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HASIL PENILAIAN SEJAWAT SEBIDANG ATAU *PEER REVIEW*
KARYA ILMIAH : JURNAL ILMIAH

Judul Jurnal Ilmiah (Artikel)	:	Effects of Heat Processing Techniques on Nutritional Value and in vitro Rumen Fermentation Characteristics of Jack bean (Canavalia ensiformis L.)
Jumlah Penulis	:	4 orang
Status Pengusul	:	penulis utama
Identitas Jurnal Ilmiah	:	a. Nama Jurnal : PAKISTAN JOURNAL OF NUTRITION b. Nomor ISSN : eISSN: 1994-7984 pISSN: 1680-5194 c. Volume, nomor, bulan tahun: Vol. 17 (6) : 294-299, 2018 d. Penerbit : Science Alert e. DOI artikel (jika ada) : http://dx.doi.org/10.3923/pjn.2018.294.299 f. Alamat web jurnal : https://scialert.net/abstract/?doi=pjn.2018.294.299 g. Terindeks di SCOPUS

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Pak J Nutr tidak memiliki nilai quartile SJR untuk tahun 2018.
Manuskrip membahas pengolahan jack bean dengan teknologi pemanasan, namun *state of the art* dan *novelty* belum dinyatakan dengan jelas, bahkan pada pendahuluan tidak mencantumkan pustaka pendukung. Pustaka yang tercantum pada daftar pustaka tidak sepadan dengan digunakan. Kualitas penerbitan sudah memadai sebagai sebuah jurnal bertaraf internasional.

Semarang, April 2020

Reviewer 1

Prof.Dr.Ir. Joelal Achmadi, M.Sc.
NIP 19590813 198603 1 002
Jabatan : Guru Besar
Unit kerja : Fak. Peternakan dan Pertanian

,LEMBAR
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Semarang, ~~April 2020~~
Reviewer 1

Prof.Dr.Ir. Vitus Dwi Y.B.I., M.S., M.Sc.
NIP 19590615 198503 1 004
Jabatan : Guru Besar
Unit kerja : Fak. Peternakan dan Pertanian

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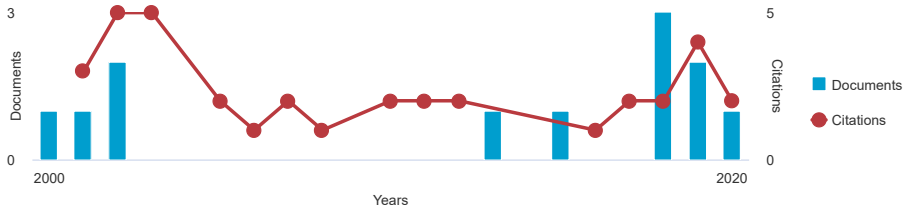

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Effects of heat processing techniques on nutritional value and in vitro rumen fermentation characteristics of Jack bean (Canavalia ensiformis L.) *Open Access*	Prasetiyono, B.W.H.E., Tampoebolon, B.I.M., Subrata, A., Widiyanto	2018	Pakistan Journal of Nutrition	1
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Pakistan Journal of Nutrition is a leading international peer-reviewed journal covering research on human and clinical nutrition, animal nutrition and basic science as applied to nutrition. The Journal recognizes the multidisciplinary nature of nutritional science and includes material from all of the specialties involved in nutrition research, including molecular and cell biology and the emerging area of nutritional genomics.

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Volume 17, Number 6, 2018

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Fate of Mimosine, Concentration of Blood Metabolites and Thyroid Hormones of Sheep Fed with Leucaena and Glyricidia Leaf Meal
Sri Suharti, Fransiscus Xaverius Shila Kurnia, Bagus Pambudi and Komang G. Wiryawan
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Composition of Functional Drinks Based on Cardamom Rhizomes (*Amomum cardamomum* Willd.) and the Benefits as an Anti-inflammatory with an Improved Lipid Profile
Hery Winarsi, Sri Sutji Susilowati and Hernayanti Oentoro
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Potential of Waste Tea Leaves (*Camellia sinensis*) in West Sumatra to Be Processed into Poultry Feed
Wingki Ari Angga, Yose Rizal, Maria Endo Mahata, Ahadiya Yuniza and Reni Mayerni
Pakistan Journal of Nutrition Volume 17, Number 6, 287-293, 2018
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Effects of Heat Processing Techniques on Nutritional Value and *in vitro* Rumen Fermentation Characteristics of Jack bean (*Canavalia ensiformis* L.)
B.W.H.E. Prasetiyono, B.I.M. Tampoebolon, A. Subrata and Widiyanto
Pakistan Journal of Nutrition Volume 17, Number 6, 294-299, 2018
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Ability of Dwarf Elephant Grass (*Pennisetum purpureum* Cv. Mott) and *Gliricidia sepium* to Capture Ammonia (NH_3) Around Chicken Cages: An *in vitro* Evaluation
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COVER LETTER FOR SUBMISSION OF NEW MANUSCRIPTS

January 06, 2018

Dr. Chih-Hung Guo
Editor-in-Chief
Pakistan Journal of Nutrition
support@scialert.com

Subject: **SUBMISSION OF NEW MANUSCRIPT FOR EVALUATION**

Dear Editor:

I am enclosing herewith a manuscript entitled "Effects of heat processing techniques on jack bean (*Canavalia ensiformis* L.) nutritional value and in vitro rumen fermentation characteristics" submitted to "**Pakistan Journal of Nutrition**" for possible evaluation.

With the submission of this manuscript I would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that my Institute's **Diponegoro University** representative is fully aware of this submission.

Type of Submitted manuscript:

- **Original Article**

For the Editor-in-Chief, I would like to disclose the following information about the project:

This research project was conducted from October 11, 2016 to January 10, 2017
Starting date Ending date
My Research Project was fully sponsored by PNBP DIPA Diponegoro University with grant number: SP DIPA-042.01.2.400898/2016 (Fiscal Year 2016).
Detail of the each author with his contribution in this paper is as under:

Name of the author and e-mail ID	Types of contribution
B.W.H.E PRASETIYONO (bambangwhep@ymail.com)	Feed Technologist
B.I.M TAMPOEBOLON (bagindaiskandar@gmail.com	Feed Technologist
A.SUBRATA (agung.subrata42@gmail.com)	Ruminologist
WIDIYANTO (widiyantowidiyanto75@yahoo.com)	Animal Nutritionist

I would also like to share the following information with Editor-in-Chief:

Jack bean is a protein rich indigenous legume grown in Indonesia, which has the potential to be used as a protein supplement for ruminants. However, information about the protein degradability of jack beans in the rumen was lacking, as was information on processing techniques that may increase the utility of jack beans as a protein supplement. Therefore, this study evaluated the effects of several heating treatments, including no treatment (H0, the control), a roasting treatment (H1), an oven treatment (H2), and an extrusion treatment (H3) on jack bean nutritional values and rumen fermentation characteristics. Heat processing techniques (H1, H2, H3) significantly increased dry matter, ash, crude fiber, and crude protein, but decreased ether extract in jack beans. Rumen fermentation characteristics, including volatile fatty acids (VFA), and ammonia (NH_3) were significantly reduced by heat processing techniques (H1, H2, H3), although rumen undegradable protein (RUP) was significantly increased. The extrusion technique (H3) was found to be the best technique for making jack beans suitable as a protein supplement for ruminants. We believe that our study makes a significant contribution to the literature because it presents novel data on how heat treatments can improve the utility of jack beans as a protein supplement for ruminants.

Further, we believe that this paper will be of interest to the readership of your journal because feedstuffs that serve as protein sources for ruminants ration are expensive, and jack beans may be an affordable source of protein for ruminants. As such, processing techniques that may increase the protein utility of jack beans as a protein supplement, especially for ruminants, are highly desirable, and in this manuscript, we present novel data on such processing techniques.

The study design was approved by the appropriate ethics review board. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these. There are no conflicts of interest to declare. The Manuscrip has been edited by the editor of English Cactus English Editing Services (certificate attached)

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

B.W.H.E Prasetiyono

Faculty of Animal and Agricultural Sciences

Diponegoro University

Semarang, Central Java, Indonesia. Email: bambangwhep@ymail.com

UNDERTAKING

[Please print out this letter on letter head, sign it and submit its signed copy via email]

Hereby I would like to submit the paper mentioned below for the publication in the

"Pakistan Journal of Nutrition"

Title of the article: Effects of Heat Processing Techniques on Nutritional Value and in vitro Rumen Fermentation Characteristics of Jack bean (Canavalia ensiformis L.)

Author's Name: B.W.H.E. Prasetiyono, B.I.M. Tampoebolon, A. Subrata and Widiyanto

Name of the corresponding author: B.W.H.E. Prasetiyono

Address of the corresponding author with phone number: Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia, Tel: +6285200971963

I, as the Corresponding Author understand and declare that:

1. The manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.
2. We have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.
3. The manuscript is original, has neither in part nor as a whole been published or submitted for publication at the same time in any other scientific journal, and if accepted, it will not be published elsewhere in the same from, in English or any other language.
4. There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.
5. Any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.
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Corresponding author: B.W.H.E. Prasetiyono

Certificate from Head of the Department/Institute

This is to certify that the information given above is correct to the best of my knowledge and I have no objection to the publication of the article cited above in Pakistan Journal of Nutrition. This study is original, scientifically sound and not misleading, it contains new and important results that significantly advance the research field.

Deputy of Dean of Faculty of Animal and Agricultural Sciences: Agus Setiadi, S.Pt.,M.Si., Ph.D.

Dear Sir / Madam

We did not receive the revised article because you did not upload/attach the file of your manuscript. Please upload your revised article in MS WORD format for further processing.

Dear “PJN” Academic Editor
Pakistan Journal of Nutrition

I apologize for the submission error on the required manuscript modification form (No: 89089-PJN-ANSI) with the title " Effects of Heat Processing Techniques on Jack bean (Canavalia ensiformis L.) Nutritional Value and In vitro Rumen Fermentation Characteristics " on 17 January 2018. Therefore, please re-submit the list of modifications mentioned in the manuscript. thank you.

Regard
Dr. Bambang Waluyo Hadi Eko Prasetiyono

Submission Receipt

Manuscript # 89089-PJN-ANSI

Title Effects of Heat Processing Techniques on Jack bean (Canavalia ensiformis L.) Nutritional Value and In vitro Rumen Fermentation Characteristics

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. To improve its potential as a protein supplement for ruminants, jack beans were processed and evaluated for nutritional value and rumen fermentation characteristics. Materials and Methods: 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.

Categories Animal feeding

Nutritional biotechnology

CONTRIBUTING AUTHOR'S

Full Name Bambang Waluyo Hadi Eko Prasetiyono

E-mail bambangwhep@ymail.com

Country Indonesia

Full Name Baginda Iskandar Moeda Tampoebolon

E-mail bagindaiskandar@gmail.com

Country Indonesia

Full Name Agung Subrata

E-mail agung.subrata42@gmail.com

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Effects of heat processing techniques on jack bean (Canavalia ensiformis L.) nutritional value and in vitro rumen fermentation characteristics

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Do go through my changes and comments in the edited file, as well as the notes in this document. Please send me your feedback or any questions. I wish you the best of luck with your manuscript!

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8. **Section headings:** Please note that the section headings should be as follows: Title and Abstract, Key words, Introduction, Materials and Methods, Results and Discussion. You have combined your Results and Discussion into one section, and added a separate Conclusions section, and I am not sure this is appropriate for this journal.

Figures and tables

(Recommended changes to embedded figures/tables, which I was unable to modify)

1. **All figures:** Please note that, according to the author guidelines, 'The same data or information given in a Table must not be repeated in a Figure and vice versa'. It seems to me that Fig. 1 contains the same VFA and NH_3 data as Table 2, and Fig. 2 contains the same

OMD and RUP data as Table 2. As such, these figures are redundant and they could be removed. Please check this.

2. **Figure 1:** Please note that this figure does not have axis labels, please include these. I also recommend including the treatment abbreviations (H0, H1, H2, H3) below the treatments for consistency.
3. **Figure 2:** Please note that this figure does not have axis labels, please include these. I also recommend including the treatment abbreviations (H0, H1, H2, H3) below the treatments for consistency. I also recommend using IVOMD instead of OMD for consistency.
4. **Table 1:** Table 1 looks good and is clear, but please note that I have made some changes to the table caption and footnote to improve clarity or English grammar. I have also ensured all the letters in the table are in superscript.
5. **Table 2:** This table looks good and is clear, but please note that I have made some changes to the table caption and footnote to improve clarity or English grammar, including explaining what the three treatments are, and listing the abbreviations below the table.

Editor's report

On the basis of changes made for coherence, logic, and flow, I have provided feedback through specific comments along with ratings for each section. The key below the table explains my ratings. I hope you find my feedback useful.

Section	Rating
Title Your title effectively describes your study, and I have made only minor changes to improve grammar and have changed it to sentence case, as per the journal's instructions.	★★★
Abstract Your abstract describes your study and your main findings well. However, I have made quite a few changes to improve English language and grammar. I have also tried to cut several words, as the Pakistan Journal of Nutrition asks that abstracts be no longer than 200 words. With my changes your abstract is 210 words, so please see if you can remove a few more words. Please also note that, when first introducing an abbreviation (such as VFA), you should spell out the full word (I have assumed you mean volatile fatty acids?). Subsequently you may use only the abbreviation. Your key words are good, but please note that I have put them in alphabetical order, as is customary.	★★
Introduction Your introduction is generally good. It provides a good background to your study, is concise, and clearly states the aim of your study. However, I recommend including a little more detail on the different treatments here, as I feel it is not completely clear what the difference is between the 'roasting' and 'oven' treatments (roasting can be done in an oven), and I am not sure what you mean by 'extrusion' in this context. Please also note that I have made a lot of changes to improve English language and grammar.	★★★
Materials and methods Your Materials and Methods are generally clear and concise, although I have made several changes to improve English language and grammar. Please note that I recommend providing some more detail on the different treatments, as it is not completely clear to me what the three treatments are. For example, I am not sure how the oven treatment is different from the roasting treatment (how were the beans roasted?), and I am not sure what the extrusion treatment was. Please note that I also recommend reporting how many beans were used for each treatment. It is also not completely clear to me how many replicates were used for each measurement. For the nutritional value analysis, how many beans in each treatment were analysed? And how many replicates were used for the rumen characteristics analysis? Please provide some more detail here.	★★
Results and discussion Please note that you have combined your Results and Discussion into one section, but the Pakistan Journal of Nutrition asks that these be in separate sections. Specifically, the guidelines state that in the Results section 'The same data or information given in a Table must not be repeated in a Figure and vice versa. It is not acceptable to repeat extensively the numbers from Tables in the text or to give lengthy explanations of Tables or Figures. The Results section should not include discussion or references to citations' and that the in the Discussion 'Statements from the Introduction and Results sections should not be repeated here. The final paragraph should highlight the main conclusions of the study'. I therefore recommend that you restructure your manuscript to have a separate Results and Discussion section. Your Results are generally clear, and you do a good job of comparing your results to those from other studies from the literature. However, please note that you should avoid starting sentences with 'According to Table X…' Please also note that I recommend that you provide more detail on the results of your statistical analyses. Instead of only saying 'Heating significantly affected DM content ($p < 0.05$)' you should write 'Heating	★★

significantly affected DM content (ANOVA, F(a, b) = y, P = x)'. I have also made quite a few changes to improve English language and grammar.

Conclusions

★★

Your Conclusions are clear (after editing for language) and sum up your results well. However, according to the journal's guidelines, the conclusions should be an integral final part of a separate Discussion, so I recommend restructuring your manuscript. However, please note that here and elsewhere I am not sure what you mean by 'bypass protein' or 'bypass supplement'. Bypass can be defined as 'a road passing around a town to provide an alternative route for traffic' or 'a secondary connection to allow a flow when the main one is blocked' or 'to go past or around'. 'Bypass protein seems to be a brand of protein supplements, but I am not sure if that is what you mean here? Please check this.

Tables and figures

★

Please see my notes on your Tables and Figures above. My main concern is that your Figures are redundant, as they include the exact same information as Table 2.

★★★ This section required only a few revisions.
★★ Most parts of this section required revision.
★ The entire section required significant revision. Please go through my comments/changes carefully.

Comments

In general your study is interesting, concise, and your results are clearly presented. However, I have had to make substantial edits to improve the use of English language and grammar throughout your manuscript. Your Abstract generally summarizes your study well, although please note that, for the Pakistan Journal of Nutrition, it should be <200 words long, and it is currently a little over this word limit, even after my changes. Your Introduction is good, and provides a good background to your study, as well as clearly stating the aims of your research. Your Materials and Methods are also clear, although I recommend including some more details on the different heat treatments, as well as providing some more details on how many replicates were used for each treatment. Your Results and Discussion and Conclusions are generally good, but please note that the Pakistan Journal of Nutrition requires the Results and Discussion to be separate sections, with the last paragraph of the Discussion being a concluding paragraph. I therefore recommend that you restructure your manuscript to meet the journal's guidelines. Your Tables are clear and present your data well, but please note that I feel the Figures are a little redundant, as they include the same information as Table 2.

Final Proof approved by the author

Respected Sir / Madam

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Dir. Publication

Pakistan Journal of Nutrition

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17 Feb jam 14.13

Dear Dr. Bambang Waluyo Hadi Eko Prasetiyono

This is with regard to your submitted manuscript, 89089-PJN-ANSI, titled Effects of Heat Processing Techniques on Jack bean (Canavalia ensiformis L.) Nutritional Value and In vitro Rumen Fermentation Characteristics, submitted to Pakistan Journal of Nutrition on January 06, 2018 for consideration as a Research Article.

The above mentioned manuscript has been finally accepted by the Reviewer for publication in Pakistan Journal of Nutrition as Research Article. You may download the final acceptance letter after log in to your account with User ID bambangwhep@ymail.com.

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Feb 02, 2018

Dr. Bambang Waluyo Hadi Eko Prasetiyono,
Dr. Bambang W.H.E. Prasetiyono
(Diponegoro University, Indonesia)
JL. TUSAM TIMUR III No. 30
BANYUMANIK, SEMARANG - 50268,
INDONESIA

Subject: Acceptance Letter for Article No. 89089-PJN-ANSI

It's a great pleasure for us to inform you that below mentioned manuscript has been accepted for publication in Pakistan Journal of Nutrition as Research Article on the recommendation of the reviewers.

Title: Effects of Heat Processing Techniques on Jack bean (Canavalia ensiformis L.) Nutritional Value and In vitro Rumen Fermen Characteristics

Author's Name: Bambang Waluyo Hadi Eko Prasetiyono, Baginda Iskandar Moeda Tampoebolon and Agung Subrata

Receiving Date: January 06, 2018

Regards

M. Imran Pasha
Publication Manager

Dear **M.Imran Pasha** (Publication Manager)

I have received a letter of acceptance for Article No. 89089-PJN-ANSI , titled “Effects of Heat Processing Techniques on Jack bean (Canavalia ensiformis L.) Nutritional Value and In vitro Rumen Fermentation Characteristics”, but there was an error in the names of authors. There was a name of the author who has not been listed, namely **Widiyanto**.

Please repair it (**Author's Name**:Bambang Waluyo Hadi Eko Prasetiyono, Baginda Iskandar Moeda Tampoebolon, Agung Subrata and Widiyanto) and send it to me immediately. thanks

Regards

Bambang Waluyo Hadi Eko Prasetiyono

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---------- Instance Type and Transmission ----------
Notification (Transmission) of Original sent to SWIFT (ACK)
Network Delivery Status : Network Ack
Priority/Delivery : Normal
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Sender : PDJGIDJAXXX
BPD JATENG
SEMARANG ID
Receiver : BNINUS33XXX
BANK NEGARA INDONESIA (PERSERO) PT., NEW YORK AGENCY
NEW YORK,NY US
---------- Message Text ----------
20: Sender's Reference
50TRJATENG0218
23B: Bank Operation Code
CRED
32A: Val Dte/Curr/Interbnk Settld Amt
Date : 22 February 2018
Currency : USD (US DOLLAR)
Amount : #250.#
50K: Ordering Customer-Name & Address
/3034 20724 4
BAMBANG WALUYO HADI EP
JL.TUSAM TIMUR III no 30
BANYUMANIK SEMARANG
57A: Account With Institution - FI BIC
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59: Beneficiary Customer-Name & Addr
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71A: Details of Charges
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72: Sender to Receiver Information
/ACC/PLS PAY TO YR
//ROYAL BANK of CANADA
//382 YONGE St UNIT 5, TORONTO,
//ON M5B 1S8, CANADA
---------- Message Trailer ----------
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Operator : SYSTEM
Text
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Correspondent Input Reference : 1912 180222BNINUS33AXXX348422905
---------- Message Header ----------
Swift Output : FIN 950 Statement Message
Sender : BNINUS33XXX
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NEW YORK,NY US
Receiver : PDJGIDJAXXX
BPD JATENG
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MUR : 6067
---------- Message Text ----------
20: Transaction Reference Number
VG00148518/P1
25: Account Identification
475
28C: Statement Number/Sequence Number
75/1
60F: First Opening Balance
Debit/Credit : Credit
Date : 22 February 2018
Currency : USD (US DOLLAR)
Amount : #725,870.06#
61: Statement Line

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				PDJGIDJAXXX	
180222	0222		NMSC	PDJGIDJAXXX	#10.#
				WITHDRAWAL TRANS	
				0654840420861001 50TRJATENG0218 FE	

62F: Closing Balance (Booked Funds)
Debit/Credit : Credit
Date : 22 February 2018
Currency : USD (US DOLLAR)
Amount : #725,610.06#
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Debit/Credit : Credit
Date : 22 February 2018
Currency : USD (US DOLLAR)
Amount : #725,610.06#
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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.)

B.W.H.E. Prasetiyono, B.I.M. Tampoebolon, A. Subrata and Widiyanto

Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia

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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Citation: B.W.H.E. Prasetiyono, B.I.M. Tampoebolon, A. Subrata and Widiyanto, 2018. Effects of heat processing techniques on nutritional value and *in vitro* rumen fermentation characteristics of jack bean (*Canavalia ensiformis* L.). Pak. J. Nutr., CC: CC-CC.

Corresponding Author: B.W.H.E. Prasetiyono, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia Tel: +6285200971963

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.

	Treatments					
Parameters	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

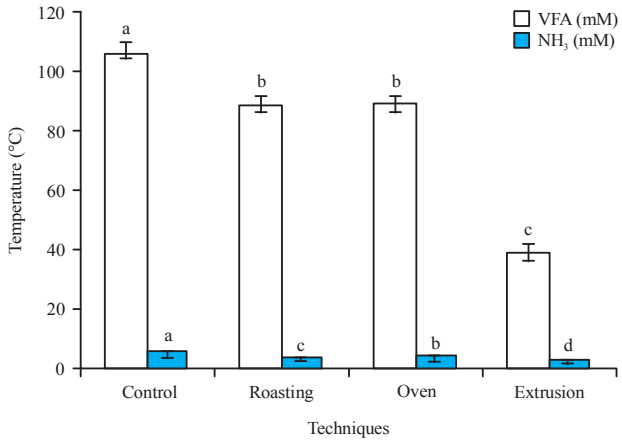

Fig. 1: Effect of heat processing techniques on VFA and NH_3 concentrations

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

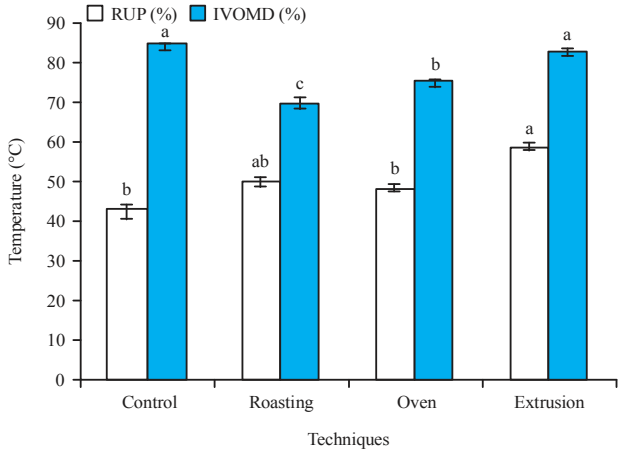

Fig. 2: Effect of heat processing techniques on RUP and IVOMD percentages

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

B.W.H.E. Prasetiyono, B.I.M. Tampoebolon, A. Subrata and Widiyanto

Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia

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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Corresponding Author: B.W.H.E. Prasetiyono, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia Tel: +6285200971963

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.

	Treatments					
Parameters	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

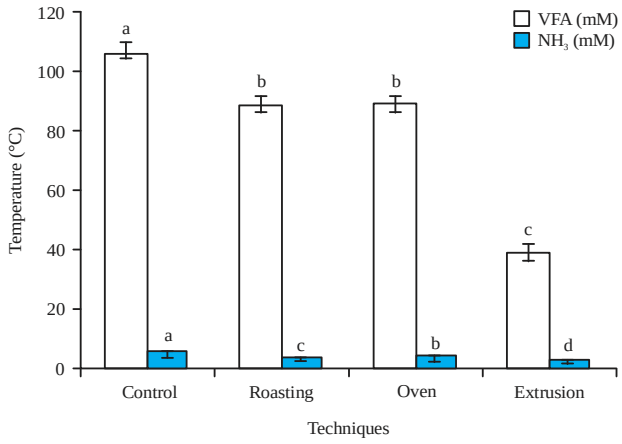

Fig. 1: Effect of heat processing techniques on VFA and NH_3 concentrations

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

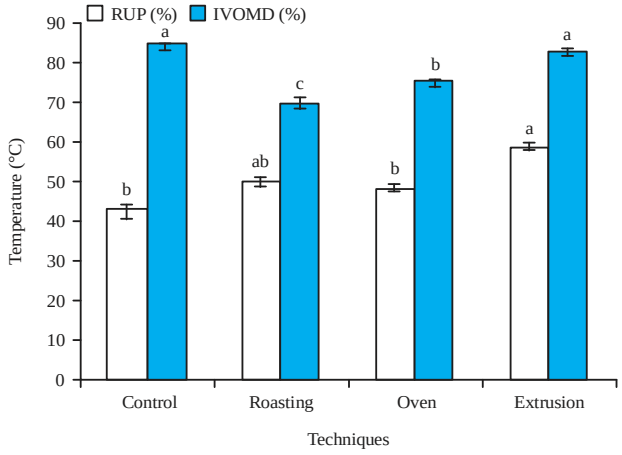

Fig. 2: Effect of heat processing techniques on RUP and IVOMD percentages

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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