Digestibility and Blood Metabolite Profiles of Chicken Fed Fermented Jatropha Seed Meal

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Abstract. This research had been conducted to obtain a feed formula that is capable of supporting optimal production performance both in broilers and laying hens fed on feed containing fermented jatropha seed meal and addition saccarides. Fifty four broiler and fifty four layer were used. Nested classification was the experimental design with bird kinds as group, levels of fermented *Jatropha curcas* meal (JCM-F) (9%, 12%, 15%) as sub group and levels of fructooligosaccharida/FOS (0.5%; 1%; 1.5%) as a sub sub group. There were 3 replications in each treatment. *Jatropha curcas* meal was fermented with lactid acid bacteria (*L. acidophilus* and *Bifidobacter spp*) for 12 x 24 hour in 37^{o}C. The objective of the research was to evaluate growth, feed consumption, nutrient digestibility (crude protein and gross energy), blood metabolites (glucose, cholesterol and trgliseride). The results showed that the type of chicken had significant effect on growth and protein retention. The level of *Jatropha curcas* meal had significant effect on blood serum triglyseride concentration. On the other hand, the level of FOS had no significant effect on all variables. It could be concluded that *Jatropha curcas* meal is better as broiler feed than layer feed with 0.5% FOS. However, to be used as feed of laying hens, it requires further optimalization of the fermentation process.

Keywords : *Jatropha curcas* meal (JCM), nutrient retention, blood serum metabolit.

Abstrak. Penelitian bertujuan untuk memperoleh formula pakan yang dapat mendukung performans produksi ayam broiler dan ayam petelur yang optimal melalui pemanfaatan bungkil biji jarak (JCM) fermentasi dan penambahan sakarida. Materi terdiri atas 54 ekor ayam broiler dan 27 ekor ayam petelur. Metode penelitian yang diterapkan adalah eksperimental, Pola Tersarang (*Nested Classification)*, dilanjutkan uji BNT bila jenis ayam nyata dan orthogonal polynomial bila pakan dan FOS nyata. Perlakuan terdiri dari jenis ayam sebagai grup, pakan mengandung bungkil biji jarak fermentasi ((9%, 12%, 15%) sebagai sub grup dan fructooligosaccarida/FOS (0,5%; 1% dan 1,5%) sebagai sub sub grup. Masing-masing perlakuan diulang 3 kali. Peubah yang diteliti adalah konsumsi pakan, pertumbuhan, kecernaan nutrien (protein dan energi), profil metabolit serum darah (glukosa, kolesterol dan trigliserida). Hasil penelitian menunjukkan bahwa jenis ayam berpengaruh nyata terhadap pertumbuhan dan retensi protein. Level penggunaan bungkil biji jarak fermentasi dalam jenis ayam berpengaruh sangat nyata terhadap kadar trigliserida darah. Kandungan FOS dalam pakan tidak berpengaruh terhadap semua peubah yang diamati. Kesimpulan penelitian adalah penggunaan bungkil biji jarak fermentasi memberi respon yang lebih baik bila digunakan sebagai pakan ayam broiler dengan kandungan FOS 0,5%, namun untuk digunakan sebagai pakan ayam petelur membutuhkan optimalisasi proses fermentasi lebih lanjut.

Kata kunci : bungkil biji jarak, retensi nutrien, metabolit serum darah

Introduction

Lectin are plants or animals or glycoproteins that bind non covalently to carbohydrates and show non immune but selective binding to specific carbohydrate moieties within cells and their membranes (Fernandez et al., 2000). The presence of lectin in the seed cake is an obstacle in its use as animal feed especially poultry, but not excessive amounts of lectins can be used as prebiotic agents capable of stimulating the activity of useful bacteria in the digestive tract of poultry. Previous studies showed that through lectin, *Lactobacillus sp* and *Bifidobacter sp* is able to form a defensive barrier in the digestive tract mucus intestinum monogastric livestock. Similarly, the addition of various other prebiotics such as fructo oligo saccharide is shown to inhibit the

hemagglutination activity of chicken blood cells (Widiyastuti and Prayitno, 2010), adding saccarides could inhibit hemaglutination activity (Gao dan Meng, 2004). *N-Acetylglucoamine* in the saccarides increasing lactid acid bacteria activity in the intestinum (Chaiton dan Trenev, 2001). Based on a through assessment (nutrient digestibility, blood profiles and the number of intestinal microbes), the results of previous study showed that the type of chicken did not significantly influence all response variables. Feeding experiments had real impact on crude fat digestibility, hemagglutination activity (agglutination time) but no significant effect was found on the digestibility of crude protein, energy and profile of erythrocytes and leukocytes. Descriptively, the experiment feed affects the amount of chicken intestinal microbe (Lactic acid bacteria and *E. coli*) (Widiyastuti and Prayitno, 2010).

Based on the previous study, this research focused on the evaluation of production performance during one production period of broiler and one month egg production for one month of layers. This research was conducted to obtain feed formula which is able to support the optimal performance in production of broiler and layers.

Materials and Methods

Preparation Materials. Fermentation of *Jatropha curcas* meal (JCM): 1500 g JSM, mixed with 500 ml of milk as a source of carbohydrate (made of 200 grams of milk dissolved in 1000 ml of distilled water). Then taken 150 ml plus 350 ml distilled water then put in a heat-resistant plastic bag, covered with plastic gutter measuring 5-10 cm and cotton. Sterilized by means of steam for 30 minutes. Once the temperature is about 40^{o}C, coupled with 150 ml of inoculum *Lactobacillus acidophilus* (in the same way for fermentation using *Bifidobacterium spp*), then incubated at 37°C for 12x24 hours. After the incubation is completed JCM fermentation samples taken for analysis of proximate and ready to be mixed into the feed (without drying) (with three level of FOS (Fructooligosaccarides)) was used into feed ingredient (fermented JCM using Lactobacillus sp and Bifidobacterium spp respectively combined in the feed with a ratio of 1:1).

Feed Ingredients and Diet Formulation. The experimental diets were formulated to be of iso-nitrogenous and iso-caloric value and in accordance to National Research Council (1994) The ingredient and nutrient composition are shown in Tables 1 and 2.

Experimental animals and Experimental Design. Broiler chickens and laying hens as many as 54 heads, finisher periods, and 27 heads, 25-week old respectively, were used. This research applied nested pattern experimental method (Nested Classification) 2x5x4, 3 times repetition. The group was type of chicken consisted of: B (broiler chickens) and L (laying hens), the sub-group was feed consisted of: R1 (Feed with JCM-F 9%), R2 (Feed with JCM-F 12%), R3 (Feed with JCM-F 15%). As sub-sub-group was FOS (0.5%, 1% and 1.5%). Variables studied were growth, feed consumption, retention of nutrients (protein and energy) (Sibbald method and Wolynetz), blood serum metabolite profiles (glucose, cholesterol and triglycerides). The data were analyzed using Microsoft Office Excel 2007 version, the significant treatment was followed by LSD and orthogonal polynomial.

Animals Management. Birds were kept for 50 days in the individual cage. Feed consumptions were measured during keeping period, average daily gains (growths) were measured weekly. Nutrient retentions were measured as nutrient digestibility. Feces collection was done on the last 7 days maintenance period with a total collection method. Before collection, the feces was sprayed with 0.3 N H_2SO_4 solution every 4 hours to reduce N evaporation. Faeces were weighed every day (day 1 to day 7), take 10%,

Table 1. Feed ingredient

Feed Stuff	Broiler			Layer		
	R1	R2	R3	R1	R2	R3
Yellow Corn	46	46	46	46	46	46
Rice bran	19	19	19	27	26	25
Fish Meal	13	10	13	6	4	2
SBM	9	9	9	8	8	8
JSM-F	9	12	15	9	12	15
Bone Meal	0.5	0.5	0.5	0.5	0.5	0.5
Mineral Mix	0.6	0.6	0.6	0.6	0.6	0.6
Oil	1.9	1.9	1.9	1.9	1.9	1.9
Methionine	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100

The addition of FOS is as much as 0.5%, 1% and 1.5% in each subgroup.
R1 (Feed with JCM-F 9%), R2 (Feed with JCM-F 12%), R3 (Feed with JCM-F 15%).

Table 2. Nutrien composition of feed *

Treatments	Moisture	Ash	Crude Protein	Crude Fat	Crude Fibre	BETN	GE
	%	(%DM)					Kal/g
LR1 0.5	13.66	6.61	19.99	11.77	6.50	55.13	3206.556
LR1 1.0	13.60	6.31	19.64	11.32	7.06	55.85	3135.677
LR1 1.5	13.75	6.19	19.98	11.33	6.26	56.24	3197.776
LR2 0.5	14.30	5.54	19.90	11.87	8.07	54.62	3173.241
LR2 1.0	14.49	5.75	19.35	11.81	7.96	55.13	3166.415
LR2 1.5	14.25	5.70	19.32	11.49	7.07	56.42	3166.597
LR3 0.5	15.42	4.99	18.93	11.75	8.66	55.67	3271.600
LR3 1.0	15.12	4.89	18.42	11.38	8.28	57.03	3202.091
LR3 1.5	15.05	4.88	18.30	11.18	8.46	57.18	3177.219
BR1 0.5	13.66	8.12	22.87	9.50	7.88	51.63	3136.921
BR1 1.0	13.73	8.03	22.70	9.68	7.94	51.65	3098.884
BR1 1.5	13.31	8.25	22.21	9.91	7.09	52.54	3180.941
BR2 0.5	14.61	7.37	21.18	9.83	7.89	53.10	3336.294
BR2 1.0	14.37	7.11	21.09	9.65	9.64	54.51	3231.861
BR2 1.5	14.38	7.03	21.88	9.46	9.10	53.53	3213.297
BR3 0.5	15.24	6.58	21.49	10.68	9.35	51.90	3200.511
BR3 1.0	15.16	6.19	21.53	10.54	9.42	52.32	3134.587
BR3 1.5	15.24	6.02	21.79	10.78	9.33	52.08	3146.994

Description: * Analized by Feedstuff Laboratory, Faculty of Animal Science, Jenderal Soedirman University (2010) LR1 0.5/1.0/1.5 (Layer ration with 9% JCM+ 0.5%/1%/1.5% FOS), : LR2 0.5/1.0/1.5 (Layer ration with 12% JCM+ 0.5%/1%/1.5% FOS), : LR3 0.5/1.0/1.5 (Layer ration with 15% JCM+ 0.5%/1%/1.5% FOS), BR1 0.5/1.0/1.5 (Broiler ration with 9% JCM+ 0.5%/1%/1.5% FOS), BR2 0.5/1.0/1.5 (Broiler ration with 12% JCM+ 0.5%/1%/1.5% FOS), BR3 0.5/1.0/1.5 (Broiler ration with 15% JCM+ 0.5%/1%/1.5% FOS)

then dried and composited. Feces sample were analyzed for levels of crude protein and gross energy according to the AOAC method (1984). 1.5 ml blood was collected at last keeping period through brachial vein with 3 mL syringes (content 0.1 ml heparin). The chickens were

fasted for 12 hours prior to blood sampling. Blood samples were analyzed for blood metabolite profiles by Pathology and Anatomy Laboratory of Veterinary Faculty of Gadjah Mada University.

Results and Discussion

The averages of daily feed consumption figures indicated the range of 65.950 to 93.373 grams/head (broiler) and 73.590 to 93.833 grams/head (layer). The mean of daily body weight gain ranged from 13.333 to 46.190 grams/head (broiler) and 3.097 to 7.380 g/head (layer). The results of analysis of variance showed that the type of chicken, JCM-F levels in feed and the level of FOS did not affect significantly ($P>0.05$) on feed consumption. However, the results showed a better growth rate in broiler than layer ($P<0.01$). The mean value of feed protein retention experiments ranged from 57.997 - 78.193% (broilers) and 14.753 - 39.407% (layer). Digestibility of protein in broiler and layer chickens differed very significantly ($P<0.01$). The results showed that the average energy retention ranged from 97.873 - 99.190% in broiler chickens and 97.763 - 98.793% in the layer. Results of analysis of variance showed that the retention of energy in broiler chickens and laying hens was not significantly different ($P>0.05$), as well as the JCM-F usage levels and levels of FOS in feed.

The results showed average serum glucose ranged from 112.960 - 190.737 mg/dl (broiler) and 148.143 - 165.737 mg/dl (layer). The mean serum cholesterol ranged from 162.500 - 216.667 mg/dl (in broiler) and 158.333 - 295.833 mg/dl (in layer). Average blood serum triglycerides ranged between 61.107 - 322.220 mg/dl (in broiler) and 55.553 - 261.110 mg/dl (in layer). Based on the results of statistical analysis indicated that the type of chicken, JCM-F level to the level of FOS had no significant effect ($P>0.05$) on glucose and cholesterol levels, whereas level of JCM-F in this type of chicken was very significant ($P<0.01$) on triglycerides in the blood serum level.

Rate of feed consumption showed no difference among treatment, it was indicated that no effect of JCM-F on palatability. But broiler showed better growth rate than layer, it indicated that broiler is more tolerant with JCM-F. This is in contrast with the results of the Wina et al. (2010) who reported that the palatability of feed containing JCM-F lower than the control diet. The fermentation process with lactic acid bacteria produce lactic acid which will cause a decrease in pH, so caused JCM fermented into acids. the presence of organic acids will dissolve the fat in the JCM will also reduce phorbolester. As stated by Goel et al. (2007) Phorbolester are defined as “polycyclic compound in which two hydroxy group on neighboring carbon atoms are esterified to fatty acid”. Ahmed and Salimon (2009) reported that total phorbolester could reduce after oil refining step.

In contrast with the results of the previous studies in which the digestibility of crude protein in broiler and layer was not significantly different, feed with JCM-F can be used to pullet layer with the level of protein digestibility ranged from 55.196 - 73.501%. In this study the use of JCM-F and the addition of FOS actually lowered the level of digestibility in laying hens production period. Low protein digestibility resulted in body weight gain and low egg production in laying hens. Pusztai (1991) stated that the lectin is a protein that is not easily degraded by proteolysis enzymes digestive tract. It also indicated that fermentation of JCM with lactic acid bacteria can degrade anti-nutrient but couldn’t reduce crude fiber of JCM. The high content of crude fiber in JCM-F limited digestibility of protein and other nutrients.

Table 3. Nutrient digestibility of feeding experiment

Treatments		Digestibility (%)		Consumption	Growth
		Protein	Energy	(g/d/bird)	(g/d/bird)
Broiler	R1(0.5%)	75.413[a]	99.190	89.887	40.713[a]
	R1 (1%)	69.037[a]	99.023	93.373	20.477[a]
	R1 (1.5%)	78.193[a]	98.983	91.780	20.717[a]
	R2(0.5%)	69.380[a]	98.313	84.770	29.763[a]
	R2 (1%)	57.997[a]	98.567	81.500	46.190[a]
	R2 (1.5%)	72.163[a]	98.910	80.140	22.617[a]
	R3(0.5%)	66.253[a]	99.127	88.177	41.670[a]
	R3 (1%)	67.527[a]	99.077	88.293	29.527[a]
	R3 (1.5%)	59.957[a]	97.873	65.950	13.333[a]
Layer	R1(0.5%)	32.827[b]	98.687	91.283	4.763[b]
	R1 (1%)	24.703[b]	98.723	93.833	4.763[b]
	R1 (1.5%)	36.260[b]	98.733	85.497	5.950[b]
	R2(0.5%)	34.100[b]	98.793	89.950	5.240[b]
	R2 (1%)	21.783[b]	98.247	81.913	5.713[b]
	R2 (1.5%)	18.613[b]	98.413	90.507	3.097[b]
	R3(0.5%)	15.617[b]	98.437	81.853	6.667[b]
	R3 (1%)	14.753[b]	98.240	73.590	5.717[b]
	R3 (1.5%)	39.407[b]	97.763	76.960	7.380[b]

[a, b] Values bearing different superscript on the same column differ significantly (P<0.01)

On the other hand, the use of JCM-F and the increased levels of FOS can increase the retention of energy than previous studies. Almost all the energy can be utilized by both broiler and layer chickens (P>0,05). Natalia and Priadi (2006) reported that the use of probiotic lactic acid bacteria was beneficial because of an enzyme activity that supports the absorption of essential nutrients and ions, bacteria coagregation can facilitate beneficial interactions among nutrition coagregation partner. Fermentation using lactic acid bacteria will increase the availability of energy in the diet, as stated by Jay (2000) that the group of lactic acid bacteria can ferment carbohydrates into energy and lactic acid. Then Hendalia et al. (2012) reported that Improved feed efficiency and protein efficiency balance shows that the probiotic is able to improve the absorption of nutrients, especially protein and amino acids, Xu et al. (2003), probiotics increase the activity of amylase and protease.

The Metabolite blood profiles of the chicken showed that the result was different to Chivandi et al. (2006), they reported that using detoxification jatropha in pig feed had negatif effects on packet cell volume (PCV), glucose, cholesterol, and trigliseride level of blood serum. It was indicated that through fermented with lactid acid bacteria can reduce negatif effect of nutrient inhibitor in jatropha seed cake.

Based on orthogonal polynomial test, JCM-F level influence on broilers showed a linear response with the equation Y = 33.333519 - 180.25296 X, with R^2 = 31.15%. JCM-F while the influence of the layer showed a linear response with the equation Y = 295.67963 to 10.802778 X R^2 = 87.845%. In broiler, JCM-F level in serum triglyceride levels of 31.15%. The higher JCM-F level was the higher blood serum triglycerides got. This is presumably because the higher the JCM-F in the feed, the higher was the addition of milk containing FOS also indicated by the

increasing energy in the feed. In contrast to the response of the JCM-F in layer, the higher JCM-F contribution the lower levels of triglycerides in the blood serum with a 87.845% rate determination. This is presumably because although the rate of energy retention in laying hens to broiler chickens was not different, the level of consumption and ADG in laying hens was lower. Therefore, high energy retention has not expressed optimal utilization of nutrients in the feed but it tends to be used for maintenance requirement. This is also supported by a decline in egg production during the feeding trial. addition of FOS until 1.5% in the feed showed no significant effect on all variables, it is consistant with the results of Xu et al. (2003) reported the application of FOS grading 4.0 g/kg of broiler chicken male improve growth, FCR and increase the population of bacteria Brevibacterium and Lactobacterium, pressing E. coli and increases the activity of amylase and protease. But adding higher than 8 kg/kg did not affect the growth of FCR, intestinal microflora and enzyme activity.

Table 4. Profile of chicken blood serum metabolites

Treatments	Broiler			Layer		
	Glucose	Cholesterol	Trigliserides	Glucose	Cholesterol	Trigliserides
	----------mg/dl----------					
R1(0,5%)	112.960	175.000	94.440	155.550	158.333	205.553
R1 (1%)	174.997	177.083	61.107	156.480	160.417	138.887
R1 (1,5%)	136.103	170.833	77.773	151.847	183.333	261.110
R2(0,5%)	143.510	170.833	316.660	165.737	158.333	166.663
R2 (1%)	190.737	172.917	322.220	149.997	204.167	188.883
R2 (1,5%)	135.180	162.500	272.220	148.143	245.833	122.220
R3(0,5%)	135.180	162.500	305.553	162.033	295.833	172.217
R3 (1%)	137.033	183.333	266.660	151.850	162.500	55.553
R3 (1,5%)	152.773	216.667	261.110	148.143	295.833	183.330

R1 (Feed with JCM-F 9%), R2 (Feed with JCM-F 12%), R3 (Feed with JCM-F 15%).

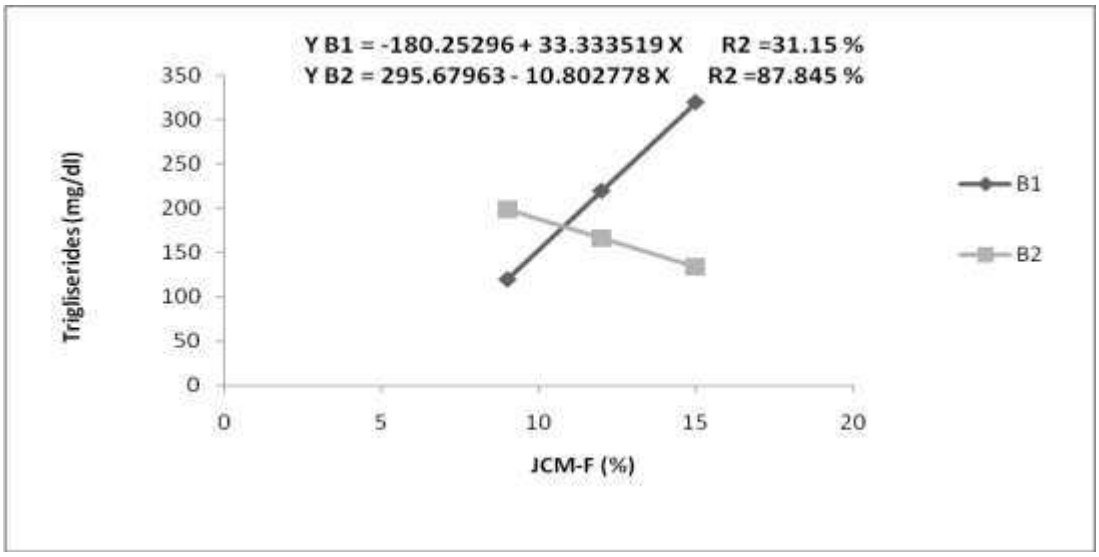

Figure 1. Concentration of triglycerides in chickens fed containing JCM-F (B1: Broiler; B2: Layer)

Conclusion

Based on the review of all variables (consumption, growth and metabolite profiles of blood serum) feed containing 15% fermented jatropha seed meal (JCM-F) and 0.5% FOS addition can be used as a constituent for broiler chicken feed but not for laying hens.

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References

Ahmed WA and J Salimon. 2009. Phorbol Ester as Toxic Constituents of Tropical *Jatropha curcas* Seed Oil. European Journal of Scientific Research. 31(3):429–436.

AOAC. 1984. Official Methods of Analysis of the Association of Official Analytical Chemis. Editor by W. Harwitz. Benjamin Franklin Station. Washington DC.

Chaiton, NDDOL and N Trenev. 2001. Probiotics. The Revolutionary 'Friendly Bacteria' Way to Vital Health and well-being. Thorsons Publishing Group. England. P: 160

Chivandi E, KH Erlwanger, SM Makuza, JS Read and JP Mtimuni. 2006. Effects of Dietary Jatropha curcas Meal on Percent Packet Cell Volume, Serum Glucose, Cholesterol and Triglyceride Concentration and Alpha-amylase Activity of Weaned Fattening Pigs. Research Journal of Animal Veteterinary Science. 1(1):18-24.

Fernandez PE, CG Barbeito, EL Portiansky and EI Gimeno. 2000. Intermediate Filament Protein Expression and Sugar Moieties in Normal Canine Placenta. Histology and Histopathology 15:1-6.

Gao, W and QX Meng. 2004. Lectin Activity and Chemical Characteristik of Escherichia coli, Lactobacillus spp dan Bifidobacterium spp from Gastrointestinal Mucosa of Growing Pigs. Asian-Australian Journal of Animal. 17(6):863–868.

Goel G, HPS Makkar, G Francis and K Becker. 2007. Phorbol Ester: Structure, Biological Activity, and Toxicity in Animals. International J. Toxicology. 26:279–288. DOI: 10.1080/10915810701464641

Hendalia, E, F Manin, Yusrizal and GM Nasution. 2012. The Application of Probiotik in Increasing Protein Efficiency and Reducng Fecal Ammonia on Broiler Chickens. Agrinak. 1(2):2012:29-35. (In Indonesia with abstract in English).

Iriyanti N, M Mufti and T Widiyastuti. 2007. Manipulasi Pakan dengan Immunostimulan Probiotik dan Prebiotik terhadap Tampilan Sistem Immunologik Berdasarkan Profil Darah dan Mikroba Saluran Pencernaan Ayam Petelur. Laporan Penelitian. Program Pascasarjana. Universitas Jenderal Soedirman. Purwokerto.

Jay, JM. 2000. Fermentation and Fermented dairy product. In: modern Food Microbiology. 6th edition. Gaithersburg: Aspen Publishers, Inc.

Natalia L and A Priadi. 2006. Sifat Lactobacilli yang Diisolasi dari Usus Ayam sebagai Probiotik. Makalah Seminar Nasional Teknologi Peternakan dan Veteriner. Balai Penelitian Veteriner. Bogor. p:801–811.

National Research Council. 1994. Nutrient Requirements of Poultry. Ninth Revised Edition, 1994. Committee on Animal Nutrition. Board on Agriculture. National Academy Press. Washington , D.C.

Pusztai A. 1991. Plant Lectins. Hangtuah Digital Library. Table 6.9:179.

Steel, RGD and JH Torrie. 1993. Prinsip dan Prosedur Statistika Suatu Pendekatan Biometrik. Edisi kedua. Translate by B. Sumantri. PT Gramedia Pustaka Utama. Jakarta.

Tranggono B and Setiaji. 1986. Kimia Lipida. PAU Pangan dan Gizi. Universitas Gadjah Mada. Yogyakarta.

Widiyastuti T and CH Prayitno. 2010. Microintestinal Environmental Manipulation Using Lectin in Jatropha seed cake as Adhesion Media of Lactic Acid Bacteria In Chicken intestine. Proceedings of the National Seminar on "Perspectives Agribusiness Development in Indonesia" in the framework of the 44th Anniversary of Animal Science Faculty of Jenderal Soedirman University. (In Indonesia with abstract in English)

Wina E, B Tangendjaja, T Pasaribu and T Purwadaria. 2010. Broiler performance were given jatropha seed cake (Jatropha curcas) detoxified by treatment Fermentation,

Physical and Chemical. JITV 15(3):174–181 (In Indonesia with abstract in English).

Xu ZR, CH Hu, MS Xia, XA Zhan and MQ Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities intestinal microflora and morphology of male broilers. Poultry Science. 82:1030–1036.