11.3±1.2	

[a,b,c,d] Different superscripts within the same parameter are significantly different at P<0.05.
NH_3 = ammonia; VFA = volatile fatty acid; C_2 = acetate; C_3 = propionate; C_4 = butyrate.

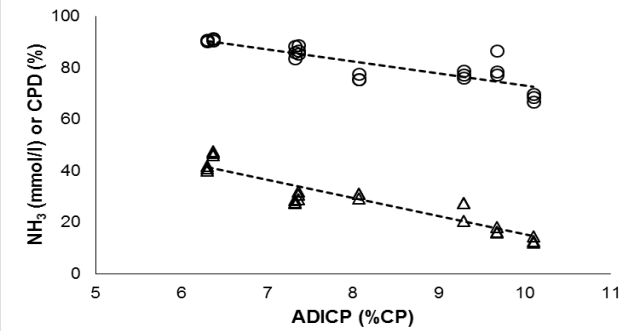

Figure 1. Relationships between acid detergent insoluble crude protein (ADICP; % dry matter) content and *in vitro* ruminal ammonia (NH_3; Δ; mmol/l) concentration and crude protein digestibility (CPD; o; %).
NH_3 = 86.0 – 7.08 ADICP (n = 24; P<0.001; R^2 = 0.851)
CPD = 120 – 4.70 ADICP (n = 24; P<0.001; R^2 = 0.733).

high in CP contents and rumen fluid was sampled at 24 h. In addition, low usage of ruminal NH_3 by microbes is apparently caused by the low energy (ATP) produced during carbohydrate fermentation as indicated by the generally low VFA concentration. Greater NH_3 concentration in the incubation of soybean is due to its greater CP and CPD in comparison to redbean. This was confirmed by a positive association (curvilinear) between NH_3 concentration and CPD (Figure 2). Lower NH_3 at higher drying temperature seems to be because of the greater accumulation of Maillard products as confirmed by the negative relationship between ADICP and NH_3 concentration (Figure 1).

In general, different beans and drying temperature did not influence total VFA concentration and its individual component (acetate and propionate) except for butyrate; the later concentration was greater in the incubation of redbean in comparison to soybean (P<0.05). Carbohydrate fermentation, either fiber or non-fiber carbohydrate by microbes in the rumen resulting in VFA and the product serves as a main energy source for the host animals after absorption (Noziere *et al.*, 2011). Range of

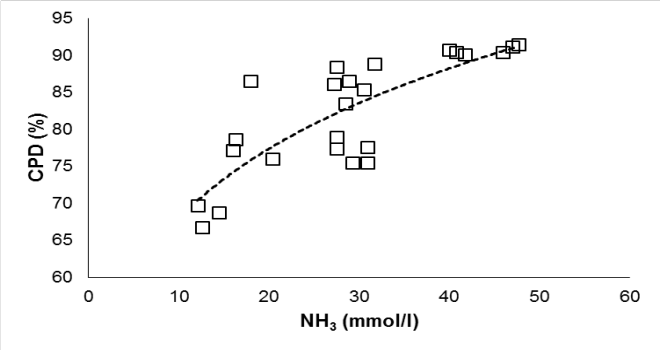

Figure 2. Relationship between *in vitro* ruminal ammonia (NH_3; mmol/l) concentration and crude protein digestibility (CPD; %).
CPD = 44.2 $(NH_3)^{0.188}$ (n = 24; P<0.001; R^2 = 0.647).

total VFA concentration in the present study was relatively low, considering that the experimental materials were not rich in fermentable carbohydrate; instead, they were high in protein and fat (only soybean). A tendency (0.05≤P<0.1) of greater total VFA in the incubation of redbean in comparison to soybean is due to its greater non-fiber carbohydrate. Main VFA present in the rumen is acetate, propionate, and butyrate; some minor VFA are also present such as isobutyrate, valerate, isovalerate, and caproate (McDonald *et al.*, 2011). Proportion of VFA, particularly acetate to propionate ration is influenced by nature of feed entering the rumen. Acetate proportion is greater when animals consume fibrous diet and, conversely, propionate proportion is greater with high concentrate diet (Gonzalez *et al.*, 2012; Li *et al.*, 2014). Apparently since both materials are beans, which are among concentrate categories, acetate and propionate proportions to total VFA in the present experiment were insignificant. This is also the reason on the insignificance effect of drying temperature on total VFA and individual VFA proportion; it seems that drying temperature causes only negligible change on these parameters. After being absorbed, apart from their roles as energy sources for the host animals, acetate is used as precursor for body fat and milk fat synthesis whereas propionate is used for milk sugar synthesis (Fievez *et al.*, 2012).

Conclusion

Drying temperature at 50 or 60°C is safe to maintain nutritional quality of soybean and redbean, and this temperature is therefore recommended for drying feed materials. At higher drying temperature, i.e. 70 or 80°C, Maillard products have been accumulated as shown by elevated NDICP and ADICP contents. Such condition leads to a decrease of protein quality present in both beans as indicated by lower CPD and ruminal NH_3 concentration. The negative relationship between ADICP and CPD and ruminal NH_3 concentration reveals the usefulness of the component as an indicator of protein quality in the feed of ruminants.

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