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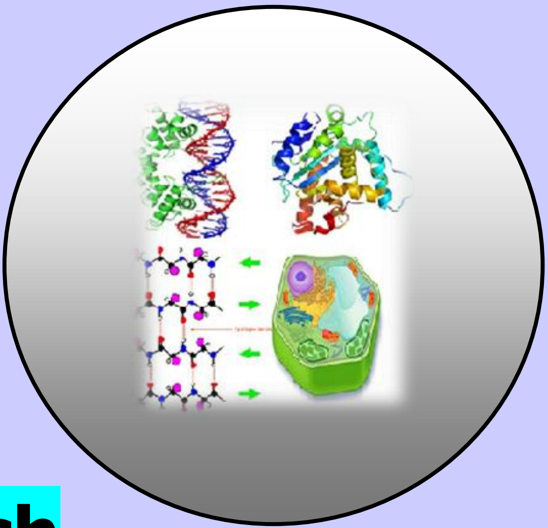

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RESEARCH PAPER

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The Effect of Fermentation Using *Cellulolytic B-6* Bacteria (Isolation from Buffalo Rumen) on Nutrition Value and Digestibility of Soybean Epidermis (Waste of Tempe) as Broiler Feed

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ABSTRACT

This research was carried out to study the increase of nutrients value and digestibility of soybean epidermis (waste of tempe) in fermenting with Cellulolytic B-6 bacteria (isolated from buffalo rumen) as broiler feeds. The research used a complete randomized design (CRD) with three treatments in six replicates. The third treatment were: soybean epidermis unfermented as control (A); Soybean seed coat with 0.20% Cellulolytic B-6 bacteria fermented (B); and soybean epidermis with 0.40% Cellulolytic B-6 bacteria fermented (C), repectively. The results of this experiment showed that soybean epidermis fermentation with 0.20-0.40% Cellulolytic B-6 bacteria were significantly different (P<0,05) increased crude protein, crude fibre, and gross energy of soybean epidermis. The soybean epidermis fermented by 0.20% to 0.40% Cellulolytic B-6 bacteria were significantly different (P<0,05) improved dry matter digestibility, organic digestibility, and protein digestibility rather than control. It can be concluded that fermentation of soybean epidermis by Cellulolytic B-6 bacteria (isolated from buffalo rumen) can improve of nutrient and digestibility of soybean epidermis (waste of tempe) as broiler feed.
Key words: Fermentation, Cellulolytic Bacteria, Digestibility and CMC-ase.

INTRODUCTION

Soybean seed coat (soybean epidermis) is an industrial waste or household waste making tempeh and often also called soybean dregs. Epidermis soybean coats produced is 20% of the soya bean seeds (Bidura, 2007). The soybean seed coat availability is quite a lot and do not compete with humans, interesting to study as broiler feed.

The main drawback of feed material use of waste (agro-industry) as poultry feed is the high crude fiber content of the material. Cellulose fraction is the component most big as a constituent of plant cell walls, which is about 40-50%, is one of the coarse fraction of the fiber plant that is very difficult/not digestible by digestive enzymes poultry. In order to use the cellulose must first be broken down into simpler compounds. The degradation involves a complex of cellulase enzymes produced by microbes (Wainwright, 2002), namely the endo-beta-glucanase and beta-glucosidase (CMC-ase). High crude fiber content of the Soybean seed coat, is limited factor in its use as poultry feed. Therefore it is necessary for the processing by fermentation. The advantage of fermentation is changing macro protein molecules into micro molecules that are easily digested by poultry, and does not produce toxic chemical compounds. It was reported, in addition to improving the protein content in rations, the fermentation process can also increase digestibility of feed from complex compounds into the compounds that are easily digested (Bidura *et al*., 2008).

BachKnudsen (2001) reported that crude fibre has been defined as the complex macromoleculer substances on food plants that are not degraded by poultry digestive enzymes. With the exception of lignin, all of the materials called CF are carbohydrates in nature. Crude fibre is thought to mediate protective effects on the colonic epithelium through their fermentation products and fecal bulking capacity (Wang *et al*., 2004). Feed high fiber resulted can a lowered rate of lipogenesis and tendency of an increased capacity to utilization of acetyl-CoA reductase enzymes (Zhu *et al*., 2003). Non starch polysaccharide (NSP) are the carbohydrate components of CF and are the predominant substrates for anaerobic fermentation.

Fermentation of fiber in feedstuffs will be able to increase the nutrient content of the feed. Fermentation can improve the digestibility of dry matter, organic matter, crude protein and crude fiber materials. According Wibawa *et al.* (2015), that the fermentation of rice bran with 0.20 to 0.40% yeast Saccharomyces sp complex can increase the nutrient content and digestibility of rice bran as feed ducks. Soybean seed coat contains antinutrition factors such as phytic acid as phytate and high content of crude fiber. Anti-nutritive factors have been reported by Kahlique *et al*. (2003), that can cause reduction of feed intake and depress performance of poultry. These anti-nutritive factors have been reported can reduce feed intake and depress performance of poultry.

The use of Soybean seed coat in poultry diets has been highly limited by the presence of phytic acid and other anti-nutritional factors as like tannin and non-starch polysaccharides (NSP). The high levels of NSP (non-starch polysaccharides) on Soybean seed coat, is restricted for using in poultry feed. These NSPs are known to increase the gut viscosity, reduce nutrient absorption on the intestine and affected indirectly the growth and performance of poultry (Rhames *et al.*, 2006; Cao *et al*., 2003). Many studies have clearly demonstrated that, supplementation of probiotics in diets rich in NSP resulted a significant reduction on the intestinal viscosity, enhances energy, and decreased protein utilization (Bidura *et al.,* 2012; Bidura *et al.,* 2009). Hong *et al*. (2004) reported that fermentation of feed using Aspergilus oryzae can increase digestibility of its dry matter (DM) and crude fiber (CP). The use of probiotics in poultry production as become interesting, because continued use of antibiotics in animal feeds may result in the presence of antibiotics residues in animal products (Han *et al*., 2001).

The intestinal tract microfloral enzymes are beneficial to the nutrition of the host because they increase the digestion of nutrients, especially in the lower intestine. Previous experiments showed that the inclusion of probiotics microorganisms in the diets improved feed conversion efficiency and digestibility (Chen *et al.,* 2005). Piao *et al.* (1999) showed that 0.10% yeast added to a diet could reduce animal wastes because probiotics microorganisms could improve posphorus utilization in growing chickens.
This research was carried out to study the increase of nutrients value and digestibility of soybean epidermis (waste of tempe) in fermenting with *Cellulolytic B-6* bacteria (isolated from buffalo rumen) as broiler feeds.

METERIAL AND METHODS

Animals and experimental design

An eighteen of broiler up to six weeks of age were assigned to three treatments in a completely randomized design. Each treatment has six replications with one bird per replication (individual cage by force feeding techniques). All of the birds were fed experimental diets for two days. The treatments were: (i) the soybean seed coat unfermented as control; (ii) soybean seed coat fermented by *Cellulolytic B-6* bacteria isolate (isolated from buffalo rumen); and (iii) soybean seed coat fermented by *Cellulolytic B-6* bacteria, respectively. This study is to determine the effect of soybean seed coat fermentation by *Cellulolytic B-6* bacteria (isolated from buffalo rumen) on nutrient value, digestibility, and metabolizable energy of soybean epidermis (waste of Tempe).

Fermented of soybean seed coat

The isolate of *Cellulolytic B-6* bacteria (isolated from buffalo rumen) which has been passed approved from bile salt test and in vitro test on poultry digestive tract. The isolate were used, both as probiotics agent and has CMC-ase activity too according to our previous study (Bidura *et al*., 2014). Fermentation of soybean epidermis (waste of Tempe) was prepared as follows: soybean epidermis was added approximately 0.40% (2×10^8 spores) *Cellulolytic B-6* bacteria into the 100 g of steamed soybean epidermis. Then, water was added until the content of water up to 35% and for 2 days for incubations. After that, soybean epidermis fermented was dried at 45^0C temperature for six hours and then soybean epidermises fermented were prepared for analysis.

Retention and excretion of nutrients

In order to determine the nutrient digestibility and metabolizable energy (ME) of the soybean seed coat/ soybean epidermis (by-product). The amount of soybean seed coat used was 50 g. This amount as based on preliminary assays with broilers aged to six week using soybean epidermis. All of the birds were deprived of feed for 24 h to ensure that their alimentary canals were empty from feed residues. They were then forced-fed with the specific amount of soybean seed coat (fermented and unfermented) by the stainless steel funnel with 40 cm stem was used in forced feeding technique (Mustafa *et al*., 2004). Water were offered ad libitum during the experimental period. The total excreta were collected in plastic trays. The excreta samples were frozen, to equilibrium with the atmospheric moisture, weighed, and grinded through a 1 mm sieve. Samples of excreta and soybean seed coat were subjected to appropriate analysis to determine dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), and energy, respectively.

Laboratory analyses

To determination of DM, OM), and CP were done according to the Assocciation of Official Analytical Chemists (l994). The content of CP samples was determined using the Kjeldahl procedure (AOAC, 1994). Crude fibres in the samples were determined using the procedure of Van Soest *et al*. (1991) on oven-dried samples and Gross energy (GE) was measured with an adiabatic oxygen bomb calorymeter (Parr, USA).

Calculations

The data were used to calculate AME value according to the following formulate (Mustafa *et al.,* 2004): AME (apparent metabolizable energy) = IE-FE. Where IE = ingested energy; FE = fecal energy voided by the fed birds.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and if it was significantly different it was further tested by Duncan's Multiple Range Test.

RESULTS

Based on the analysis of research on the dry matter content of the soybeans epidermis (soybean seed coat) without fermented as a control (A) was 88.66% (Table 1). The average of dry matter epidermis soybeans contents that fermented with 0.20% *Cellulolytic B-6* bacteria isolated from buffalo rumen (B) and 0.40% *Cellulolytic B-6* bacteria were isolated from buffalo rumen (C) were: 2.03% and 1.81% lower but not significantly different (P>0.05) than the control, respectively. The Chemical composition of soy beans epidermis (soybean seed coat) (fermented compared to the unfermented) were shown in Table 1 as below:

Table 1. Effect of soybean seed coat (soybeans epidermis/waste of tempe) fermentation with *Cellulolytic B-6* bacteria (isolated from buffalo rumen) on the content of dry matter, organic matter, crude protein, crude fiber, and gross energy.

Variable	Treatment[1)]			SEM[2)]
	A	B	C	
Dry matter (%)	86.66a[3)]	88.42a	88.23a	0.158
Organic matter (%)	91.96b	92.61a	92.73a	0.116
Crude protein (%)	19.13b	21.29a	21.24a	0.100
Crude fibre (%)	23.91a	22.51b	22.30b	0.163
Gross energy (GE kcal/kg	3142.83b	3364.00a	3301.33a	31.596

Note:

1. Soybean seed coat without fermented as a control (A), soybean seed coat fermented by *0.20% Cellulolytic B-6* bacteria (B), and fermented by 0.40% *Cellulolytic B-6* bacteria (C)
2. SEM: Standart Error of The Treatment Means
3. The different superscript at the same row is significantly different (P<0.05)

Organic matter of epidermis soybeans fermented by 0.20% *Cellulolytic B-6* bacteria (isolated from buffalo rumen) and epidermis soybeans fermented by 0.40% *Cellulolytic B-6* bacteria (C) were 0.71% and 0.84%, respectively higher significantly different (P<0.05) compared with controls (unfermented). Crude protein of epidermis soybeans fermented by 0.20% bacterial culture *cellulolytic B-6* isolates buffalo rumen (B) and epidermis soybeans fermented by 0.40% *cellulolytic B-6* bacteria isolates buffalo rumen (C) were: 11.29% and 11.03%,respectively higher significantly different (P<0.05) than the control (unfermented).

Crude fibre of epidermis soybeans fermented by 0.20% *cellulolytic B-6* bacteria (B) and 0.40% of cellulolitic B-6 bacteria (C) were: 5.86% and 6.73%, respectively lower significantly different (P< 0.05) than the control (A). Gross energy content of fermented soy bean husk by 0.20% *cellulolytic B-6* bacteria isolates buffalo rumen (B) and 0.40% *cellulolytic B-6* bacteria isolates buffalo rumen (C) were: 7.04% and 5.04%, respectively higher significantly different (P<0.05) compared than control groups (A).

The results of the study to measure the digestibility of soybeans epidermis with force feeding method showed that dry matter digestibility of soybean epidermis without fermented as a control (A) was 55.34% (Table 2). While, dry matter digestibility of soybeans fermented by 0.20% bacterial culture *cellulolitic B-6* isolates buffalo rumen (B) and epidermis soybeans fermented by 0.40% bacterial culture *cellulolitic B-6* isolates buffalo rumen (C) were: 9.63% and 9.72%, respectively higher significantly different (P<0.05) compared with controls (A).

Organic matter digestibility of epidermis soybeans fermented by 0.20% *cellulolitic B-6* bacteria (B) and 0.40% fermented by bacteria *cellulolitic B-6* (C), were: 9.98% and 10.04%, respectively higher significantly different (P<0.05) than the control group. Crude protein digestibility of soybean epidermis (soybean seed coat) fermented by 0.20% *cellulolitic B-6* bacteria (B) and 0.40% fermented by bacteria *cellulolitic B-6* (C), were: 5.89% and 6.59%, respectively higher significantly different (P<0.05) than the control group. Crude fibre digestibility of epidermis soybeans (soybean seed coat) fermented by 0.20% *cellulolitic B-6* bacteria(B) and 0.40% fermented by bacteria *cellulolitic B-6* (C), were: 16.66% and 17.41%, respectively higher significantly different (P<0.05) than the control group. The digestibility of soy beans epidermis (soybean seed coat) (fermented compared to the unfermented) were shown in Table 2 as below:

Table 2. The digestibility of dry matter, organic matter, crude protein, crude fiber, and metabolizable energy of soybean seed coat with and without fermented by *Cellulolytic B-6* bacteria (isolated from buffalo rumen).

Variable	The treatment[1)]			SEM[2)]
	A	B	C	
Dry matter digestibility (%)	55.34b[3)]	60.67a	60.72a	0.298
Organic matter digestibility (%)	57.84b	63.61a	63.65a	0.218
Crude protein digestibility (%)	62.42b	66.33a	66.80a	0.249
Crude fibre digestibility (%)	57.39b	66.95a	67.38a	0.272
Metabolizable energy (kcal/kg)	1747.16b	2001.14a	2102.13a	47.053

Note:

1. The soybean seed coat without fermented as a control (A), soybean seed coat fermented by *0.20% Cellulolytic B-6* bacteria (B), and fermented by 0.40% *Cellulolytic B-6* bacteria (C)
2. SEM: Standart Error of The Treatment Means
3. The different superscript at the same row is significantly different (P<0.05)

The results showed that the content of metabolizable energy of soybean seed coat without fermentation (A) was 1747.16 kcal/kg (Table 2). The average content of metabolizable energy from soybean seed coat fermented in treatment B and C were 14.54% and 20.32% significantly different (P<0.05), respectively higher than the metabolizable energy content of soybean seed coat without fermented (A).

DISCUSSION

Soybean epidermis (waste of tempe) fermented with microba can increase the microbial biomass, so that the crude protein content of soybean seed coat were increased. The fermentation process and those product was affected by the type and number of microbes, substrat types, pH, and temperature during the fermentation process. Fermentation feed purpose is to improve the usability of feed and elimination of anti-nutritional substances, as well as utilize biomass formed (Utama *et al*., 2006). Biomass is a form of mass from the biological processes of microorganisms. Microorganisms capable of converting material into proteins. According Jaelani *et al.* (2008), that fermented palm kernel cake can markedly increase the crude protein content of palm kernel cake compared to without fermentation. The fermentation process has the objective to produce a product (material feed) that have a nutrient content, texture, better biological availability, and reduce substance antinutrisi. Suparjo *et al*. (2003) stated that the fermentation of rice bran with 0.20% *Aspergillus niger* cultured for 72 hours can markedly increase protein and phosphorus content of rice bran, but can reduce crude fiber content and acid phytat of rice bran.

Bidura *et al.* (2012) reported that fermentation of rice bran by *Saccharomyces spp.Gb-7* and *Gb-9* may increase microbial biomass, so the crude protein content of rice bran increased. The success of the fermentation process is influenced by the type and number of microbes are used, the type of substrate, pH, and temperature during fermentation. Microorganisms capable of converting the material into microbial protein better known as single cell protein. In the same report, fermentation process has the objective to produce a product (material feed) that have nutritional content, texture, and better biological value, as well as lowering antinutrisi substances. Jaelani *et al*. (2008) reported that fermentation of feedstuffs with *Trichoderma reesei* can increase the energy content, total soluble sugars, and crude protein content. Increase the energy content of fermented coffee pulp was due to the formation of sugars derived from the breakdown of crude fiber, that caused crude fibre content in this research were decreased significantly.

According Sumarsih *et al*. (2007), the longer the storage time of feed ingredients when fermented, the more the loss of dry matter and organic matter material of feed, but increasing the crude protein content and decreased crude fiber content of feed fermented). Also reported that the longer the fermentation process or storage time, can increase of dry matter digestibility and organic matter materials.

The energy content of fermented soybean seed coat was significantly increased compared with no fermented. As reported by Jaelani *et al.* (2008), the increase in energi content of palm kernel cake (palm kernel cake/meal) as a result of fermentation by the fungus *T. reesei* of 1824.13 kcal/kg to 1930.44 kcal/kg suspected because of the degradation of mannan polysaccharides exist on palm kernel by fungus *T. reesei* into simpler forms (monosaccharides) that produces enough energy value better than in the form of polysaccharides mannan. The same thing was reported also by Sabini *et al.* (2000) which states that the fungus *T. reesei* is able to degrade a polysaccharide mannan mannotriosa, mannobiosa, and monnosa, converted to simpler form. Fermented palm kernel cake can markedly increase the crude protein content compared to palm kernel cake without fermentation (Jaelani *et al*., 2008).

The digestibility of dry matter, organic matter, crude protein, crude fibre, and metabolizable energy of soybean seed coat, were increased by fermented of *Cellulolytic-B-6* bacteria (isolated from buffalo rumen). These results indicated that carbohydrates other than fibres were used for microbial growth (*Cellulolytic B-6* bacteria) and the reduction of nitrogen free extract resulted in increased concentration of the other components. The same report, Yi *et al*. (1996) that supplementation of microbial in diets can improved N retention in broiler chickens and protein digestibility by *in vitro*. Also, Chen *et al*. (2005) reported that addition of 0.20% complex microbial (*L. acidophilus* and *S. cerivisae*) in diets can improve dry matter digestiblities of feed. Reported by Utama *et al*. (2006) and Howard *et al.* (2003), that the yeast *Saccharomyces cerevisiae* capable of producing the enzymes amylase and selulolase, so as to increase the digestibility of protein and crude fiber, such as cellulose and hemicellulose has overhauled in the form of a simple monosaccharide. Cellulolytic microbia capable of producing the enzyme 1,4 beta-endo-glukonase, 1,4 beta-exo glukonase, and beta-glucosidase that can degrade components of crude fiber into soluble carbohydrates. Chen *et al*. (2005), suggested that probiotics in the gastro intestinal tract can improve digestibility of dry matter, crude protein, and energy retention on the body of birds. These fungal are effective in degrading of the complex compounds such as beta-glucans and arabinoxylans.

The soybean seed coat fermented by *Cellulolytic B-6* bacteria isolates from buffalo rumen will be able to soften and break down the cell walls of soybean seed coat and microba capable of releasing microfibrils, so that the structure of the cell wall of feedstuff become brittle and more open. The microba work gradually in breaking the cell wall components. Through fibrils and hyphae of microba secrete extracellular peroxidase enzymes. The extracellular peroxidase enzymes actively working on lignolisis activity, thus lignocellulose and lignin fraction breaks down into CO_2. Fermentation using microbes can improve the digestibility of feed nutrients (Bidura and Suastina, 2002).

The administration of microba (*S. cerevisiae*) in the diet can increase the digestibility of protein and crude fiber components, such as cellulose and hemicellulose, overhauled in the form of simple monosaccharide (Utama, 2011). Reported by Bidura *et al.* (2014), that the pollard fermentation by *Saccharomyces spp* isolated from colon of bali cattle can markedly increase the digestibility of dry matter, organic matter, protein and crude fiber of pollard, and can significantly increase the metabolizable energy of pollard. The same thing was reported by Candrawati *et al*. (2014), that the use of *Saccharomyces spp* isolated from feces of Bali cattle in the process of fermentation of rice bran, can markedly increase the digestibility of dry matter, organic matter, and crude fiber of rice bran, and can significantly increase metabolizable energy of rice bran.

Hong *et al*. (2004) reported that feed fermented using *Aspergillus oryzae* significantly increased dry matter digestibility and crude protein. According to Jaelani *et al.* (2008), an increase in metabolizable energy content of palm kernel cake as a result of fermentation by the fungus *T. reesei*. This is due to the degradation of mannan on the polysaccharides in palm kernel cake by the fungus *T. reesei* into a simpler form (monosaccharides) which generates enough energy value better than in the form of polysaccharides mannan. The same thing was reported also by Sabini *et al.* (2000), that the fungus T.reesei capable of degrading polysaccharides mannan to form mannotriosa, mannobiosa, and monnosa.

Cho *et al.* (2007) reported that supplementation of microbe in diet could improve the bioavailability of dietary. According Wang *et al.* (2004), that the inclusion of fiber sources such as wheat bran or potato starch can reduce energy for maintenance requirement. These microba are effective in degradating the complex compounds such as beta-glucans and arabinoxylans (Dubey, 2006). Reported that dietary supplementation of complex probiotic slightly improved digestibility of nutrients. Inconsistent reports about the effect of probiotics microba may be due to several aspects, such as strains of bacteria, dose level, diet composition, feeding strategy, feed form, and interaction with other dietary feed additives (Chen *et al.,* 2005).

Many studies have clearly demonstrated that the addition of probiotics culture or enzymes in diets rich in NSP resulted in a significant reduction of intestinal viscosity enhances energy and protein utilization. Degree of microbial fermentation in the large intestine improves the bioavailability of feed (Wang *et al.,* 2004). According Hong *et al.* (2004), that fermentation of feed using *Aspergilus oryzae* can increase digestibility of its DM and CP (Jaelani *et al*., 2008; Wang *et al*., 2004; Suprapti *et al.,* 2008), increased both of metabolizable energy and crude protein contents of palm kornel meal.

CONCLUSION

It can be conclude that fermentation of soybean epidermis (waste of tempe) by 0.20-0.40% *Cellulolytic B-6 bacteria* isolates from buffalo rumen, might improve quality of feed and increase metabolizable energy of soybean epidermis (soybean seed coat) as feed.

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