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Research Article
Effects of Heat Processing Techniques on Nutritional Value and *in vitro* Rumen Fermentation Characteristics of Jack bean (*Canavalia ensiformis* L.)

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Abstract
Background and Objective: Feedstuffs that serve as protein sources for ruminants are expensive. Jack bean (*Canavalia ensiformis* L.) is an indigenous legume grown in Indonesia, which is rich in protein (23.95%), but is not used for protein supplementation in ruminants. This study on jack bean was conducted to improve its potential as a protein supplement for ruminants, jack beans were processed and evaluated for nutritional value and rumen fermentation characteristics. **Methodology:** Effects of no treatment (H0), compared to a roasting treatment (H1), an oven treatment (H2) and an extrusion treatment (H3) were investigated. **Results:** Heat processing techniques (H1, H2, H3) significantly ($p<0.05$) increased dry matter, ash, crude fibre and crude protein (CP), but decreased ether extract. Comparison of CP concentrations under the different treatments indicated that jack beans treated with the H3 method had the highest CP (26.89%). Rumen fermentation characteristics, including volatile fatty acids (VFA) and ammonia (NH_3) were significantly ($p<0.05$) reduced by heat processing techniques (H1, H2, H3). However, rumen undegradable protein (RUP) was significantly ($p<0.05$) increased. Jack beans treated with the H3 treatment had the highest RUP (59.16%), although the *in vitro* dry matter digestibility (IVDMD) was not significantly different from jack beans in the control (H0) group. **Conclusion:** The extrusion technique (H3) was found to be the best technique for making jack beans suitable as a protein supplement for ruminants.

Key words: Protein supplement, heat processing technique, jack bean, nutritional values, rumen fermentation characteristics

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jack bean (*Canavalia ensiformis* L.) is a legume which tolerates the Indonesian climate, but is not used yet as protein supplement in cattle feed. The jack bean has several excellent nutritional properties, including a high protein content. The nutritional composition of jack beans is as follows: Total protein 34.6%, total fat 2.4%, fibre 1.2%, ash 2.8% and moisture 12.5%[1]. However, more information about the protein degradability of jack beans in the rumen is needed and processing techniques that may increase the protein utility of jack beans as a protein supplement, especially for ruminants, are highly desirable.

Several simple and easily applied heat processing techniques are available, including roasting, oven and extrusion. However, the most optimum heating process technique for improving the nutrient quality of jack beans and its use in ruminants, needs to be determined through investigating rumen fermentation characteristics. Heat treatment of protein rich feedstuffs can increase the protein utilization efficiency for ruminants, because heating can result in peptide chain and carbohydrate bonding, which decreases the protein degradation in the rumen and increases the availability of crude proteins and amino acids passing to the intestine. However, overheating can result in indigested protein fractions in the intestine, which decreases the protein value[2]. This study aimed to determine the optimum heating process technique, using *in vitro* rumen characteristics, in order to find the best method for the efficient utilization of jack bean proteins by ruminants.

MATERIALS AND METHODS

This study was conducted at the Feed Technology Laboratory of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. The jack bean samples were divided into four treatment groups namely, H0: Unprocessed (control = no heating) jack beans, H1: Jack beans roasted at 115°C for 10 min[3], H2: Jack beans heated in an oven at 110°C for 30 min[3] and H3: Extrusion at 120°C for 10 sec, using a single crew[4]. Jack beans in each group were ground to meal with a hammer mill and were subsequently sieved using a 1 mm diameter sieve.

The *in vitro* fermentation techniques were conducted with batch culture methods[5] using rumen fluids from beef cattle slaughtered at a local slaughter house as inoculation sources. The rumen fluids were placed in a thermos (39°C), immediately brought to the laboratory and then filtered using a thin cloth and placed in a glass flask. The filtered rumen fluids were subsequently mixed with McDougall Buffer solution at a 1:2 ratio (v/v) and then flushed with oxygen free CO_2 and used as mixed rumen microbe inoculum. The anaerobic buffered rumen fluid (50 mL) was poured into a 250 mL tube containing 2 g samples of the jack bean suitable to the treatment. The filled tubes were covered (under continuous flushing of CO_2) with rubber and aluminium foil and tubes were placed in a shaking water bath at 39°C for 3 h. The fermentation process was stopped using saturated $HgCl_2$. Analyses were conducted for ruminal ammonia (NH_3), volatile fatty acid (VFA) production, rumen undegradable protein (RUP), *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). These parameters were analyzed according to Tilley and Terry[5]. The dry matter, ash, ether extract, crude fibre and crude protein were analyzed by AOAC standard methods[6].

The data were analyzed by one way analysis of variance (ANOVA) and mean differences between treatments were analysed by Duncan's Multiple Range Tests (DMRT) with procedures of SAS[7].

RESULTS AND DISCUSSION

Nutritional value: The nutritional values of the experimental jack beans treated with several heat processing techniques are presented in Table 1.

Dry matter: Heating processes H1, H2 and H3 increased ($p<0.05$) the dry matter (DM) content of the jack beans

Table 1: Nutritional values of experimental jack beans (dry matter basis)

	Treatments					
Parameters	H0	H1	H2	H3	SEM	Significance
Dry matter (%)	86.93^{d}	97.59^{a}	95.50^{b}	89.97^{c}	2.11	p<0.05
Ash (%)	2.23^{c}	2.76^{a}	2.60^{b}	2.49^{b}	1.20	p<0.05
Ether extract (%)	3.61^{ab}	3.29^{b}	3.89^{a}	2.30^{c}	0.15	p<0.05
Crude fibre (%)	7.33^{b}	8.30^{a}	8.21^{a}	7.59^{b}	0.13	p<0.05
Crude protein (%)	23.95^{d}	26.29^{b}	25.87^{c}	26.89^{a}	0.12	p<0.05

^{a,b,c,d}Different superscripts in the same row indicate significant differences (p<0.05). Means and Standard Error of the Means (SEM) are shown. Treatments include H0 (no heating=control), H1: Roasting, H2: Heated in an oven and H3: Extrusion

(Table 1). Not heating jack beans at all (H0) resulted in the lowest DM content (86.93%), whereas heating process H1 resulted in the highest DM content (97.59%). Of the three heating process techniques, process technique H3 resulted in jack beans that had the best texture and a DM content that was similar to the control (H0) treatment, as well as a fragrant scent. This may have been caused by a browning reaction between the protein and sugar content in the jack beans during the extrusion process[8]. This result is consistent with a previous study conducted by Prasetiyono *et al.*[4], which showed that extrusion processes resulted in a fragrant scent in soybeans. The DM concentration of jack beans processed by extrusion (H3) was 89.97%.

Ash: Heating processes significantly increased ($p<0.05$) ash concentrations of jack beans (Table 1). Jack beans treated with all heating processes, including roasting (H1), oven (H2), as well as extrusion (H3), had higher ash concentrations, while jack beans in the unheated treatment group (H0) had the lowest ash concentration (2.23%). The organic matter (OM) content was reduced because a part of the OM was converted to volatile compounds. For example: Polyunsaturated fatty acids undergo depolymerization and become volatile products, such as the conversion of linoleic acid to decadienoic acid. Legumes including jack beans have high linoleic acid contents, therefore, a decrease in linoleic acid led to a significant decrease in organic matter and therefore, increased the ash concentration[9].

Ether extract: The H1 and H3 heating process techniques significantly ($p<0.05$) decreased the ether extract (EE) concentration of jack beans (Table 1). The H3 heating process technique resulted in the lowest EE concentration (2.30%). The significant reduction of EE by the extrusion process was due to volatility as well as lipid extraction, which resulted from the combination of high pressure and high temperatures during the heating process (i.e. high temperature short time, HTST)[9].

Crude fibre: Heating process techniques H1, H2 and H3 significantly ($p<0.05$) increased the crude fibre (CF) concentration of jack beans (Table 1). The H1 and H2 processing techniques significantly ($p<0.05$) increased the CF concentration, as in these techniques, the heating resulted in lignin artefact formation through non-enzymatic browning reactions[8]. The formed compound was included in the fibre analysis, because of its lignin-like chemical properties. This phenomenon did not occur in the extrusion heating technique treatment (H3), even if this technique involved higher temperatures than the roasting (H1) and oven heating treatment (H2). This is because, the duration of the extrusion heating technique was much shorter (10 sec) than the roasting (10 min) and oven heating (30 min) techniques, although the temperature used in the extrusion heating technique was higher[4].

Crude protein: Heating technique processes significantly ($p<0.05$) increased the crude protein (CP) concentration in jack beans (Table 1). The H0 heating process technique (unheated jack beans) resulted in the lowest CP concentration (23.95%), whereas technique H3 resulted in the highest CP concentration (26.89%). On the other hand, the CP concentration (23.95%) of jack beans without heat treatment (H0) was consistent with results found by Doss *et al.*[10], namely in the range of 23.8-27.6%.

The CP analysis procedure by proximate analysis included the nitrogen in the lignin artefacts. Lignin was formed through destruction processes in proximate analysis and covered in CP calculation, so that the CP concentration does not decrease, but increases significantly in the heating technique treatment groups. The increase in CP concentration may be caused by the volatility of lipid components, which generally decreased, although in the extrusion heating technique treatment, CP was significantly ($p<0.05$) increased. This may be because of the combination between high tension and temperature in short time (HTST). The increase of CP due to the extrusion process was similar to results reported by Sanders[11], who showed that the extrusion process could increase the CP concentration in Kapok seeds. Parand *et al.*[12] also reported that the extrusion process could increase the CP concentration of soybeans.

Rumen fermentation characteristics: The rumen fermentation characteristics of the experimental jack beans treated with several heat processing techniques are presented in Table 2.

***In vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD):** Heating process techniques by roasting (H1) and oven (H2) significantly ($p<0.05$) decreased IVDMD and IVOMD of jack beans, whereas IVDMD and IVOMD in jack beans treated with the extrusion process were not significantly different from the control (H0). The IVDMD in H0 and H3 were 82.87 and 81.71%, respectively, while the IVDMD in H1 and H2 were 67.62 and 74.03%, respectively. The IVOMD had a similar pattern as IVDMD, in this case H0 and H3 treatments resulted in higher IVOMD than H1

Table 2: Rumen fermentation characteristics of jack beans treated with different heating processes.

Parameters	Treatments H0	H1	H2	H3	SEM	Significance
IVDMD (%)	82.87[a]	67.62[c]	74.03[b]	81.71[a]	0.41	p<0.05
IVOMD (%)	84.67[a]	69.81[c]	75.38[b]	83.33[a]	0.65	p<0.05
NH_3 (mM)	5.28[a]	3.39[c]	3.83[b]	2.71[d]	1.01	p<0.05
VFA (mL moL^{-1})	105.00[a]	87.50[b]	87.50[b]	37.50[c]	2.60	p<0.05
RUP (%)	43.35[b]	50.36[ab]	48.69[b]	59.16[a]	2.67	p<0.05

[a,b,c,d]Different superscripts in the same row indicate significant difference (p<0.05). Means and Standard Error of the Means (SEM) are shown. Treatments include H0 (no heating = control), H1: Roasting, H2: Heated in an oven and H3: Extrusion. IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic matter digestibility, NH_3: Ammonia, VFA: Volatile fatty acids, RUP: Rumen undegradable protein

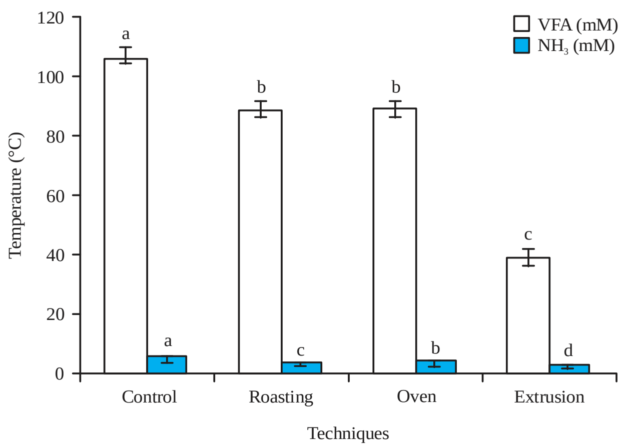

Fig. 1: Effect of heat processing techniques on VFA and NH_3 concentrations

and H2 treatments, namely: 84.67 and 83.33% in H0 and H3, respectively vs. 69.81 and 75.38% in H1 and H2, respectively. The decrease in IVDMD and IVOMD in jack beans treated with treatments H1 and H2 may have occurred because of the artefact lignin formation due to the bonding between free carbonyl groups in carbohydrates and the amino groups in proteins. This would have meant that these could not be digested, which would have inhibited the digestibility of other components of dry matter and organic matter. On the other hand, the higher proportion of IVDMD and IVOMD in jack beans treated with heating technique H3, compared to jack beans treated with techniques H1 and H2, indicates that the extrusion process, which included heating jack beans for a short time (10 sec), did not result in significant lignin artefact formation.

Ammonia concentration: Heating process techniques significantly (p<0.05) decreased the ammonia concentration in jack beans (Table 2). The highest ammonia concentration was found in the H0 treatment group (unheated jack beans), (5.28 mM), whereas the lowest ammonia concentration was found in jack beans in the H3 treatment group (2.71 mM). In general, heating process techniques decreased the rumen NH_3 concentration in all three heating process technique treatment groups (H1, H2, H3). In H1 and H2 heating process techniques, the decrease in ammonia concentration was caused by the decrease in protein degradability due to decreasing protein solubility in rumen fluid[13]. On the other hand, the decrease in ammonia concentration (2.71 mM) found in the extrusion heating process technique (H3), may be due to high use of ammonia for microbial protein synthesis. This result is supported by the high IVOMD and the low VFA concentration in jack beans treated with the H3 heating technique (Fig. 1). These experimental results are similar to those found by Prasetiyono *et al.*[4] and Soltan[14], who reported a decrease in rumen ammonia concentrations due to extrusion processes on soybean seed. Pena *et al.*[15], also found that extrusion processes on Kapok seed resulted in a decrease in rumen ammonia concentrations.

VFA concentration: There were significant effects (p<0.05) of heating processes of jack beans on the rumen VFA concentration (Table 2). The highest VFA concentration (105 mM) was found in the unheated jack bean group (H0), whereas the lowest VFA concentration (37.5 mM) was found in the extrusion treatment (H3). Generally, VFA concentration in the three heating technique treatment groups (H1, H2 and H3) significantly (p<0.05) decreased. The decrease in VFA in treatments H1 and H2 may have occurred due to the decrease in IVOMD[16], as VFA would have been formed as a degradation product from organic matter. The VFA concentration in H3 was lowest, although the IVOMD was higher than those of treatments H1 and H2 and not significantly different from the control group (H0). This could be due to the use of the carbon skeleton (alpha-keto acids, which are the intermediary compound in VFA production) for microbial protein synthesis. This result is supported by lower ammonia concentrations in the H3 treatment group compared to the H1 and H2 treatment groups (Table 2 and Fig. 1). The decrease in rumen

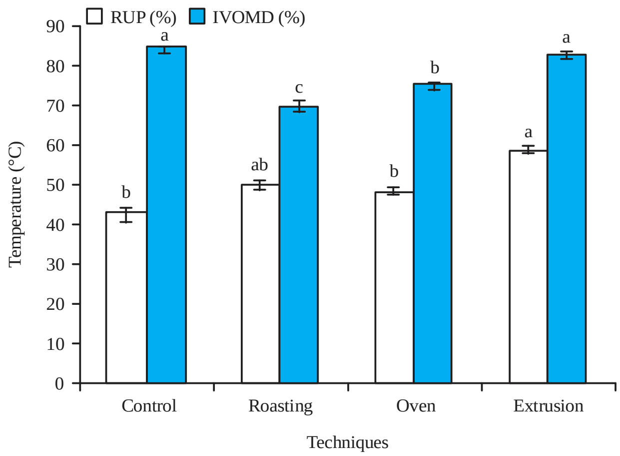

Fig. 2: Effect of heat processing techniques on RUP and IVOMD percentages

VFA concentration may also occur as a result from extrusion processes (H3), which could decrease protein and carbohydrate degradation in the rumen, while carbohydrates and proteins support the VFA production in the rumen.

Rumen undegradable protein: Heating process techniques significantly ($p<0.05$) increased the percentage of rumen undegradable protein (RUP) (Table 2). The increase in RUP may have been due to the low degradation rate in the rumen, which was indicated by the decrease in ammonia concentration in the rumen (Table 2)[17]. This result agreed with the finding of Prasetiyono *et al.*[4], who demonstrated that RUP had high biological value because of its high essential amino acid content, which could be absorbed in the intestine. The RUP in H1 and H2 heating technique treatment groups tended to be higher than the RUP in H0 treatment group. This could be due to the decrease in protein solubility[18]. That was also supported by a decrease in NH_3 concentration in these heating technique treatment groups. These results are in agreement with the findings of Karlsson *et al.*[19], who reported that heat treatment decreased crude protein solubility in heat treated hempseed cakes compared with untreated controls. Similarly, Solanas *et al.*[20] found that extrusion treatment of protein source feedstuffs could decrease protein degradability in the rumen.

The highest RUP (59.16%) was found in the extrusion heating treatment (H3), whereas the lowest RUP (43.35%) was found in the group that did not receive heat treatment (H0). This finding is consistent with the findings of Karlsson *et al.*[19] and Chantiratikul and Chumpawadee[21], who reported that heat treatment increased RUP in heat treated hempseed cakes compared to untreated controls. An interesting result was found in extrusion heating technique treatment groups (H3), namely that RUP in this treatment group was higher than the other treatment groups. That could be due to an increase in proteins and due to the combination of protein denaturation and increase in microbial protein synthesis. This result was supported by the low concentrations of rumen VFA and NH_3 concentration in treatment H3 compared to treatments H1 and H2, although IVOMD in treatment H3 was higher than that in treatments H1 and H2. The role of protein denaturation and microbial protein synthesis were reflected in the high concentrations of RUP in the H3 treatment group, although its IVOMD was highest (Fig. 2).

CONCLUSION

The roasting and oven heating process techniques tested decreased the IVDMD and IVOMD of jack beans, whereas the extrusion heating process did not significantly change IVDMD and IVOMD of jack beans. The VFA and NH_3 concentrations decreased due to heating processes. The RUP increased due to heating processes and the highest RUP was found in jack beans treated with the extrusion process. The heating process technique by extrusion was found to be the best technique to increase the protein bypass supplement and improve rumen fermentation characteristics, without decreasing the utility of jack beans as a protein supplement for ruminants.

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