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

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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: Suplementation, N source, Mineral, Protein Concentrate, Jatropha seed cake

Abstrak. Tujuan penelitian adalah menghasilkan bahan pakan alternatif sumber protein dengan mengoptimalkan potensi bungkil biji jarak (*Jatropha curcas*) yang merupakan limbah agroindustri sebagai bahan pakan lokal. Penelitian ini merupakan bagian dari eksplorasi bungkil biji jarak, dengan mengkaji optimalisasi proses fermentasi menggunakan *Lactobacillus acidophilus* dengan suplementasi sumber N yang berasal dari tepung bungkil kedelai dan tepung ikan. Percobaan menggunakan Rancangan Acak Lengkap (RAL) pola faktorial, faktor pertama adalah suplementasi (F) dan faktor kedua adalah waktu inkubasi (W), optimasi fermentasi terdiri : F_0 (Konsentrat protein bungkil biji jarak), F_1 (F_0 + 2,5% tepung bungkil kedelai), F_2 (F_0 + 2,5% tepung ikan), F_3 (F_1 + 0,45% *Dicalsium Phosphat*) dan F_4 (F_2 + 0,45% *Dicalsium Phosphat*). Waktu inkubasi dibedakan W1: 3 hari, W2:5 hari dan W3: 7 hari. Berdasarkan hasil penelitian dapat disimpulkan bahwa:Kadar BK, gross energi, calsium dan phospor dipengaruhi oleh interaksi antara jenis sumber N + DCP dengan lama waktu fermentasi, kadar lemak hanya dipengaruhi oleh lama waktu fermentasi dengan waktu optimal penurunan kadar lemak adalah 5,92 hari dan kadar lemak terendah. Kadar protein dan asam amino total hanya dipengaruhi oleh jenis suplementasi yang berbeda. Kadar antinutrisi phorbolester dipengaruhi oleh lama waktu fermentasi. Dapat disimpulkan bahwa perlakuan Fermentasi Konsentrat protein dengan suplementasi sumber N asal tepung ikan dan mineral DCP dengan waktu fermentasi 5 hari (F4W5) merupakan perlakuan terbaik, sehingga dapat digunakan sebagai penyusun pakan.
Kata Kunci : Suplementasi sumber N, Mineral, *Concentrate Protein*, Bungkil Biji Jarak

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.acidophillus* 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 Phospor (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.

Table 1. Nutrient Average of Concentrate Protein Jatropha Seed Cake Post Fermented

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	43.83 ± 1.236	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

F_1 : F0 + Soybean. F_2 : F0+ Fish Meal. F_3 : F_1 + 0.45 % *Dicalsium Phosphat.* F_4 : F_2 + 0.45% *Dicalsium Phosphate*

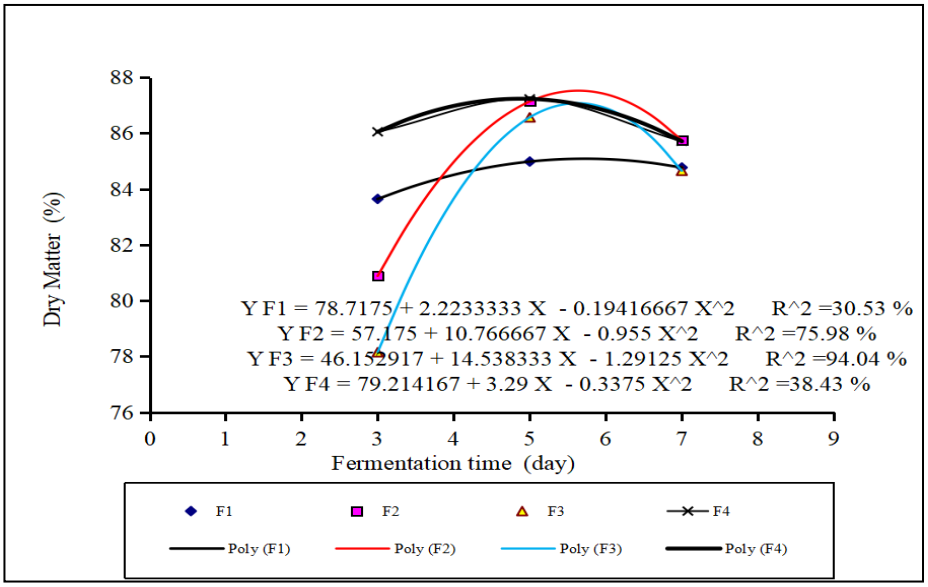

Figure 1. Effect of fermentation time on the type of N and Mineral source supplementation to PC-JSC post Fermentation

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 0^{th} hour until the 12^{th}-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 $Y = 13.796 - 3.814X + 0.322 X^2$. with r: 0.7816 and R2: 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

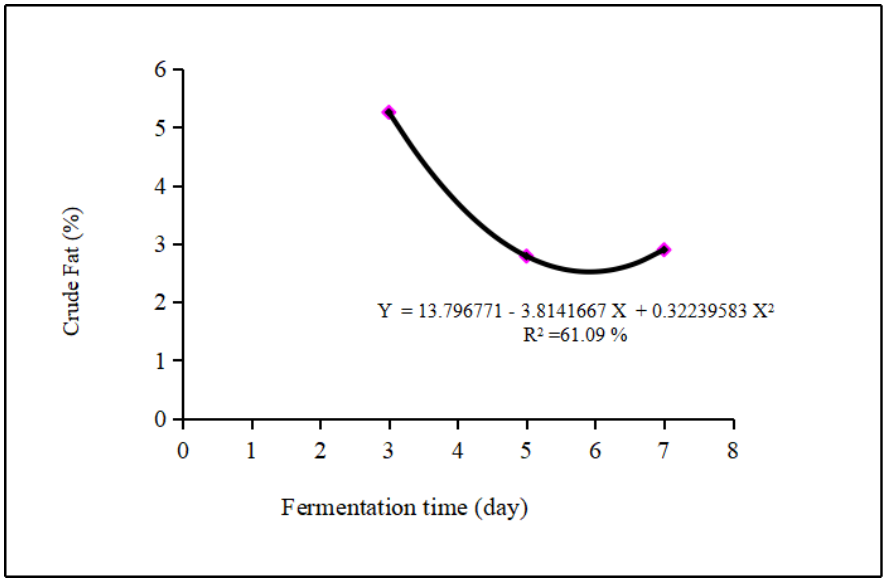

Figure 2. Response time of fermentation to CP-JSC post-Fermentation on Crude fat content

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).

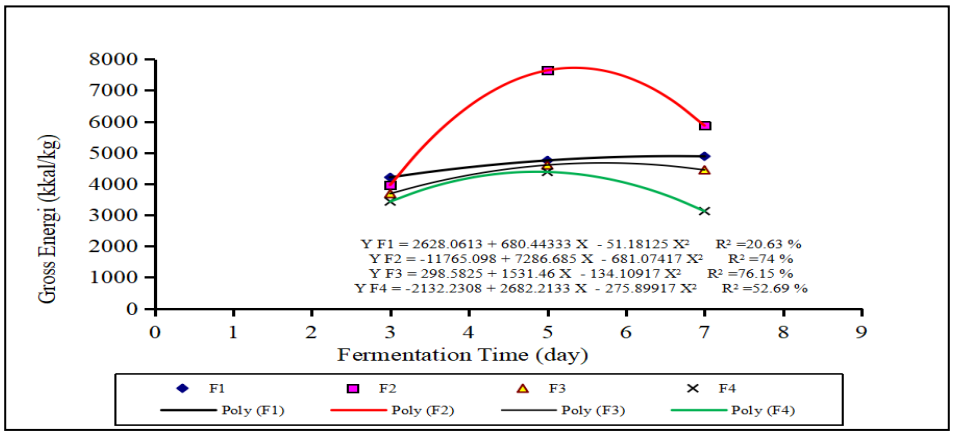

Figure 3. Interaction response between fermentation treatments at different types of supplementation on CP-JSC Gross Energy post Fermentation

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 R2: 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 R2: 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 R2: 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 R2: 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 - 0.07500000X^2. r: 0.9769. R2: 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

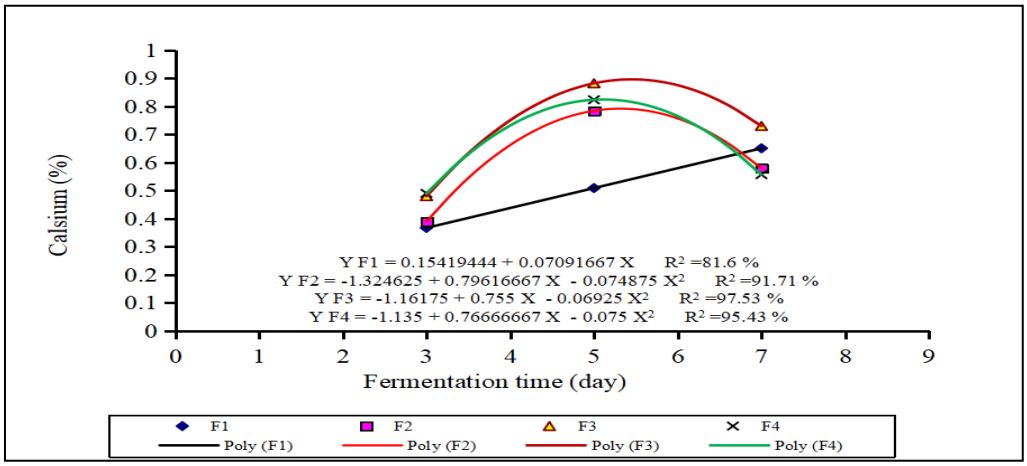

Figure 4. Interaction response between fermentation treatments at different types of supplementation on CP-JSC calcium post Fermentation

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 - 0.06375000X2. 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 R2: 86.6385%. (4)) the effect of fermentation time on fish meal supplementation + DCP (F4) shows linear response with the equation Y = 0.22250000 + 0.067500000X. r: 0.8499 and R2: 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.

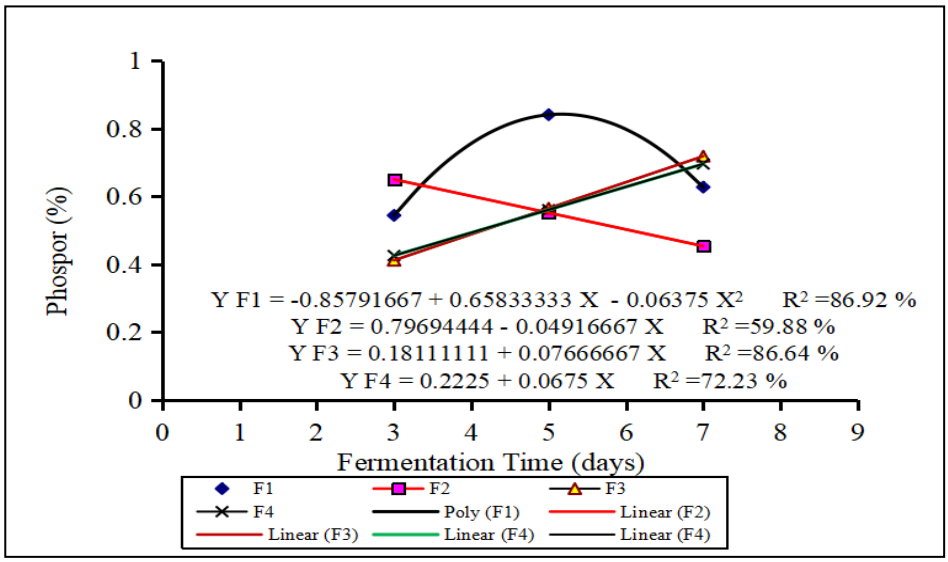

Figure 5. Interaction response between fermentation treatments at different types of supplementation on PC-JSC Phospor content post Fermentation

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

Table 3. Amino Acid of PC-JSC Post fermented

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 dicalsium 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. Acidophillus. Makkar (2016) stated that the decrease in Phorbolester content until below 3 mg/kg is safe for animal feed.

Table 4. Phorbolester Content of CP-JSC post fermented

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

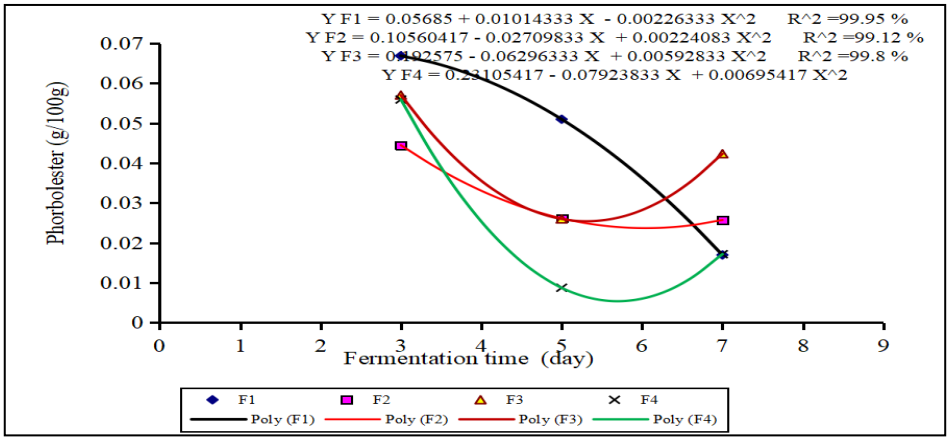

Figure 6. Interaction response between fermentation treatments on different types of supplementation to post-fermentation phorbolester level

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.

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