Optimization on Fermentation Process of Protein Concentrate of Jatropha Seed Cake with N Sources and Minerals Supplementation

Titin Widiyastuti and Nur Hidayat

Animal Science Faculty, Jenderal Soedirman University, Purwokerto
Corresponding author email: dyast72@yahoo.com

Abstract. The objective of this research is to produce alternative feed sources of protein by optimizing the potential of Jatropha curcas which is agroindustry waste. This study is a series of jatropha seed exploration through fermentation using Lactobacillus acidophilus through optimization of the fermentation process by supplementing N source (soybean meal and fish meal). The experiments using Completely Randomized Design (CRD) factorial pattern with the first factor was supplementation (F) and the second factor was incubation time (W), fermentation optimization consisted of: F1 (F0 + 2.5% soybean meal flour), F2 (F0 + 2.5% fish meal), F3 (F1 + 0.45% Dicalcium Phosphate) and F4 (F2 + 0.45% Dicalcium Phosphate). The incubation time differentiated W1: 3 days, W2: 5 days and W3: 7 days. It can be concluded that: DM concentration, gross energy, calcium and phosphorus are influenced by interaction between type of supplementation of source of N + DCP with fermentation time, whereas fat content is only influenced by fermentation time with optimal time decrease of fat content is 5.92 days. Total protein and amino acid levels are only influenced by different types of supplementation. Phorbolester anti-nutrition levels are influenced by the duration of the fermentation. Based on antinutritive as a limiting factor. Fermentation of protein concentrate with fish meal as N source and DCP at 5 days (F4W5) is the best treatment and can be used as a feed ingredient.

Keywords: Supplementation, N source, Mineral, Protein Concentrate, Jatropha seed cake

Introduction

Efforts to eliminate the negative effects of antinutrition of jatropha seed meal have been widely used among experts by adding sodium butyrate to feed (Arnouts and Vandendriessche, 2007), heating and chemical treatment (Aregheore et al., 2003; Herrera et al., 2005; Chivandi et al., 2006), precipitation techniques in alkaline (Makkar et al., 2008), fermented with lactic acid bacteria (Lactobacillus spp and Bifidobacter spp) and addition of FOS up to 1.5% have been done and applied to broiler and layer chicken feed (Widiyastuti et al., 2013). Widiyastuti et al. (2014) showed that the complete feeding treatment containing
fermented jatropha seed cake can be tolerated/safely used in Rex rabbit ration up to 12% percentage, but has not yet produced optimal production rate, prior research has been obtained the best quality protein concentrate of jatropha seed meal (CP-JSC) with the highest quality of antinutrition (lectin, phorbol ester, and antitrypsin), optimal nutrient content (moisture content, crude fat, crude protein, crude fiber, Ca, P, gross energy, ADF and NDF, amino acids), the optimal biological value of proteins (protein solubility in pepsin) using *Lactobacillus acidophilus* (Widiyastuti et al., 2015a). However, some nutrients such as methionine and lysine, Ca and P were decreased. Widiyastuti et al. (2015b) also reported that the protein concentrate of jatropha seed cake could be used to substitute soybean meal, but further research is needed to assess the production performance and reproduction of rabbits. This requires a comprehensive step in order to obtain a protein concentrate that has optimal nutrient quality in addition to eliminate various anti-nutrients contained so, to support maximum livestock production, through the optimization of the fermentation process by enriching the substrate with the necessary nutrients under conditions of fermentation conditions. Many factors affect the fermentation process such as nitrogen (N2), minerals, sugar, oxygen, pH, medium, CO2 and air pressure can directly utilize free N2 from air so that the requirement is given in the form of salt, Nitrogen is needed by microorganisms in fermentation process as base material for protein, nucleic acid and vitamins for growth. Microbes can utilize N source in media derived from inorganic material (urea) or organic ingredients such as fish meal and soybean meal flour. In addition to the protein requirement of some inorganic salts become essential for microorganisms ie Ca and P, Ca and P minerals are needed by microorganisms for growth, cell forming and synthesis of metabolite products. In this study, the optimization of CP-JSC fermentation process using *Lactobacillus acidophilus* was done through supplementation of nitrogen source (fish meal and soybean meal) and addition of dicalcium phosphate to obtain protein concentrate with low biological value of high anti-nutrition.

**Materials and Methods**

The research design used is Completely Randomized Design (RAL) Factorial Pattern, the first factor is fermentation optimization (F) as follows: F1: F0 + 2.5% soybean meal meal, F2: F0 + 2.5% fish meal, F3: F1 + 0.45% Dicalcium Phosphate, F4: F2 + 0.45% Dicalcium Phosphate, while the second factor is the incubation time (W) consists of W1: 3 days, W2: 5 days and W3: 7 days, Each treatment combination is repeated 3 times so that there are 36 units of treatment.

Variables observed: Fermented nutrient content (moisture content, crude fat, crude protein, Ca, P, gross energy) and amino acid content, anti-nutrition content of phorbol ester. The obtained data were analyzed using variance analysis (ANOVA), if the treatment had significant effect followed by Orthogonal Polynomial test or BNJ test to know the optimal treatment (Steel and Torrie, 1993). The obtained data were analyzed using Microsoft Excel software ver. 2007. The data was taken through the research stages as follows:

Step 1, Processing of protein concentrate jatropha seed meal. This stage is the first step to obtain the protein concentrate of jatropha seed by using precipitation method according to Makkar et al. (2008) which has been modified by Widiyastuti et al. (2015a).

Step 2, CP-JSC Fermentation. This stage consists of rejuvenating *L.acidophilus* culture, making inoculum and fermentation according to different treatment and supplementation time.

Step 3, Testing sample. Measurement of nutrient levels was performed according to AOAC method (2005), Phorbolester Analysis.
using HPLC (Munarso, 2010), Analysis of amino acids using HPLC.

**Results and Discussions**

Nutrient concentrate protein contain of jatropha seed meal which obtained supplementation of N source of soybean meal and calcium and Phosphor (DCP) mineral in fermentation is shown in Table 1. The results showed that post-fermentation CP-JSC nutrient level from lowest to highest is as follows: the range of DM is 88.10% (F2) - 86.92% (F4), the crude fiber range is 1.89% (F4) - 4.44% (F1), the crude protein content range is 49.97% (F3) - 50.34% (F1), the range of crude fat content is 16.84% (F3) - 18.11% (F2), the gross energy level range is 4007 kcal / kg (F2) - 4190 kcal / kg (F1), the calcium content is 0.46 is (F4) - 0.50% (F3), the phosphorus level is 0.37% (F4) - 0.62% (F2).

**Dry matter**

The results showed that the interaction between treatment of N and mineral supplementation with incubation or fermentation time had a significant effect on the content of CP-JSC post-fermentation (P < 0.01). The result of orthogonal polynomial test of incubation time at F1 (F0 + 2.5% soybean meal) was not significant. Incubation time at F2 (F0 + 2.5% fish meal) showed quadratic response with equation Y = 57.175 + 10.767X - 0.955X^2, with r: 0.872 and R^2: 75.98%. Max point (5.64; 87.52). The response time of fermentation in the supplementation treatment of F3 is shown by quadratic curve with the equation Y = 46.153 + 14.54 X - 1.29X^2. with r: 0.97 and R2: 94.04 and maximum point (5.63; 82.08).

Based on the result of research indicated that CP-JSC fermentation with 2.5% fish meal supplementation showed the highest DM content in fermentation 5.64 day with 87.52% DM. While fermentation with supplementation using soybean meal + 0.45% DCP shows highest level of DM reached at the time of fermentation 5.63 day with level DM 87.08%. This indicates that the maximum dry matter increases until fermentation time 5.63 - 5.64 day, then decrease of DM.

| Treatments | Dry Matter (%) | Crude Fat (%) | Crude Protein (%) | GE (kcal/kg) |
|------------|----------------|---------------|-------------------|--------------|
| F1W3       | 83.64 ± 0.974  | 5.68±0.067    | 45.01 ± 0.600    | 4209 ± 1026  |
| F1W5       | 84.98 ± 0.861  | 2.94±0.555    | 42.39 ± 6.517    | 4751 ± 254   |
| F1W7       | 84.77 ± 1.360  | 3.94±1.019    | 42.79 ± 1.524    | 4883 ± 597   |
| F2W3       | 80.88 ± 3.178  | 5.21±0.237    | 42.79 ± 1.524    | 3965 ± 583   |
| F2W5       | 87.13 ± 0.268  | 2.92±1.697    | 37.52 ± 1.876    | 7641 ± 1154  |
| F2W7       | 85.75 ± 0.246  | 2.22±1.046    | 40.16 ± 1.960    | 5869 ± 1375  |
| F3W3       | 78.15 ± 1.389  | 5.33±0.174    | 43.77 ± 0.331    | 3686 ± 312   |
| F3W5       | 86.56 ± 0.508  | 2.70±1.054    | 45.22 ± 0.782    | 4603 ± 213   |
| F3W7       | 84.65 ± 1.230  | 3.62±0.809    | 44.49 ± 0.323    | 4447 ± 288   |
| F4W3       | 86.05 ± 0.807  | 4.79±0.081    | 40.15 ± 0.368    | 3431 ± 898   |
| F4W5       | 87.23 ± 1.246  | 2.59±0.987    | 36.59 ± 4.127    | 4381 ± 498   |
| F4W7       | 85.71 ± 0.925  | 1.80±1.030    | 40.52 ± 1.335    | 3124 ± 322   |

F1 : F0 + Soybean. F2 : F0+ Fish Meal. F3 : F1 + 0.45 % Dicalcium Phosphat. F4 : F2 + 0.45% Dicalcium Phosphate

Table 1. Nutrient Average of Concentrate Protein Jatropha Seed Cake Post Fermented
The decrease in DM indicates that the production of water content is higher along with the development of lactic acid bacteria. Increasing time of fermentation will increase water production, the decrease in DM is also caused by the use of nutrients by lactic acid bacteria, the longer the fermentation time will increase the bacterial population, where the consumption of nutrients by bacteria is also higher. As stated by Zubaidah et al. (2010; 2012) that during the fermentation of lactic acid bacteria will utilize nutrients such as carbohydrates, proteins, and dietary fiber as an energy source for growth, cell formation, and biosynthesis of metabolite products. Subsequently expressed on the bran fermentation medium of the crude fiber content at the 0th hour until the 12th-hour decreases and the higher the total lactic acid bacteria the more lactic acid bacteria that utilize the fibers for cell metabolism and hydrolyzes them into simple compounds to be fermented by lactic acid bacteria through glycolysis to acid. Changes in dry matter content during fermentation with different supplementation are shown in Figure 1.

Crude Fat

The interaction between treatment of supplementation and fermentation time had no significant effect nor was the influence of type of supplementation in fermentation. Fermentation time had highly significant effect (P <0.01) on CP-JSC post-fermentation fat content. The result of orthogonal polynomial test on the fermentation time showed the quadratic response (P <0.01) with the equation \[ Y = 13.796 - 3.814X + 0.322X^2 \] with \( r: 0.7816 \) and \( R^2: 61.094\% \). The minimum point is at (5.915; 2.516). The cell membrane of microorganism contains lipid, organic, inorganic compounds and some elements such as lipid component consisting of triglyceride. The fat content of the naturally and pretreated fermented cassava peels increased with increase in fermentation time. The increase in the fat content might due to the increase in the microbial mass, activities of lipolytic microorganism to secrete extracellular enzyme (lipase), secretion of microbial oil into the fermenting medium and other products from metabolism (Oboh et al., 2002). Fermentation causes a decrease in fat content until minimum point is at 5.915; 2.516, which is due to the production of lactic acid L. acidophilus during fermentation. Hajar and Hafidi (2014) stated that the fermentable substrates (glucose, fructose, mannitol, sucrose, etc) are the main energy...
source of fermentative microorganisms, which will provide organic acids (mainly lactic acid) essential for the stability and preservation during fermentation and storage.

**Crude Protein**

The result of variance analysis showed that the interaction between treatment type of supplementation and fermentation time had no significant effect. As well as the simple effect of fermentation time. While the supplementation treatment had a highly significant effect on the crude protein content of CP-JSC post-fermentation (P <0.01). The result of BNJ test shows that F1 treatment is very different with F2 and F4 but not different with F3 (F1 and F3 are not different with F2 and F4). The result of BNJ test shows that F1 treatment is very different with F2 and F4 but not different with F3 (F1 and F3 are not significantly different with F2 and F4). As Jamila et al. (2009) that during the fermentation process it uses Lactobacillus sp will increase the number of microbes that can increase protein synthesis and produce amino acids. Jude-Ojei (2010) states that during the fermentation process there will be microbial growth used in fermentation to increase biomass on fermentation products (Itelima et al., 2013).

**Gross Energy**

The interaction between the treatment of N and mineral supplementation with fermentation time had a significant effect on Gross Energy content of post-fermented protein concentrate JSC (P <0.05). Treatment response showed that the interaction of fermentation time in supplementation of N source of soybean meal (F1) (F2) has highly significant effect with quadratic response. as shown by the equation $Y = -11765.098 + 7286.89X - 681.074X^2$. r: 0.8602 and R2: 74.0006%. Max point (5.349; 7724.62). The influence of interaction between source S source N from soybean meal + DCP with fermentation time was not significant. Meanwhile, the interaction between the length of time of fermentation with supplementation of N source of fish meal was very significant (P <0.01) with quadratic response. As shown by the equation of line $Y = -2132.2308 + 2682.2133X - 275.8992 X^2$. r: 0.7259 and R2: 52.6903%. The maximum point is at the point (4.8608; 4386.698).
Figure 3. Interaction response between fermentation treatments at different types of supplementation on CP-JSC Gross Energy post Fermentation

The effect of interaction between N source of soybean meal + DCP with fermentation time was not significant. Meanwhile, the interaction between the length of time of fermentation with supplementation of N source of fish meal was very significant (P <0.01) with quadratic response, as shown by the equation of line Y = -2132.2308 + 2682.2133X - 275.8992 X^2, r: 0.7259 and R^2: 52.6903%, the maximum point is at the point (4.8608; 4386.698). Increased of GE occurs in the treatment of F2 and F4 up to day 5. The increase of GE in F2 is higher than F4, on day 5 fermentation of the most optimal JSC protein concentrate group of lactic acid bacteria, fermented carbohydrate into energy and lactic acid (Jay, 2000).

Increased metabolic energy content due to fermentation is a reflection of the existence decomposition of crude fiber components that are difficult to digest into easily digestible components (Sukaryana, 2010).

**Mineral Content (Calcium dan Phosphor) of Protein Concentrate –JSC post-fermented**

**Calcium**

The results showed that the interaction between N and mineral source supplementation with different fermentation time had a significant effect on CP-JSC calcium post-fermentation (P <0.01). The long response time of fermentation in the supplementation treatment is as follows: (1) The effect of fermentation time on soybean meal supplementation (F1) shows linear response with the equation of line Y = 0.1542 + 0.07092 X. r:0.903 R^2: 81.595%. (2) the effect of fermentation duration on fish meal supplementation shows the quadratic response with the equation Y= - 1.3246 + 0.7962X - 0.0749 X^2. r: 0.9576 R^2: 91.7082% maximum (5.317; 0.792). (3) the influence of fermentation time on soybean meal supplementation + DCP (F3) with equation Y = - 1.1617500 + 0.7550000X - 0.0693 X^2. R: 0.9876 AND R^2: 97.5287% with maximum point (5.45; 0.896). (4) the effect of fermentation time on fish meal powder + DCP (F4) shows the quadratic response with the equation Y= -1.1350000 + 0.7667 X - 0.0750000X^2. r: 0.9769. R^2: 95.4329% maximum (5.1111; 0.824). The ash content is always a rough measure of inorganic mineral elements in a sample (Olaniyi et al., 2010). The increase in ash content could b as a result of the growth and multiplication of the microorganism in the fermentation medium (Ahaotu et al., 2013).
Table 2. Minerals Content of Concentrate Protein-JSC post-fermented

| TREATMENTS | Calcium (%) | Phosphor (%) |
|------------|-------------|--------------|
| F1W3       | 0.37 ± 0.010 | 0.54 ± 0.071 |
| F1W5       | 0.50 ± 0.094 | 0.84 ± 0.050 |
| F1W7       | 0.65 ± 0.067 | 0.63 ± 0.055 |
| F2W3       | 0.39 ± 0.010 | 0.63 ± 0.095 |
| F2W5       | 0.78 ± 0.097 | 0.59 ± 0.030 |
| F2W7       | 0.58 ± 0.033 | 0.43 ± 0.078 |
| F3W3       | 0.48 ± 0.010 | 0.41 ± 0.053 |
| F3W5       | 0.88 ± 0.023 | 0.57 ± 0.060 |
| F3W7       | 0.73 ± 0.050 | 0.72 ± 0.067 |
| F4W3       | 0.49 ± 0.010 | 0.41 ± 0.050 |
| F4W5       | 0.82 ± 0.015 | 0.58 ± 0.093 |
| F4W7       | 0.55 ± 0.064 | 0.68 ± 0.093 |

Phosphor

The results showed that the interaction between treatment of supplementation and incubation duration had significant effect (P <0.01) on CP-JSC post phosphorus content of fermentation with the following response: (1) the effect of fermentation length on the source N source soybean meal (F1) shows the quadratic response with the equation $Y = -0.85791667 + 0.658333X - 0.06375000X^2$. with r: 0.9323 and $R^2$: 86.9224%. (2) the effect of fermentation duration on fish meal supplementation (F2) shows linear response with equation $Y = 0.79694444 - 0.04927X$. r: 0.7738 and $R^2$: 59.8796%. (3) the effect of fermentation on the source of N soybean meal + DCP (F3) showed linear response with the equation $Y = 0.181111 + 0.07666667X$. with r: 0.9308 and $R^2$: 86.6385%. (4) the effect of fermentation time on fish meal supplementation + DCP (F4) shows linear response with the equation $Y = 0.22250000 + 0.06750000X$. r: 0.8499 and $R^2$: 72.2259%.

CP-JSC fermentation with supplementation of N and mineral sources can improve the quality of phosphor, both on source N from soybean meal and fish meal and DCP minerals showed significant increases in phosphorus levels. This is thought to be because of L.
Acidophilus produces phytase enzymes that can increase the availability of phosphorus. As reported by Askelson et al. (2014) that Phytate-degrading activity has been reported in *Lactobacillus* species and has been suggested to improve the nutritional quality of fermented cereal grains. Phytate degradation has been attributed to nonspecific acid phosphatases in other lactobacilli.

**Amino Acid Content**

The results showed that the interaction between the treatment of supplementation with fermentation time was not significant (P> 0.05) to the total amino acid content of CP-JSC after fermentation, also the duration of fermentation is not significant. While the treatment of supplementation had highly significant effect (P <0.01) on total amino acid levels. BNJ test results show that F1 treatment is different from all other supplementation treatments. Acknowledgment of F2 is different from F3 but not different from F4. This shows that the effect of adding soybean meal is significantly different from the addition of fish meal in fermentation. While

| TREATMENTS | Total Amino Acid (%) | Methionine (% AA) | Lysine (% AA) |
|------------|----------------------|-------------------|---------------|
| F1W3       | 25.99 ± 0.447^a      | 0.340 ± 0.010     | 1.013 ± 0.488 |
| F1W5       | 25.75 ± 0.547^a      | 0.310 ± 0.030     | 1.057 ± 0.327 |
| F1W7       | 26.19 ± 1.144^a      | 0.293 ± 0.023     | 1.270 ± 0.200 |
| F2W3       | 21.25 ± 0.528^c      | 0.280 ± 0.026     | 1.053 ± 0.025 |
| F2W5       | 21.31 ± 1.006^c      | 0.200 ± 0.114     | 0.953 ± 0.045 |
| F2W7       | 22.01 ± 0.719^c      | 0.207 ± 0.021     | 1.070 ± 0.072 |
| F3W3       | 24.62 ± 1.070^b      | 0.330 ± 0.026     | 1.230 ± 0.061 |
| F3W5       | 24.97 ± 1.227^b      | 0.257 ± 0.074     | 1.167 ± 0.060 |
| F3W7       | 23.26 ± 1.122^b      | 0.270 ± 0.052     | 1.107 ± 0.145 |
| F4W3       | 19.81 ± 1.065^c      | 0.203 ± 0.023     | 1.023 ± 0.091 |
| F4W5       | 21.05 ± 0.653^c      | 0.273 ± 0.085     | 0.913 ± 0.075 |
| F4W7       | 19.93 ± 1.771^c      | 0.190 ± 0.000     | 0.890 ± 0.149 |
the addition of dicalcium phosphate minerals in supplementation with N source of fish meal origin did not affect post-fermentation amino acid levels. D’Este et al. (2018) stated that amino acids are at present produced through three different routes, namely, extraction from protein-hydrolysates, chemical synthesis and microbial processes (enzymatic synthesis and fermentation). In selected lactic acid bacteria might be used for developing functional beverages with improved characteristics such as reduced β-lactoglobulin (BLG) content and increased branched-chain essential amino acid.

**Phorbolester Content**

The results showed the average phorbolester levels in CP-JSC post-fermentation with different types of supplementation of N sources and the source of calcium and phosphorus ranged from 0.0087g/100g (F4W5) to 0.0669g/100g (F3W3). This average indicates a very high variation and shows that the longer the fermentation time of phorbolester levels is lower. Widiyastuti and Sutardi (2016) reported phorbolester post-optimization content with 0.5% amino acid methionine + lysine supplementation with 3 days incubation time of 0.05 g / 100 g. While the results of the study with fish / DCP fish meal supplementation showed levels lower phorbolester ie 0.01 g / 100 g. Phorbolester’s decrease was 87.5% higher than Munarso (2010) who reported that phorbolester content in jatropha seed meal could be reduced by 32-38% after boiling or fermenting with A. oryzae. Meanwhile Widiyastuti et al. (2015a) reported phorbolester decrease in post-precipitation and fermented seed meal using *Lactobacillus acidophilus* (40.79916%).

The decrease in Phorbolester levels is due to the lower fat content with the longer fermentation time. Which is due to the higher production of lactic acid by *L. Acidophilus*. Makkar (2016) stated that the decrease in Phorbolester content until below 3 mg/kg is safe for animal feed.

| TREATMENTS | PE (g/100g)   |
|------------|---------------|
| F1W3       | 0.0669 + 0.0008 |
| F1W5       | 0.0510 + 0.0005 |
| F1W7       | 0.0170 + 0.0003 |
| F2W3       | 0.0445 + 0.0017 |
| F2W5       | 0.0261 + 0.0001 |
| F2W7       | 0.0257 + 0.0001 |
| F3W3       | 0.0570 + 0.0010 |
| F3W5       | 0.0260 + 0.0002 |
| F3W7       | 0.0423 + 0.0006 |
| F4W3       | 0.0559 + 0.0005 |
| F4W5       | 0.0087 + 0.0001 |
| F4W7       | 0.0171 + 0.0002 |
Conclusions

Based on the result of the research. It can be concluded that: Dry matter, gross energy, Calcium and phosphorus are influenced by interaction between type of supplementation of source of N + DCP with fermentation time. Whereas fat content is only influenced by fermentation time with optimal time decrease fat content is 5.915 days and lowest fat content. Total protein and amino acid levels are only influenced by different types of supplementation. Phorbolester anti-nutrition levels are influenced by the duration of the fermentation. Based on antinutritive as a limiting factor. Fermentation of protein concentrate with fish meal as N source and DCP at 5 days (F4W5) is the best treatment and can be used as a feed ingredient.

References

Ahaotu I, Ogueke CC, Owuamanam CI, Ahaoutu NN, Nwosu JN. 2013. Fermentation of under watered cassava pulp by linamarase producing microorganism: Effect of nutritional composition and residual cyanide. Am J Food Nutr. 3:1-8
Akintayo ET. 2004. Characteristics and composition of Parkia biglobbossa and Jatropha curcas oils and cakes. Bioreasour. Technol.. 92: 307-310.
AOAC. 2005. Official Methods of Analysis of AOAC International. 18th Edition. Published by: AOAC International. Maryland. USA.

Aregheore, EM, K Becker and HPS Makkar. 2003. Detoxification of A Toxic Variety of Jatropha curcas Using Heat and Chemical Treatments. and Preliminary Nutritional Evaluation with Rats. S.Pac.J.Nat.Sci. 21 : 50 – 56
Armouts, S. and J Vandendriessche. 2007. The Effect of lectin in Combination with Sodium Butyrate on The Performance of Broilers. INVE Technologies. Hoogveld 93. 9200 Dendermonde. Belgium.
Askelson, TE, A Campasino, JT Lee, and T T Vuong. 2014. Evaluation of Phytate Degrading Lactobacillus Culture Administration to Broiler Chickens. Appl Environ Microbiol. 80(3): 943–950. doi: 10.1128/AEM.03155-13.
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 Anim. Vet. Sci. 1 (1): 18-24.
Despal, N Sigit dan P Hasanah. 2007. Nutrient Content and In Vitro Digestibility of Detoxified Jatropha Meal (Jatropha curcas L.). Proceeding National Seminar of AlNI VI. Local Wisdom in the Supply and Development of Feed and Livestock in the Era of Globalization. 26-27 Juli 2007. Yogyakarta. (In Indonesian with abstract in English).
D’Este, M, MAI Morales and I Angelidaki. 2018. Amino acids production focusing on fermentation technologies – A review.
Biotechnology Advances. 36:14-23.
doi.org/10.1016/j.biotechadv.

Hajar, K and A Hafidi. 2014. Chemical composition changes in four green olive cultivars during spontaneous. LWT- Food Science and Technology. 57:663-670

Herera, MJ, P Siddhuraju, G Francis, GD Ortiz and K Becker. 2006. Chemical composition toxin/antimetabolic constituens. and effects of different treatments on their levels. in four provenances of Jatropha curcas L From Mexico. Food Chem. 96: 80-89.

Itelima, J, F Onwuliri, E Onwuliri, I Onyimba and S Oforji. 2013. Bio-ethanol production from banana. plantain and pineapple peels by simultaneous saccharification and fermentation process. Int. J. Environ. Sci. Dev. 4(2):213 - 216

Jamila, F, K Tangdilintin dan R Astuti. 2009. The content of crude protein and crude fiber in chicken feces fermented with Lactobacillus Sp. National Seminar of Livestock and Veterinary Technology. Center for Livestock Research and Development. Bogor.

Jay, JM. 2000. Fermentation and fermented dairy products. In: Jay JM (ed) Modern food microbiology, 6th edn. Aspen Publication, Springer US, New York, pp. 113–130. doi:10.1007/978-1-4615-4427-2_7

Jude-Ojei, B S. 2010. Effect of fermentation on the proximate composition of ripe pineapple (Ananas comosus). Thesis. Department of Microbiology. Federal University of Technology. Akure.

Makkar, HPS, G Francis and K Beckers. 2008. Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. Journal of the Science of Food and Agriculture. 88(9). pp. 1542-1548(7)

Makkar, HPS. 2016. State-of-the-art on detoxification of Jatropha curcas products aimed for use as animal and fish feed: A review. Anim. Feed Sci. and Tech. Vol. 222 p: 87 – 99

Monnet, V. 1986. Purification et caractérisation d’une protéase de paroi chez Streptoeoeus laetis. In Boyaval P. 1989. Review article - Lactic acid bacteria and metal ions. Elsevier. 69 (2) : 87-113.

Munarro, S J. 2010. Detoxification of Jatropha Seed Cake through Fermentation and Its Utilization To Suppress The Use Of Lagung And Soy> 20% In Complete Feed Formulation With Cheaper Cost 15%. Research Report. Center for Plantation Research and Development. Department of Agricultural Research and Development. Ministry of Agriculture. Jakarta. (IN Indonesian with abstract in English).

Oboh G, Akindahunsi AA., Oshodi AA. 2002. Nutrient and anti-nutrient content of Aspergillus niger fermented cassava products (flour and gari). J Food Comp Anal. 15:617-622

Olaniyi, OO, Akinyele Bj, Arotupin Dl. 2010. Purification and characterization of α-amylase from Volvariella volvacea. Nigerian Journal of Microbiology. 24:76-82

Sukaryana, Y. 2010. Increase of Metabolism Energy of Palm Kernel Cake and Rice Bran Mix Fermentation Products. Jurnal Penelitian Pertanian Terapan. Vol.10 (2): 138-143 ISSN 1410-5020

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

Widiyastuti, T, CH Prayitno and N Iriyanti. 2013. Digestibility and Blood Metabolite Profiles of Chicken Fed Fermented Jatropha Seed Meal. J. of Animal Production 15 (2): 98 - 105

Widiyastuti, T, M Indradji, A Wibowo and R Hendroko. 2014. Biorefinery of Jatropha Seed Cake by Lactid Acid Bacteria and The Effects on Hematological Profile of Rex Rabbit. Energy Procedia (Elsevier) 47(1):290-294

Widiyastuti, T, TR Sutardi and M Indradji. 2015a. Quality of Protein Concentrate from Jatropha curcas Seed Cake Produced by Chemical and Biological Processing. The 5th International Conference on Sustainable Animal Agriculture for Developing Countries October 27-30. 2015 Pattaya. Thailand www.saadc2015.com.

Widiyastuti, T, T R Sutardi and RH Setyobudi. 2015b. Evaluation of Protein Concentrate from Jatropha Seed Cake as a Soybean Meal Substitution in The Rabbit Feed. Energy Procedia. Science Direct (Elsevier) 65(2015):362-367.

Widiyastuti, T and T R Sutardi. 2016. Amino Acid And Mineral Supplementation In Fermentation Process Of Concentrate Protein Of Jatropha Seed Cake (Jatropha Curcas L.). J. of Animal Production. 18 (3): 141 -148
Zubaidah, E, E Saparianti dan J Hindrawan. 2012. Studies of antioxidant activity in rice bran and fermented probiotic skim milk (Lactobacillus plantarum B2 and Lactobacillus acidophilus). Jurnal Teknologi Pertanian 13: 111-118.

Zubaidah, E, N Aldina dan F C Nisa. 2010. Study of antioxidant activity of bran and fermented skim milk of probiotic lactic acid bacteria (Lactobacillus plantarum J2 and Lactobacillus casei). Jurnal Teknologi Pertanian 11: 11-17.