ISSN 1682-8356 ansinet.org/ijps



POULTRY SCIENCE



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

International Journal of Poultry Science

ISSN 1682-8356 DOI: 10.3923/ijps.2018.51.56



Research Article Using Physical and Chemical Methods to Improve the Nutrient Quality of Dragon Fruit (*Hylocereus polyrhizus*) Peel for Use as Feed for Laying Hens

¹Yusuf Mahlil, ²Husmaini, ³Warnita, ²Mirzah and ²Maria Endo Mahata

¹Universitas Andalas, Kampus Limau Manis, 25163 Padang, Indonesia

²Magister and Doctoral Faculty of Animal Science, Universitas Andalas, Kampus Limau Manis, 25163 Padang, Indonesia ³Magister and Doctoral Faculty of Agricultural Science, Universitas Andalas, Kampus Limau Manis, 25163 Padang, Indonesia

Abstract

Background and Objective: The processing of dragon fruit (Hylocereus polyrhizus) into a food product leaves fruit peel waste, which is a potential feed for laving hens. Dragon fruit peel contains phytochemical compounds such as anthocyanins, beta-carotene and lycopene. which are reported to have antioxidant functions and to lower cholesterol in blood serum. Previous study showed that the problem with using dragon fruit peel as feed for laying hens was due to its high content of crude fiber, which inhibited the amount of its utilization in the poultry diet and lowered its phytochemical absorption in the digestive tract. Physical and chemical methods, such as steaming and soaking in acid solution, could reportedly degrade and lower the crude fiber content in feed containing high levels of crude fiber. The goal of this study was to evaluate the nutrient content of dragon fruit peel, particularly its crude fiber content, after processing with physical and chemical methods, to determine its use in feed for laying hens. Materials and Methods: The dragon fruit peels in this experiment were collected from local restaurants in Indonesia. The experiment consisted of 2 parts. In part one, dragon fruit peels were steamed in boiling water at a temperature of 98°C. It was performed in a completely randomized design, with different steaming times (0, 5, 10, 15 and 20 min) and each treatment was replicated 4 times. Then the dry matter, crude fiber and crude protein were measured. In the second part, the dragon fruit peels were treated by soaking in 7.5% acetic acid (pH 4). The experiment was performed in a completely randomized design with different soaking times (0, 2, 4, 6 and 8 h) and each treatment was replicated 4 times. The dry matter, crude fiber and crude protein were measured. **Results:** The experiment showed that both the physical and chemical methods highly significantly reduced the crude fiber content of dragon fruit peel (p<0.01) but did not significantly affect the dry matter or protein content of dragon fruit peel (p>0.05). Conclusion: Processing dragon fruit peels by using a physical method (steaming for 20 min) and a chemical method (soaking in acetic acid solution for 4 h) lowered the crude fiber content in dragon fruit peel from 24.01-19.81% and from 24.01-20.39%, respectively, without altering the protein and dry matter content of dragon fruit peel.

Key words: Dragon fruit peel, steaming, soaking, crude fiber, feed for laying hens

Received: September 05, 2017

Accepted: January 05, 2018

Published: January 15, 2018

Citation: Yusuf Mahlil, Husmaini, Warnita, Mirzah and Maria Endo Mahata 2018. Using physical and chemical methods to improve the nutrient quality of dragon fruit (*Hylocereus polyrhizus*) peel for use as feed for laying hens. Int. J. Poult. Sci., 17: 51-56.

Corresponding Author: Maria Endo Mahata, Magister and Doctoral Faculty of Animal Science, Universitas Andalas, Kampus Limau Manis, 25163 Padang, Indonesia Tel+6287895056126

Copyright: © 2018 Yusuf Mahlil *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

Agricultural waste can be used as an alternative poultry feed because it contains nutrients that can be utilized by poultry. A mixture of juice wastes from various fruits and vegetables, such as carrot, apple, mango, avocado, orange, melon and dutch eggplant (juice waste mixture), could reportedly account for as much as 20% of a broiler ration¹. The utilization of an improved juice waste mixture by soaking up to 40% of the rice hull ash filtrate in broiler ration in the juice or replacing as much as 80% of the corn with the juice maintained broiler performance². Furthermore, boiled tomato waste can be utilized as an alternative feed material for broiler and laying hens. The use of boiled tomato wastes (up to 7%) in broiler rations lowered the cholesterol, low-density lipoprotein (LDL) and triglycerides and maintained the high-density lipoprotein (HDL) in broiler blood serum. The utilization of boiled tomato in laying hen rations (as much as 12%) decreased the egg yolk cholesterol and increased the egg yolk index^{3,4}. Pineapple waste was also reported as a potential feed for poultry, but its use was constrained due to its high fiber content, which could not be digested by the poultry. According to Adrizal et al.5, the fermentation of pineapple waste for 1 week with a solution of local microorganisms from bamboo sprout, at a dosage of 375 mL/500 g fresh pineapple waste, reduced the pineapple waste crude fiber from 24-17.16%.

Dragon fruit peel is a waste obtained from the processing of fresh dragon fruit for juice or material for use in cakes, syrups and jams. This waste is also a potential poultry feed because it contains the nutrients required by poultry. No statistical data on the availability of fresh dragon fruit peel are available. Previous study showed that dragon fruit peel accounted for 22% of a whole fresh dragon fruit^{6,7}. According to Wahyu⁸, 1 Ha of dragon fruit plantation will produce 50 t of fresh dragon fruit and they predicted that the same plantations would produce as much as 11 t of dragon fruit peel (calculated as 22% of the 50 t of fresh dragon fruit being dragon fruit peel). Mahlil⁷ reported that 1 kg of fresh dragon fruit peel would produce 100 g of powdered dragon fruit peel after drying under sunlight until the water content reached 14%. Therefore, it could be predicted that 1 ha of dragon fruit plantation would produce as much as 1.1 t (calculated as $22\% \times 50,000$ kg $\times 0.1$ kg) of dragon fruit peel powder. Based on this description, dragon fruit peel is available as a potential poultry feed.

According to Bakar *et al.*⁶, fresh dragon fruit peel contains 92.65% water, 0.95% protein, 0.10% lipid, 0.10% ash, 6.20% carbohydrates, 150.46 mg/100 g beta-cyanin and 10.8%

pectin. Furthermore, on a dry weight basis, red dragon fruit peel powder contains 9.47% water, 16.22% ash, 8.90% crude protein, 3.18% lipid, 24% crude fiber, 0.68% Ca, 0.84% available P and EM 2,031 Kcal g⁻¹, 8.84% lignin, 699.14 mg/100 g tannin, 108.55 mg/100 g anthocyanin, 3.033 ppm lycopene and 5.569 ppm β -carotene⁹.

Anthocyanin in dragon fruit peel plays an important role in the reduction of cholesterol. According to Lewis and Rader¹⁰ and Anggraeni¹¹, the mechanism by which anthocyanin reduces LDL cholesterol in mice and humans is by inhibiting the enzyme HMG-CoA reductase from producing mevalonate. In addition, anthocyanin increases the activity of lecithin cholesterol acyltransferase (LCAT) in the conversion of free cholesterol to hydrophobic cholesterol ester. Then, the cholesterol ester binds the lipoprotein core to produce new HDL. This reaction increases the blood plasma HDL in humans and mice.

Previous study on the utilization of dragon fruit peel as poultry feed has been reported by Wulandari¹², who showed that dragon fruit (*Hylocereus undatus*) peel could be included in layer rations at rates as high as 6% without affecting performance. Mahata *et al.*⁹ reported that as much as 15% of dragon fruit (*Hylocereus polyrhizus*) peel in broiler rations did not affect their performance and that the inclusion of dragon fruit peel at 5% of the ration is an effective level for reducing cholesterol, LDL and triglycerides in broiler blood serum.

The problem when utilizing dragon fruit (*Hylocereus polyrhizus*) peel in poultry rations is the high crude fiber content, which inhibits the absorption of nutrients in the poultry digestive tract⁹. Therefore, it is necessary to find a method for lowering the crude fiber while retaining the content and structure of anthocyanin in dragon fruit peel as feed for laying hens.

High levels of crude fiber in feed can be lowered by both physical and chemical methods. The physical method for degrading crude fiber in shrimp waste by boiling in water (temperature 100° C) and steaming in an autoclave was an effective method in degrading the β -(1,4)-glycoside bond of crude fiber fraction in shrimp waste¹³. Mahata *et al.*¹⁴ reported that the crude fiber content in a juice waste mixture decreased from 17.10-12.02% after steaming in an autoclave for 30 min. The effective chemical method for lowering the crude fiber in plants is soaking the plant in acetic acid or vinegar. This method degrades the polysaccharide bonds in the plant cell wall and reduces the crude fiber content^{15,16}.

There are no reports on the processing of dragon fruit (*Hylocereus polyrhizus*) peel by steaming (physical method) or soaking with acetic acid or vinegar (chemical method) to reduce the crude fiber content and retain the anthocyanin

content and its structure in dragon fruit peel as feed for laying hens. The purpose of this study was to evaluate the nutrient content of dragon fruit peel, particularly the crude fiber content, by using a physical method (steaming) and a chemical method (soaking in acetic acid) for use in feed for laying hens.

MATERIALS AND METHODS

Collection of dragon fruit peel: Dragon fruit peels (*Hylocereus polyrhizus*) were obtained from various juice counters and restaurants in Padang City, West Sumatra Province, Indonesia.

Experimental design: The experiment was performed in two parts. In part one, dragon fruit peel was treated by steaming at 98°C. The experiment had a completely randomized design with different steaming durations (0, 5, 10, 15 and 20 min) and each treatment was replicated 4 times. In part two, dragon fruit peel was treated by soaking in 7.5% acetic acid (pH 4). The dragon fruit peel was soaked in acetic acid for various durations (0, 2, 4, 6 and 8 h) and each treatment was repeated 4 times. Variables measured in both parts of the experiment were the dry matter, crude protein and crude fiber contents of dragon fruit peel, which were evaluated by a proximate analysis¹⁷.

Treatment of dragon fruit peel by the physical method:

Dragon fruit peel was chopped to ± 3 cm and put into a steamer with a steam temperature of 98°C for steaming durations of 0, 5, 10, 15 and 20 min. After the steaming was stopped, the dragon fruit peel was dried in an oven for 2 days, at a temperature of 60°C and then ground into powder for analysis of the nutrient content.

Treatment of dragon fruit peel by chemical method: Acetic acid solution (vinegar) was diluted with a quades to reach a concentration of 7.5 and 0.05% lime betel solution was added to adjust the pH of the acetic acid solution (vinegar) to pH 4. Then, dragon fruit peel was soaked in the acetic acid solution (pH 4) for various soaking durations (0, 2, 4, 6 and 8 h). After the soaking was finished, the dragon fruit peel was dried in an oven for 2 days, at a temperature of 60°C and then ground into powder for analysis of the nutrient content.

Statistical analysis: All data were statistically analyzed by a one-way analysis of variance (completely randomized

design). The differences between treatments were determined using Duncan's multiple range test, with a 1% significance level¹⁸.

RESULTS AND DISCUSSION

Effects of physical method and chemical method on crude fiber, dry matter and crude protein: The effects of the physical method and the chemical method on crude fiber, dry matter and crude protein are shown in Table 1-3, respectively. Processing dragon fruit peel by both physical and chemical methods highly significantly decreased the crude fiber content of dragon fruit peel (p<0.01) but did not reduce the dry matter or protein content (p>0.05).

Crude fiber parameter: The mean amounts of total crude fiber in dragon fruit peel after treatment by the physical and chemical methods are depicted in Table 1. Treating dragon

Table 1: Crude fiber content of dragon fruit peel after processing by physical and chemical methods

	Replication				
Duration	1	2	3	4	Means
Physical method (min)					
0	22.99	24.31	24.34	24.38	24.01ª
5	22.08	22.65	20.77	22.34	21.96 ^b
10	21.78	23.10	21.62	21.88	22.10 ^b
15	22.21	21.95	21.82	22.14	22.03 ^b
20	19.76	20.63	19.59	19.26	19.81°
Chemical method (h)					
0	22.99	24.31	24.34	24.38	24.01ª
2	22.65	22.65	22.68	22.71	22.67 ^b
4	19.69	20.33	20.65	20.86	20.38 ^c
6	21.21	21.27	20.75	20.50	20.93 ^c
8	21.32	20.57	20.33	21.60	20.95°
^{a-c} Values in the same co	lumn with	different	letters are	highly sig	nificantly

^{a-c}Values in the same column with different letters are highly significantly different (p<0.01)

Table 2: Dry matter content of dragon fruit peel after processing by physical and chemical methods

	Replication				
Duration	1	2	3	4	Means
Physical method (min)					
0	92.10	93.00	91.63	90.92	91.91
5	91.91	91.13	92.10	92.61	91.94
10	92.45	92.36	92.53	91.17	92.13
15	92.20	93.30	93.30	93.00	92.95
20	92.09	91.63	93.34	93.39	92.61
Chemical method (h)					
0	92.10	93.00	91.63	90.92	91.91
2	93.34	92.97	92.45	92.20	92.74
4	93.30	91.13	93.39	94.42	93.06
6	91.91	92.53	93.30	91.17	92.23
8	93.49	92.61	92.36	92.09	92.64

Table 3: Crude protein content of dragon fruit peel after processing by phy	/sical
and chemical methods	

Duration	Replica	Replication			
	1	2	3	4	Means
Physical method (min)					
0	7.16	7.48	7.32	7.98	7.49
5	6.59	5.92	8.37	9.28	7.54
10	6.95	8.04	5.57	6.52	6.77
15	6.63	7.42	7.81	6.70	7.14
20	6.49	7.92	7.77	8.43	7.65
Chemical method (h)					
0	7.16	7.48	7.32	7.98	7.49
2	7.46	7.67	7.85	7.81	7.70
4	6.95	7.13	7.69	7.31	7.20
6	7.15	8.55	7.64	8.18	7.80
8	7.79	6.96	6.46	7.65	7.22

fruit peel by steaming in boiled water at the temperature of 98°C for various durations (0, 5, 10, 15 and 20 min) highly significantly reduced the crude fiber content (p<0.01). In the steaming duration of 0 min, the crude fiber content was different (p < 0.01) from those in the treatments with 5, 10, 15, or 20 min of steaming. Further, although the crude fiber content did not differ among the steaming durations of 5, 10 and 15 min (p>0.05), it was different (p<0.01) from that in the duration of 20 min. After processing with 20 min of steaming, the crude fiber content of dragon fruit peel decreased from 24.01-19.81%. According to Marjuki¹⁹, the steaming process degrades the bonds among cellulose, hemicellulose and lignin. The steaming method for processing feed material can change the chemical structure of the cell wall, break bonds between the cell walls and degrade hemicellulose²⁰⁻²². Soaking dragon fruit peel in 7.5% acetic acid for various durations (0, 2, 4, 6 and 8 h) highly significantly reduced the crude fiber content (p<0.01). The crude fiber content after soaking for 0 h was different (p<0.01) from those after 2, 4, 6 and 8 h and the crude fiber content after soaking for 2 h was different (p<0.01) from those after 4, 6 and 8 h, however, the crude fiber content did not differ among the treatments with soaking for 4, 6 or 8 h (p>0.05). The most effective treatment for decreasing the crude fiber content in dragon fruit peel was soaking it in acetic acid for 4 h, which decreased the crude fiber from 24.01-20.38%. According to Nathan et al.23, when natural dry grass was hydrolyzed by using food vinegar for 1 day, there was increased disruption of the bond in lignocellulose, such that the coarse fiber value decreased and that, in turn, decreased the value of NDF. Furthermore, the crude fiber content of KUME grass decreased from 43-36% and the NDF content decreased from 73-68%. Here, both physical (steaming) and chemical (soaking in acetic acid) treatments could decrease the crude fiber content in dragon fruit peel.

Dry matter content: The mean values of the total dry matter in dragon fruit peel after treatments with both physical and chemical methods are shown in Table 2, which demonstrates that treating dragon fruit peel by steaming in boiled water at a temperature of 98°C and steaming durations of 0, 5, 10, 15 and 20 min did not affect the dry matter content in dragon fruit peel (p>0.05). According to Onyeike et al.²⁴, the dry matter content of African elemi was significantly increased at all levels of processing. The range was 97.0-98.5% (1.13-1.55% increase) in African walnut and 97.3-95.8 (1.75-1.54% reduction) in African elemi. In this study, steam processing maintained the dry matter content, likely because the material was not heated for too long or at too high a temperature. Similar to the study by Okibe et al.25, the steaming did not affect the moisture content or dry matter content of fluted pumpkin (Telfairia occidentalis) leaves. Furthermore, soaking dragon fruit peel in 7.5% acetic acid for durations of 0, 2, 4, 6 and 8 h did not significantly increase the dry matter content (p>0.05). According to Martinson et al.²⁶, soaking hay affects dry matter loss in immature orchard grass and alfalfa hay, depending on both the water temperature and soaking time. In this study, the soaking duration maintained the dry matter content, likely because the soaking duration was short. Similarly, in the study by Collins²⁷, dry matter loss was not significant until 12 h of soaking for all hays, except for that of immature orchard grass, which showed a significant loss of dry matter within 1 h of soaking in warm water. In this study, however, neither physical (steaming) nor chemical (soaking in acetic acid) treatments decreased the dry matter content of dragon fruit peel.

Crude protein content: The mean values of the total crude protein in dragon fruit peel after treatments with both physical and chemical methods are shown in Table 3. This demonstrates that treating dragon fruit peel by steaming in boiled water at a temperature of 98°C for durations of 0, 5, 10, 15 and 20 min did not reduce the crude protein in dragon fruit peel (p>0.05). According to Okibe et al.25, steaming reduced the crude protein of fluted pumpkin (Telfairia occidentalis) leaves from 31.74-18.20%. In this study, steam processing maintained the crude protein content, likely because the material was not heated for too long or at an overly high temperature. Similar to the study by Onyeike et al.²⁴, steaming at a temperature of 99°C and at durations of 45 and 90 min did not reduce the crude protein in African walnut. Furthermore, soaking dragon fruit peel in 7.5% acetic acid for durations of 0, 2, 4, 6 and 8 h did not significantly reduce the crude protein content (p>0.05). According to Udensi et al.²⁸, soaking for 24 h followed by boiling decreased the protein content of *Mucuna flagellipes* flour. In this study, the soaking duration did not decrease the protein content, likely because of the short soaking duration. The acetic acid did not affect the hydrolysis of the peptide bonds. In this study, neither physical (steaming) nor chemical (soaking in acetic acid) treatments decreased the crude protein content of dragon fruit peel.

CONCLUSION

Processing dragon fruit peel by a physical method (steaming for 20 min) and by a chemical method (soaking in acetic acid solution for 4 h) decreased the crude fiber content in dragon fruit peel from 24.01-19.81 and 20.39%, respectively, while maintaining the protein and dry matter contents of dragon fruit peel.

SIGNIFICANCE STATEMENT

This study revealed that steaming (physical method) and soaking with acetic acid (vinegar, the chemical method) improved the nutrient quality of dragon fruit (Hylocereus polyrhizus) peel by reducing its crude fiber content while retaining the content and structure of anthocyanin for use as feed for laying hens. This study can be beneficial by providing new information about the nutrient contents and utilization of dragon fruit peel in feed for laying hens. This study will help researchers uncover the nutrient contents of dragon fruit peel, particularly its crude fiber content, by using a physical method (steaming) and a chemical method (soaking in acetic acid) to increase its utilization as feed for laying hens, which could not previously be explored. Thus, a new approach for using physical and chemical methods to improve the nutrient quality of dragon fruit (Hylocereus polyrhizus) peel as feed for laying hens may be determined.

ACKNOWLEDGMENTS

This study was funded by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through PMDSU No: 059/SP2H1LT/DRPM/IV 2017. Authors are very grateful to the Minister of Research, Technology and Higher Education of the Republic of Indonesia and the Rector of Universitas Andalas for their support.

REFERENCES

1. Rizal, Y., M.E. Mahata, M. Andriani and G. Wu, 2010. Utilization of juice wastes as corn replacement in the broiler diet. Int. J. Poult. Sci., 9: 886-889.

- Mahata, M.E., M.J. Sasti, R.S. Aryani, Y. Rizal and G. Wu, 2013. The effect of Improved Juice Wastes Mixture (IJWM) for corn substitution on broiler's performance. Int. J. Poult. Sci., 12: 102-106.
- Mahata, M.E., J. Manik, M. Taufik, Y. Rizal and Ardi, 2016. Effect of different combinations of unboiled and boiled tomato waste in diet on performance, internal organ development and serum lipid profile of broiler chicken. Int. J. Poult. Sci., 15: 283-286.
- Mahata, M.E., Y. Rizal, Ardi, D. Hermansyah and G.A. Nurhuda, 2016. Effects of boiled tomato waste utilization in the diet on serum lipid profile and egg quality of laying-hens. Int. J. Poult. Sci., 15: 493-496.
- 5. Adrizal, Y. Heryandi and M.E. Mahata, 2017. Evaluation of pineapple [ananas comosus (l.) merr] waste fermented using different local microorganism solutions as poultry feed. Pak. J. Nutr., 16: 84-89.
- Bakar, J., C.E. Shu, S. Muhammad, S. Kharidah and D.M. Hashim, 2011. Physico-chemical characteristics of red pitaya (*Hylocereus polyrhizus*) peel. Int. Food Res. J., 18: 279-285.
- Mahlil, Y., 2015. Pengaruh penggunaan tepung kulit buah naga daging merah (*Hylocereus polirhizus*) dalam ransum terhadap profil lemak darah broiler. [The effect of dragon fruit peel powder (*Hylocereus polyrhizus*) in diet on broiler blood lipid profile]. Master's Thesis, Fakultas Peternakan, Universitas Andalas, Padang.
- 8. Wahyu, 2010. Panen dan Pascapanen Buah Naga (Dragon fruit). http://blog.umy.ac.id/renidesmayanti/ pertanian/%20panen-dan-pascapanen-buah-naga/
- 9. Mahata, M.E., Y. Mahlil, Y. Fajri, R. Aditiya and Y. Rizal, 2015. The utilization of red dargon fruit (*Hylocereus polyrhizus*) peel as broiler feed: Abstract summary. Faculty of Animal Science, University of Andalas, Padang, Indonesia.
- 10. Lewis, G.F. and D.J. Rader, 2005. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ. Res., 9: 1221-1232.
- Anggraeni, C.L., 2010. Pengaruh pemberian ekstrak etanol ketan hitam (*Oryza sativa*Linn. var. glutinosa) terhadap kadar LDL dan HDL tikus yang diberi minyak goreng bekas pakai. [The effect of black glutinous ethanol extract (*Oryza sativa* Linn. Var. Glutinosa) against LDL and HDL rats provided used cooking oil]. Master's Thesis, Universitas Jember.
- 12. Wulandari, M., 2011. Efektifitas penggunaan limbah kulit buah naga (*Hylocereus undatus*) dalam ransum sebagai alternatif suplemen alami untuk meningkatkan kualitas telur ayam komersil. [Effectiveness of using dragon fruit peel waste (*Hylocereus undatus*) in rations as alternative natural supplements to improve the quality of commercial chicken eggs]. http://mahaw08.student.ipb.ac.id/2011/12/07/

- 13. Mirzah, M., 2006. Efek pemanasan limbah udang yang direndam dalam air abu sekam terhadap kandungan nutrisi dan energi metabolis pakan. [Effect of heating of shrimps waste soaked in rice hull filtrate to nutrition and energy metabolic feed]. J. Peternakan, 3:47-54.
- 14. Mahata, M.E., Y. Rizal and G. Wu, 2012. Improving the nutrient quality of juice waste mixture by steam pressure for poultry diet. Pak. J. Nutr., 11: 172-175.
- Ibrahim, M.M., 1983. Physical, chemical, physico-chemical and biological treatments of crops residues: An overviews. Proceedings of the 1st Workshop Affair, May 18-21, 1981, Los Banos, pp: 78-80.
- Bolsen, K.K., 1993. Prinsip Dasar Silase [Basic Principal of Silage]. In: [Pioner Seeds], Bahasa, A., S. Rini and Martoyoedo (Eds.)., Teknologi Silase, Dalam, Indonesia. (In Indonesian).
- 17. AOAC., 1990. Official Methods of Analisys of AOAC International. Association of Official Agricultural Chemists, Washington, DC., USA.
- Steel, R.G.D. and J.H. Torrie, 1991. Prinsip dan Prosedur Statistika [Principal and Statistical Procedure]. Diterjemahkan oleh Bambang Sumantri, PT. Gramedia Pustaka Utama, Jakarta.
- 19. Marjuki, 2013. Metode pengolahan limbah untuk pakan ternak [Wastes processing method to animal feed]. Master's Thesis, Universitas Brawijaya, Malang.
- Hart, M.R., H.G. Walker, Jr., R.P. Graham, P.J. Hanni, A.H. Brown and G.O. Kohler, 1980. Steam treatment of crop residues for increased ruminant digestibility. I. Effects of process parameters. J. Anim. Sci., 51: 402-408.

- 21. Pearce, G.R., 1982. Plant cell wall structure and the effect of pre-treatment on digestibility of fibrous residue. Proceedings of the of the FAO/ILCA Workshop, September 21-25, 1982, Dakar, Sinegal.
- 22. Doyle, P.T., C. Devendra and B.R. Pearce, 1986. Rice Straw as a Feed for Ruminant. IDP., Canberra, pp: 54-74.
- 23. Nathan, G.F.K., D.K. Hau, J. Nulik, J.I. Manafe and D. Amalo, 1997. Sifat fisik dan komposisi kimia standing hay rumput kume yang diolah dengan cuka makanan dan urea. [Physical Properties and chemical composition standing hay kume grass processed with vinegar food and urea]. Fakultas Peternakan-Universitas Nusa Cendana, Balai Pengkajian Teknologi Pertanian. Kupang, Nusa Tenggara Timor.
- Onyeike, E.N., E.A. Anyalogbu and M.O. Monanu, 2015. Effect of heat processing on the proximate composition and energy values of African walnut (*Plukenetia conophora*) and African Elemi (*Canarium schweinfurthi*) consumed as masticatories in Nigeria. Int. J. Scient. Technol. Res., 4: 295-301.
- 25. Okibe, F.G., B. Jubril, E.D. Paul, G.A. Shallangwa and Y.A. Dallatu, 2016. Effect of cooking methods on proximate and mineral composition of fluted pumpkin (*Telfairia occidentalis*) leaves. Int. J. Biochem. Res. Rev., 9: 1-7.
- Martinson, K., H. Jung, M. Hathaway and C. Sheaffer, 2012. The effect of soaking on carbohydrate removal and dry matter loss in orchardgrass and alfalfa hays. J. Equine Vet. Sci., 32: 332-338.
- 27. Collins, J., 2015. The effect of hay type and soaking on glycemic response in horse. Master's Thesis, Middle Tennessee State University, Murfreesboro, Tennessee.
- 28. Udensi, E.A., N.U. Arisa and E. Ikpa, 2010. Effects of soaking and boiling and autoclaving on nutritional quality of *Mucuna flagellipes* (Ukpo). Afr. J. Biochem. Res., 4: 47-50.