

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 (NH₃) 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 Manuscript 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 Prasetyono

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. Prasetyono, B.I.M. Tampobolon, A. Subrata and Widiyanto

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

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:

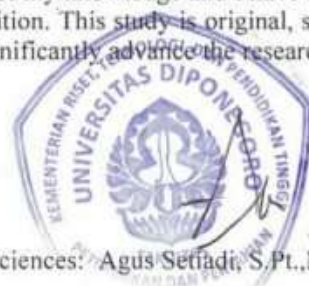
1. The manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.
2. We have given due consideration 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 form, 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.
6. We (all the authors of the manuscript) understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We (all the authors of the manuscript) confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from support@scialert.com
7. If the above article is published in Pakistan Journal of Nutrition, the copyright of this article will vest with the Pakistan Journal of Nutrition, who will have the right to enter into any agreement with any organization. Neither we nor our legal heirs will have any claims on royalty.



Corresponding author: B.W.H.E. Prasetyono

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 Prasetyono

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 (NH₃) 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 Prasetyono

E-mail bambangwhep@gmail.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

Country Indonesia

Dear B.W.H.E. Prasetyono

Thanks for being registered with Science Alert Online Manuscript Submission and Peer Reviewed System.

Science Alert integrated with the liveDNA to accurately identify and disambiguated all authors of all articles in Science alert Database.

LiveDNA is dedicated to provide a transparent and improvised tracking of the research and researcher by issuing each researcher a Numerological ID called his/her LiveDNA. It's one of the basic objective is to establish and develop strong relationship between research and researchers, contributors and the work. Having a LiveDNA will help the researcher in his/her manuscript submission, grant applications, professional society membership, to have association with other identifiers or profiles and to display it on CV, webpage and more. LiveDNA is devoted to the openness, transparency and the protection of scholars' privacy.

Therefore, please send us your CV to be the identified member of the Science Alert.

Regard
Science Alert

CERTIFICATE OF ENGLISH EDITING

This document certifies that the paper listed below has been edited to ensure that the language is clear and free of errors. The logical presentation of ideas and the structure of the paper were also checked during the editing process. The edit was performed by professional editors at Editage, a division of Cactus Communications. The intent of the author's message was not altered in any way during the editing process. The quality of the edit has been guaranteed, with the assumption that our suggested changes have been accepted and have not been further altered without the knowledge of our editors.

TITLE OF THE PAPER

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

AUTHORS

B.W.H.E PRASETIYONO

JOB CODE

SISUS_7



Signature

Vikas Narang

Vikas Narang,
Vice President, Author Services, Editage

Date of Issue
November 22, 2017

Editage, a brand of Cactus Communications, offers professional English language editing and publication support services to authors engaged in over 500 areas of research. Through its community of experienced editors, which includes doctors, engineers, published scientists, and researchers with peer review experience, Editage has successfully helped authors get published in internationally reputed journals. Authors who work with Editage are guaranteed excellent language quality and timely delivery.



Contact Editage

Worldwide
request@editage.com
+1 877-334-8243
www.editage.com

Japan
submissions@editage.com
+81 03-6868-3348
www.editage.jp

Korea
submit-
korea@editage.com
1544-9241
www.editage.co.kr

China
fabiao@editage.cn
400-005-6055
www.editage.cn

Brazil
contato@editage.com
0800-892-20-97
www.editage.com.br

Taiwan
submitjobs@editage.com
02 2657 0306
www.editage.com.tw

Dear Author,

It was a pleasure working on your document. In general I found your study very interesting, and you present your results clearly. However, I have made quite extensive changes to improve the English language and grammar, so please check the edited document carefully to ensure your original meaning is maintained throughout the document. Please also note that I have looked up the requirements for the Pakistan Journal of Nutrition, and have made several changes to ensure your manuscript meets the author guidelines of this journal. This includes inserting page and line numbers, and making some changes to the title page.

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!

Formatting

(To ensure that your paper conforms to the formatting requirements of the target publication)

1. **General comment:** Although I was not specifically instructed to format your manuscript according to a specific journal's author guidelines, I saw the note at the end of your manuscript saying that you plan to submit your manuscript to the Pakistan Journal of Nutrition.
2. As such, I have looked up the guidelines for this journal and tried to format your manuscript accordingly. This includes changing your font to Times New Roman (double spaced) and including line and page numbers.
3. **Title page:** Please note that, according to the author guidelines, the title page should 'contain the full title in sentence case, the full names (last names fully capitalised) and affiliations (in English) of all authors, and the contact e-mail address for the clearly identified corresponding author. Acknowledgements or disclaimers should be included on the title page, as well.' I have therefore made some changes to your title page, please check these.
4. **Author information:** As outlined above, I have put all author last names in all caps.
5. **Abstract:** Although your abstract describes your study well, 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.
6. **In-text citations:** Your in-text citations (as numbers) are suitable for the Pakistan Journal of Nutrition
7. **References:** You have not included your references, but please note that you will need to include these in your final version. Please ensure they meet the author guidelines
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 NH₃ 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 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

It is intimated that you did not submit the undertaking for the publication of your article mentioned above. Please submit the required undertaking immediately otherwise it will delay the publication process of your article. Copy of undertaking is also attached with this e-mail, please submit it after filling for further processing of your article. Please note, this undertaking is compulsory for the publication in Pakistan Journal of Nutrition.

Dir. Publication

Pakistan Journal of Nutrition

Science Alert <support@scialert.com>
Kepada: Bambang Waluyo Hadi Eko Prasetyono
17 Feb jam 14.13

Dear Dr. Bambang Waluyo Hadi Eko Prasetyono

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@gmail.com.

Before we can continue with final production, it is mandatory to pay Article Processing Charges. All articles published in our journals are open access and freely available online, immediately upon publication. This is made possible by an article-processing charge (APC) that covers the range of publishing services we provide. This includes provision of online tools for editors and authors, article production and hosting, liaison with abstracting and indexing services, and customer services. The APC, payable when your manuscript is editorially accepted and before publication, is charged to either you, or your funder, institution or employer.

You may download the invoice after log in to your account with User ID: bambangwhep@gmail.com.

If you have forgot your password, you may retrieve your password from the following link by providing your User ID bambangwhep@gmail.com.

http://scialert.com/forgot_password.php

Therefore, It is requested to please pay the Article Processing Charges urgently so that we may send your article to the production department for final publication.

We look forward to hearing from you.

Regard
PJN
Academic Editor
Pakistan Journal of Nutrition



Feb 02, 2018

Dr. Bambang Waluyo Hadi Eko Prasetyono,
Dr. Bambang W.H.E. Prasetyono
(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 Fermentation Characteristics

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

Receiving Date: January 06, 2018

Regards

A handwritten signature in blue ink, appearing to read 'Imran Pasha', is written over a large, stylized blue circular mark.

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 Prasetyono, Baginda Iskandar Moeda Tampoebolon, Agung Subrata and Widiyanto) and send it to me immediately. thanks

Regards

Bambang Waluyo Hadi Eko Prasetyono

SCIENCE ALERT

www.scialert.com

P.O.Box 126208 Deira Dubai, UAE.

Invoice

Billing Address	Invoice Generated on	Payment Due Date	Invoice Number
Dr. Bambang Waluyo Hadi Eko Prasatyo Diponegoro University, Indonesia	06 January, 2018	IMMEDIATE	89089INV18

Order Number	Description	Amount	Net Amount
89089-PJN-ANSI	Article Processing Charges (Article Processing Charges)	250 USD	250 USD
		Amount Payable	
		250 USD	

You have the following options to pay your dues. Please select method of payment as per your convenience.



Pay via Credit Card

If you want to pay by Credit Card, please click the below link:

https://secure.innovatepayments.com/gateway/gs/13/ScienceAlert_3670.html



Pay via PayPal

If you want to pay via PayPal, please click the below link:

https://www.paypal.com/cgi-bin/webscr?cmd=_s-xclick&hosted_button_id=8XS72XX8AE8Y

Pay Via Telegraphic Transfer (Bank to Bank Transfer)

If you wish to pay by Telegraphic Transfer, please use the following




information and send us the bank transaction document by email
(support@scialert.com)

Title of Account: SCIENCE ALERT
Account No. 02157 1000371
Bank Name: Royal Bank of Canada
Full Address of Bank: 382 Yonge St Unit 5, Toronto, ON M5B 1S8, Canada
SWIFT Code: ROYCCAT2
Office Address: 1 Yonge Street, Suite 1801, Toronto, ON M5E 1W7,
Canada

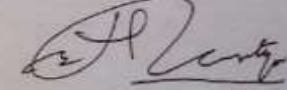


PENYETOR

Ditulis dengan huruf cetak / write in block letters

PERMINTAAN UNTUK APPLICATION FOR		<input type="checkbox"/> Telex Transfer <input type="checkbox"/> Mail Transfer <input type="checkbox"/> Demand Draft	
Jumlah Amount USD 250,00		Tgl. Val / Val. Date 22/02/2018	
Dg. Huruf : Dua ratus lima puluh in words : US Dollar			
Diisi oleh Bank / For Bank Use Only Nominasi : Commission : USD 250,00 Changes : USD 35,00 (Pay in Full) Total Cover : USD 0,00			
Dg. Huruf : in words : 285,00			
Telah dilaksanakan hari ini dengan nomor Executed today with serial number			
Dalam hal wesel unjuk, kolom di atas sebagai tanda terima wesel yang diserahkan kepada pemohon / In case of demand draft signed receipt in the above column for the draft issued.			
PT. BANK PEMBANGUNAN DAERAH JAWA TENGAH Cabang Utama 			
No. Urut		No. Rek	
Kd. tr		Nama	
Tanggal		Parap	
Memo		Jumlah	
Keterangan :		Parap	

Penerima / Beneficiary
SCIENCE ALERT
1 Yonge Street, suite 1801, Toronto
ON MSE 1W7, Canada
 Rekening pada / Account with
ROYAL BANK of CANADA
SWIFT Code : ROYCCAT2
ACC : 021571000371
 Keterangan / Payment details
Payment Article 89089-PJN-ANSI
 Atas permintaan / By Order of
BAMBANG WALUYO HADI ET
JL. Tusam Timur III no 30
 Banyumanik Semarang
 HP : 085200700000 / In reimbursement
3-034-20724-4
SAYA SETUJU DENGAN SYARAT-2 DI BALIK INI
I AGREE TO TERMS & CONDITIONS REVERSESIDE


 (Tanda tangan pemohon)
 (Applicant signature)

Sah kalau ada cetakan data computer dan tanda tangan yang berwenang

----- Instance Type and Transmission -----
Notification (Transmission) of Original sent to SWIFT (ACK)
Network Delivery Status : Network Ack
Priority/Delivery : Normal
Message Input Reference : 1721 180222PDJGIDJAAXXX4249007224

----- Message Header -----
Swift Input : FIN 103 Single Customer Credit Transfer
Sender : PDJGIDJAAXX
 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
 ROYCCAT2
 ROYAL BANK OF CANADA
 (HEAD OFFICE)
 TORONTO CA
59: Beneficiary Customer-Name & Addr
 /021571000371
 SCIENCE ALERT
 1 YONGE STREET, SUITE 1801, TORONTO
 ON M5E 1W7, CANADA
70: Remittance Information
 PAYMENT ARTICLE 89089-PJN-ANSI
71A: Details of Charges
 OUR
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 -----
(CHK:D73E4174F424)
PKI Signature: MAC-Equivalent

----- Interventions -----
Category : Network Report
Creation Time : 22/02/18 17:21:19
Application : SWIFT Interface
Operator : SYSTEM
Text
{1:F21PDJGIDJAAXXX4249007224}{4:{177:1802221721}{451:0}}

/02/18-07:12:25

FileOutputNT-7848-000001

----- Instance Type and Transmission -----
Original received from SWIFT
Priority : Normal
Message Output Reference : 0712 180223PDJGIDJAAXXX4249055245
Correspondent Input Reference : 1912 180222BNINUS33AXXX348422905

----- Message Header -----
Swift Output : FIN 950 Statement Message
Sender : BNINUS33XXX
BANK NEGARA INDONESIA (PERSERO) PT., NEW YORK AGEN
NEW YORK, NY US
Receiver : PDJGIDJAXXX
BPD JATENG
SEMARANG ID

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
Value Entr F Code Reference Amount
180222 0222 NMSC S06NYA0000144618 #250.#
50TRJATENG0218
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#
64: Closing Avail Bal (Avail Funds)
Debit/Credit : Credit
Date : 22 February 2018
Currency : USD (US DOLLAR)
Amount : #725,610.06#

----- Message Trailer -----
(CHK:6F3DF387CA71)



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. Prasetyono, 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

Received:

Accepted:

Published:

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

Copyright: © 2018 B.W.H.E. Prasetyono *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

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 value². 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 min³, H2: Jack beans heated in an oven at 110°C

for 30 min³ and H3: Extrusion at 120°C for 10 sec, using a single crew⁴. 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 methods⁵ 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 CO₂ 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 CO₂) 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 HgCl₂. Analyses were conducted for ruminal ammonia (NH₃), 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 Terry⁵. The dry matter, ash, ether extract, crude fibre and crude protein were analyzed by AOAC standard methods⁶.

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 SAS⁷.

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)

Parameters	Treatments				SEM	Significance
	H0	H1	H2	H3		
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 process⁸. This result is consistent with a previous study conducted by Prasetyono *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 concentration⁹.

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 reactions⁸. 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 higher⁴.

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 Sanders¹¹, 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				SEM	Significance
	H0	H1	H2	H3		
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, NH₃: Ammonia, VFA: Volatile fatty acids, RUP: Rumen undegradable protein

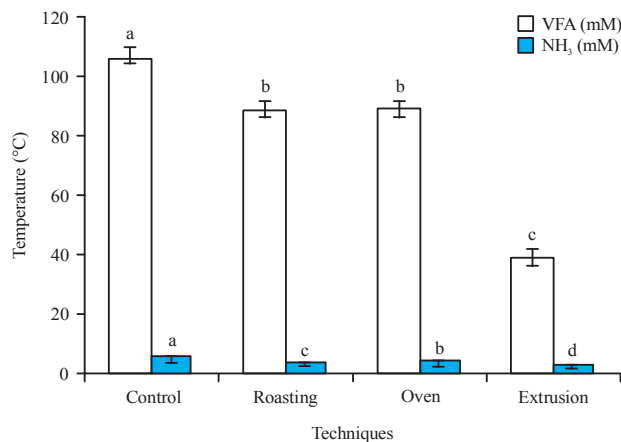


Fig. 1: Effect of heat processing techniques on VFA and NH₃ concentrations

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 NH₃ 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 fluid¹³. 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 Prasetyono *et al.*⁴ and Soltan¹⁴, 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 IVOMD¹⁶, 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

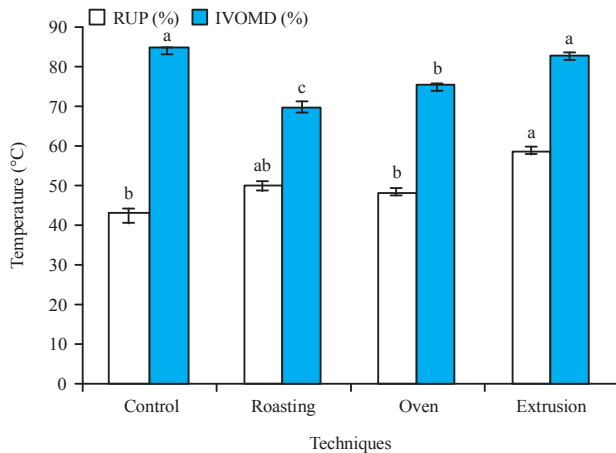


Fig. 2: Effect of heat processing techniques on RUP and IVOMD percentages

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 solubility¹⁸. 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 Chumpawadee²¹, 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.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Diponegoro University, who funded this research through a PNBP-DIPA Diponegoro University research grant, with Research Contract Number: SP DIPA-042.01.2.400898/2016 (Fiscal Year 2016). The authors also wish to thank the Feed Technology Laboratory of the Faculty of Animal and Agricultural Sciences, Diponegoro University, for collaboration in this project.

REFERENCES

- Hudiyanti, D., A.P. Arya, P. Siahaan and L. Suyati, 2015. Chemical composition and phospholipids content of Indonesian Jack Bean (*Canavalia ensiformis* L.). *Orient. J. Chem.*, 31: 2043-2046.
- Dakowski, P., M.R. Weisbjerg and T. Hvelplund, 1996. The effect of temperature during processing of rape seed meal on amino acid degradation in the rumen and digestion in the intestine. *Anim. Feed Sci. Technol.*, 58: 213-226.

3. Rafiee-Yarandi, H., M. Alikhani, G.R. Ghorbani and A. Sadeghi-Sefidmazgi, 2016. Effects of temperature, heating time and particle size on values of rumen undegradable protein of roasted soybean. *S. Afr. J. Anim. Sci.*, 46: 170-179.
4. Prasetyono, B.W.H.E., Suryahadi, T. Toharmat and R. Syarif, 2017. Strategi suplementasi protein ransum sapi potong berbasis jerami dan dedak padi. *Media Peternakan*, 30: 207-217.
5. Tilley, J.M.A. and R.A. Terry, 1963. A two stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.*, 18: 104-111.
6. AOAC., 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., Pages: 684.
7. SAS., 2009. SAS User's Guide. SAS Institute Inc., SAS Campus Drive, Cary, NC.
8. Khan, N.A., H. Booker and P. Yu, 2015. Effect of heating method on alteration of protein molecular structure in flaxseed: Relationship with changes in protein subfraction profile and digestion in dairy cows. *J. Agric. Food Chem.*, 63: 1057-1066.
9. Onyeike, E.N. and T.T. Omubo-Dede, 2002. Effect of heat treatment on the proximate composition, energy values and levels of some toxicants in African yam bean (*Sphenostylis stenocarpa*) seed varieties. *Plant Foods Hum. Nutr.*, 57: 223-231.
10. Doss, A., M. Pugalenthil and V. Vadivel, 2011. Nutritional evaluation of wild Jack bean (*Canavalia ensiformis* DC) seeds in different locations of South India. *World Applied Sci. J.*, 13: 1606-1612.
11. Sanders, K.J., 1998. The effects of extrusion on ruminal digestion and performance of ruminants. Ph.D. Thesis, The Graduate Faculty of Texas Tech University, USA.
12. Parand, E., A.R. Vakili and M.D. Mesgaran, 2016. Rumen degradability and model prediction of nutrient supply to ruminants from different processed soybean meals. *Iran. J. Applied Anim. Sci.*, 6: 41-46.
13. Hristov, A.N., R.P. Etter, J.K. Ropp and K.L. Grande, 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. *J. Anim. Sci.*, 82: 3219-3229.
14. Soltan, M.A., 2009. Rumen fermentation characteristics and lactation performance in dairy cows fed different rumen protected soybean meal products. *Pak. J. Nutr.*, 8: 695-703.
15. Pena, F., H. Tagari and L.D. Satter, 1986. The effect of heat treatment of whole cottonseed on site and extent of protein digestion in dairy cows. *J. Anim. Sci.*, 62: 1423-1433.
16. Rangel, F.C., G.V. Villalobos, D.D. Diaz and J.A.O. Gutierrez, 2017. Effect of the dietary level of cull pinto beans (*Phaseolus vulgaris*) on ruminal fermentation, kinetics and digestibility of hair lambs. *R. Bras. Zootec.*, 46: 405-412.
17. Wang, C., J.X. Liu, S.W. Zhai, J.L. Lai and Y.M. Wu, 2008. Effects of rumen-degradable-protein to rumen-undegradable-protein ratio on nitrogen conversion of lactating dairy cows. *Acta Agric. Scand. Sect. A: Anim. Sci.*, 58: 100-103.
18. Tacoma, R., J. Fields, D.B. Ebenstein, Y.W. Lam and S.L. Greenwood, 2017. Ratio of dietary rumen degradable protein to rumen undegradable protein affects nitrogen partitioning but does not affect the bovine milk proteome produced by mid-lactation Holstein dairy cows. *J. Dairy Sci.*, 100: 7246-7261.
19. Karlsson, L., M. Ruiz-Moreno, M.D. Stern and K. Martinsson, 2012. Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake. *Asian-Aust. J. Anim. Sci.*, 25: 1559-1567.
20. Solanas, E., C. Castrillo, J. Balcells and J.A. Guada, 2005. *In situ* ruminal degradability and intestinal digestion of raw and extruded legume seeds and soya bean meal protein. *J. Anim. Physiol. Anim. Nutr.*, 89: 166-171.
21. Chantiratikul, A. and S. Chumpawadee, 2011. Effect of heat-treatment on ruminal protein degradability of wolffia meal (*Wolffia globosa* L. Wimm). *Asian J. Anim. Sci.*, 5: 183-189.

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com



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. Prasetyono, 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

Received: January 06, 2018

Accepted: April 02, 2018

Published: May 15, 2018

Citation: B.W.H.E. Prasetyono, 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., 17: 294-299.

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

Copyright: © 2018 B.W.H.E. Prasetyono *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

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 value². 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 min³, H2: Jack beans heated in an oven at 110°C

for 30 min³ and H3: Extrusion at 120°C for 10 sec, using a single crew⁴. 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 methods⁵ 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 CO₂ 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 CO₂) 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 HgCl₂. Analyses were conducted for ruminal ammonia (NH₃), 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 Terry⁵. The dry matter, ash, ether extract, crude fibre and crude protein were analyzed by AOAC standard methods⁶.

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 SAS⁷.

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)

Parameters	Treatments				SEM	Significance
	H0	H1	H2	H3		
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 process⁸. This result is consistent with a previous study conducted by Prasetyono *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 concentration⁹.

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 reactions⁸. 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 higher⁴.

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 Sanders¹¹, 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				SEM	Significance
	H0	H1	H2	H3		
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, NH₃: Ammonia, VFA: Volatile fatty acids, RUP: Rumen undegradable protein

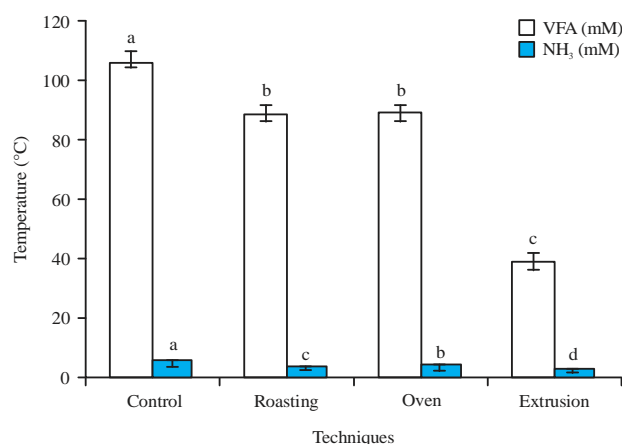


Fig. 1: Effect of heat processing techniques on VFA and NH₃ concentrations

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 NH₃ 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 fluid¹³. 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 Prasetyono *et al.*⁴ and Soltan¹⁴, 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 IVOMD¹⁶, 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

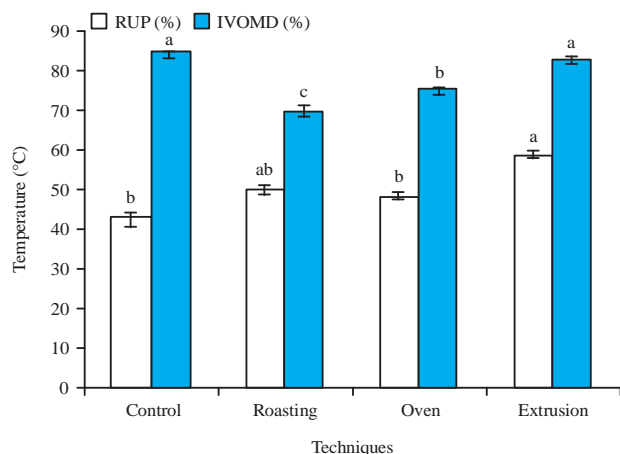


Fig. 2: Effect of heat processing techniques on RUP and IVOMD percentages

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 solubility¹⁸. 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 Chumpawadee²¹, 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.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Diponegoro University, who funded this research through a PNBPDIPA Diponegoro University research grant, with Research Contract Number: SP DIPA-042.01.2.400898/2016 (Fiscal Year 2016). The authors also wish to thank the Feed Technology Laboratory of the Faculty of Animal and Agricultural Sciences, Diponegoro University, for collaboration in this project.

REFERENCES

- Hudiyanti, D., A.P. Arya, P. Siahaan and L. Suyati, 2015. Chemical composition and phospholipids content of Indonesian Jack Bean (*Canavalia ensiformis* L.). *Orient. J. Chem.*, 31: 2043-2046.
- Dakowski, P., M.R. Weisbjerg and T. Hvelplund, 1996. The effect of temperature during processing of rape seed meal on amino acid degradation in the rumen and digestion in the intestine. *Anim. Feed Sci. Technol.*, 58: 213-226.

3. Rafiee-Yarandi, H., M. Alikhani, G.R. Ghorbani and A. Sadeghi-Sefidmazgi, 2016. Effects of temperature, heating time and particle size on values of rumen undegradable protein of roasted soybean. *S. Afr. J. Anim. Sci.*, 46: 170-179.
4. Prasetyono, B.W.H.E., Suryahadi, T. Toharmat and R. Syarif, 2017. Strategi suplementasi protein ransum sapi potong berbasis jerami dan dedak padi. *Media Peternakan*, 30: 207-217.
5. Tilley, J.M.A. and R.A. Terry, 1963. A two stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.*, 18: 104-111.
6. AOAC., 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., Pages: 684.
7. SAS., 2009. SAS User's Guide. SAS Institute Inc., SAS Campus Drive, Cary, NC.
8. Khan, N.A., H. Booker and P. Yu, 2015. Effect of heating method on alteration of protein molecular structure in flaxseed: Relationship with changes in protein subfraction profile and digestion in dairy cows. *J. Agric. Food Chem.*, 63: 1057-1066.
9. Onyeike, E.N. and T.T. Omubo-Dede, 2002. Effect of heat treatment on the proximate composition, energy values and levels of some toxicants in African yam bean (*Sphenostylis stenocarpa*) seed varieties. *Plant Foods Hum. Nutr.*, 57: 223-231.
10. Doss, A., M. Pugalenth and V. Vadivel, 2011. Nutritional evaluation of wild Jack bean (*Canavalia ensiformis* DC) seeds in different locations of South India. *World Applied Sci. J.*, 13: 1606-1612.
11. Sanders, K.J., 1998. The effects of extrusion on ruminal digestion and performance of ruminants. Ph.D. Thesis, The Graduate Faculty of Texas Tech University, USA.
12. Parand, E., A.R. Vakili and M.D. Mesgaran, 2016. Rumen degradability and model prediction of nutrient supply to ruminants from different processed soybean meals. *Iran. J. Applied Anim. Sci.*, 6: 41-46.
13. Hristov, A.N., R.P. Etter, J.K. Ropp and K.L. Grande, 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. *J. Anim. Sci.*, 82: 3219-3229.
14. Soltan, M.A., 2009. Rumen fermentation characteristics and lactation performance in dairy cows fed different rumen protected soybean meal products. *Pak. J. Nutr.*, 8: 695-703.
15. Pena, F., H. Tagari and L.D. Satter, 1986. The effect of heat treatment of whole cottonseed on site and extent of protein digestion in dairy cows. *J. Anim. Sci.*, 62: 1423-1433.
16. Rangel, F.C., G.V. Villalobos, D.D. Diaz and J.A.O. Gutierrez, 2017. Effect of the dietary level of cull pinto beans (*Phaseolus vulgaris*) on ruminal fermentation, kinetics and digestibility of hair lambs. *R. Bras. Zootec.*, 46: 405-412.
17. Wang, C., J.X. Liu, S.W. Zhai, J.L. Lai and Y.M. Wu, 2008. Effects of rumen-degradable-protein to rumen-undegradable-protein ratio on nitrogen conversion of lactating dairy cows. *Acta Agric. Scand. Sect. A: Anim. Sci.*, 58: 100-103.
18. Tacoma, R., J. Fields, D.B. Ebenstein, Y.W. Lam and S.L. Greenwood, 2017. Ratio of dietary rumen degradable protein to rumen undegradable protein affects nitrogen partitioning but does not affect the bovine milk proteome produced by mid-lactation Holstein dairy cows. *J. Dairy Sci.*, 100: 7246-7261.
19. Karlsson, L., M. Ruiz-Moreno, M.D. Stern and K. Martinsson, 2012. Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake. *Asian-Aust. J. Anim. Sci.*, 25: 1559-1567.
20. Solanas, E., C. Castrillo, J. Balcells and J.A. Guada, 2005. *In situ* ruminal degradability and intestinal digestion of raw and extruded legume seeds and soya bean meal protein. *J. Anim. Physiol. Anim. Nutr.*, 89: 166-171.
21. Chantiratikul, A. and S. Chumpawadee, 2011. Effect of heat-treatment on ruminal protein degradability of wolffia meal (*Wolffia globosa* L. Wimm). *Asian J. Anim. Sci.*, 5: 183-189.