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Calcium and phosphorus excretion and retention in diets of laying hens fed soybean waste fermented by *Trichoderma viride*

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Abstract

A 19-day experiment was conducted to determine the effect of soybean waste (SBW) fermented at several duration time by *Trichoderma viride* in diets on calcium (Ca) and phosphorus (P) excretion and retention of laying hens. The experiment was designed to completely randomized design (CRD) with 5 treatments and 4 replications. Dietary treatments were SBW unincubated-based diet, fermented SBW 3 days, 5 days, 7 days and 9 days-based diet, as SBW-0, SBW-3, SBW-5, SBW-7 and SBW-9, respectively. The method of total excreta collection was applied to determine Ca and P excretion and retention.

The statistical evaluation of the chicks' dietary response indicated no difference in Ca intake, in Ca excretion and in P excretion of the birds fed SBW fermented *T. viride* 3-9 days in diets compared to fermented SBW in the diet. There were differences in Ca retention, in P intake and in P retention. It could be concluded that SBW fermented *T. viride* 9 days was the best. Feed produced by microbial fermentation of SBW could be successfully used as poultry feed.

Key words: *by-product, digestibility, fungi, poultry*

Introduction

Tempe (or tempeh) is a solid fermented soybean product that is consumed widely in Indonesia, and in recent years, there has been considerable interest in the West, especially the USA. In the processing of tempeh, the seed coat or hull of soybean will be discarded after soaking and dehulling of tempeh processing as industrial waste (Nelwida 2011). Iriyani (2001) reported that soybean hulls contained 18.0% crude protein, 5.5% crude fat, 24.8% crude fibre and 2989 Kcal/kg metabolizable energy, and were considered to be exellent source of calcium (Ca) and phosphorus (P). And, although the Ca and P levels are variable, most products contain between 7 to 10% Ca and 3.5 to 5.0% P.

Soybean waste (SBW) can be an alternative feed ingredient for poultry, because it was available and do not compete with human needs. However, feed ingredients derived from agro waste are usually very limited use for poultry diet, because these materials generally contain high crude fiber. According to Ward and Reichert (1986) the dietary fiber in canola and soybean seeds are in the outter hull and the cell wall material. These fibers may affect nutrients and especially mineral digestion in animals (Van der Aar et al 1983). The absorption of certain dietary minerals depends on their bioavailability from digestible plants, interaction of the minerals with other dietary nutrients and physiological state of the animal. Higher fiber in feed ingredients has been shown to have negative effects on digestion and absorption of nutrients in chickens (Krogdahl 1986). Therefore, the necessary efforts to overcome the weaknesses of the materials through bioconversions process which can be made with fermentation technology, that is one alternative and inexpensive method to improve the nutritional value of a waste. Fermentation with the fungus *Trichoderma viride* has been used in various substrates, especially the high crude fiber such as SBW. The fungus *Trichoderma viride* have potency to produce cellulase in relatively large quantities in order to degrade cellulose (Sukaryana et al 2010), and also have potency to produce pentonase and xylanase (Hardjo et al 1984).

The digestion of Ca and P in poultry have been generally measured over the total digestive tract (Common et al 1948) and the inter-relationship between dietary Ca and P concentrations in poultry nutrition and metabolism has been recognized for many years. The importance of the identification of the Ca bioavailability in laying hens has been of great interest to researchers for a long time (De Witt et al 2008). Calcium is an important feedstuff for shell strength (William et al 2006), and is the main mineral component of the egg shells which responsible of the internal egg quality (Roudybush and Grau 1987). Roland et al (1996) reported that Ca deficiency lead to decreased egg production, feed consumption and bone density and strength. While excess calcium significantly reduced egg production, and feed consumption (Harms and Waldroup 1971), and high dietary Ca have negative effect on P absorption in different species (Liu et al 2000).

Reduction of P excretion into environment has become one of the most important tasks in modern poultry production. Poultry diets are usually based on cereals in which the mineral P is bound in the form of phytate, and also phytate can makes complexes with mineral Ca (Pintar et al 2005). Utilization of plant P by chickens is poor because a significant proportion of the total P in the vegetable feed ingredients is bound to phytate (Eeckhout and Paepe 1994). Several studies have shown that dietary P concentration is an important factor that affects apparent P digestibility by poultry (Qian et al 1996; Rodehutscord and Dieckmann 2005).

A high ratio of dietary Ca to P reduces the digestibility and absorption of Ca and P due to increased precipitation of Ca-P complexes (Plumstead et al 2008; Selle et al 2009). However, because Ca is the mineral added in highest concentrations in poultry diets, it has a greater impact in forming mineral-phytate chelates than other dietary minerals, making both Ca and P unavailable for absorption (Tamim et al 2004).

The research was conducted to investigate the chemical composition and mineral Ca and P retention of broiler chickens fed diets containing varying duration time of fermentation of soybean waste.

Materials and methods

Collecting and processing of soybean waste

The tempeh was prepared according to traditional methods such as soaking, boiling/heating, drying and fermentation (Hedger 1982; Wei 1991; Egounlety and Aworh 2003). Soaking is necessary to remove inhibitors from the soybeans and to loosen the hulls, and dehulling is necessary for successful fermentation (Hedger 1982). Clean soybean grains were dehulled, and the separated cotyledons were cooked in boiling water for 10 min. After the phase of soaking and dehulling soybean waste were collected and processing for treatments.

Soybean wastes were dried and ground and then covered with alumunium foil. The substrates then were autoclaved at 121 ^{0}C for 15 minutes. The autoclaved samples were allowed to cool to ambient temperature before inoculation. All experiments were performed in triplicates.

Inoculum was made by the suspension of filamentous fungi *Trichoderma viride* (contain of *T. viride* and destilation water in 1:9 ratio) and nutrients (31.25 g (NH4)2PO4; 6.25 g NaH2PO4; 0.3 g KCl; 2.08 g MgSO4; 0.31 g FeSO4 and 0.35 g urea in 1 L destilation water) in 1:4 ratio. Soybean waste meal then was inoculated with inoculum of *T. viride* on 5L per 50 kg SBW for 3 days, 5 days, 7 days and 9 days of incubation. Then, the fermented soybean waste was dried and ground for treatments.

Diets

The experiment was designed to completely randomized design (CRD) with 5 treatments and 4 replications. Dietary treatments were unfermented SBW-based diet, fermented SBW 3 days, 5 days, 7 days and 9 days-based diet, as SBW-0, SBW-1, SBW-2, SBW-3 and SBW-4, respectively. The feed ingredients and composition of based diet were shown in Table 2.

Chicks were raised in 20 metabolic batteries constitutes of cages, which were equipped with feeder and drinker. Feed in collection period was made 80% restriction and water was made available for *ad libitum* consumption. The Chemical Composition of SBW is shown in Table 1 and compositions and calculated analysis of diets are shown in Table 2.

Table 1. The chemical composition of soybean waste

Nutrients	SBW-0 (unfermented)	Fermented SBW		
		SBW-3	SBW-5	SBW-7
Crude Protein	16.6	18.0	18.4	22.2
Crude Fiber	43.9	42.5	41.1	38.1
Ash	0.83	3.87	4.00	4.76
Ca	0.66	0.66	0.66	0.78

SBW = soybean waste

Table 2. Composition and Calculated Analysis of the Diets

Ingredients	Diets (%)				
	SBW-0	SBW-1	SBW-2	SBW-3	SBW-4
Soybean Waste	15	15	15	15	15
Yellow Corn	54	54	54	54	54
Rice bran	5	5	5	5	5
Coconut Cake	4.5	4.5	4.5	4.5	4.5
Soybean Cake	5	5	5	5	5
Fish Meal	10	10	10	10	10
Bone Meal	2	2	2	2	2
Shell-fish Meal	4	4	4	4	4
Premix A	0.5	0.5	0.5	0.5	0.5
Soybean Waste	15	15	15	15	15
Calculated analysis					
Crude Protein	19.6	19.8	19.9	20.4	20.9
Crude Fiber	9.34	9.12	8.93	8.47	8.03
Ca	2.69	2.69	2.69	2.71	2.73
P	0.84	0.84	0.85	0.86	1.28

Retention and Excretion

The *in vivo* digestibility trial was conducted by using 7 months old Strain Brown laying hens. Twenty layers having similar body weight (1700 – 1725 g) were obtained from a commercial layer farm and were reared in individual metabolic cages.

The experiment was conducted using total collection method, period lasted 19 days: a 12-day precollection period and a 7-day collection period, and during experiment birds were fed same amount of feed. The collected excreta were sprayed by 2% boric acid solution to prevent any loss in ammonia. The excreta of all experimental units were collected daily on trays covered with plastic and then dried in an oven at 60 ^{0}C for 24 hours.

At the end of the experimental period, the twenty samples of excreta were weighed and ground prior to analysis for calcium and phosphorus as described by AOAC (1990).

Retention was calculated as follows:

% Retention = (consumed – excreted) x 100/consumed (Pintar et al 2005)

Statistical Analysis

The data were subjected to analysis of variance technique using Completely Randomized Design (CRD) whereas, Duncan's multiple range test (Snedecor and Cochran, 1967) was used for comparison of the treatment means. Software package Genstat 12.2 was used for statistical calculation.

Results and discussion

Data on calcium and phosphorus excretion and retention of laying hens affected by soybean waste (SBW) in diet is shown in Table 3.

Table 3. Effect of soybean waste fermented in diet on Ca and P excretion and retention of laying hens

Variables	Duration time of fermentation P				
	0-d	3-d	5-d	7-d	9-d
Ca Intake	1.91a	1.78a	1.90a	1.84a	2.14a
Ca Excretion	0.52a	0.37a	0.43a	0.38a	0.27a
Ca Retention	1.40a	1.41a	1.48a	1.74ab	1.86b
Ca Retention (%)	73.0a	79.4b	77.5b	79.2b	87.1c
P Intake	0.60a	0.55a	0.60a	0.66a	0.92b
P Excretion	0.24a	0.21a	0.22a	0.18a	0.21a
P Retention	0.36a	0.36a	0.38a	0.48b	0.71c
P Retention (%)	60.3a	62.0a	62.8a	72.1b	76.9c
Ca Intake	1.91a	1.78a	1.90a	1.84a	2.14a
Ca Excretion	0.52a	0.37a	0.43a	0.38a	0.27a
Ca Retention	1.40a	1.41a	1.48a	1.74ab	1.86b
Ca Retention (%)	73.0a	79.4b	77.5b	79.2b	87.1c
P Intake	0.60a	0.55a	0.60a	0.66a	0.92b
P Excretion	0.24a	0.21a	0.22a	0.18a	0.21a

abc Means in the same row without common letter are different at P<0.05)

There were no difference among treatments in Ca intake and Ca excretion, however, the diets containing fermented SBW 7 and 9 days showed increased Ca retention when compared to those fed unfermented (SBW-0) and fermented 3 and 5 days diets. The diets containing SBW-9 were increased in mineral P intake when compared to those fed SBW-0 and fermented SBW-3, SBW-5 and SBW-7 diets. And the diets containing fermented SBW-7 and SBW-9 were increased in mineral P retention when compared to those fed SBW-0 and fermented SBW 3 and 5 days diets. However, there was no difference among treatments in P excretion.

The lowest retention of these minerals was exhibited by the birds fed diet containing SBW-0 followed by the diets containing fermented SBW 3 and 5 days. It means that the lower the fiber the higher the retention of Ca and P. The result of the study revealed that the digestibility of minerals Ca and P were affected in the birds fed different term of fermentation of SBW. There was a corresponding increase in the mean digestibility values with increase the term of fermentation of SBW in the diets.

Cellulose in fiber produced the bulkiest fecal material and had the fastest transit rate. This may indicate that cellulose affects absorption of minerals mainly through increased bulkiness and decreased transit time will decrease the frequency of mucosal-mineral interactions necessary for absorption. Harmuth-Hoene and Schelenz (1980) reported that the inclusion of various fibers into the rat diets produced lower apparent availability of copper, iron, zinc, magnesium, calcium and phosphorus. These decreases were depending on the type of fiber added to the diet and the particular mineral measured, that were intestinal transit time was shortened and fecal bulk was greater in comparison to the control diet, chelation of the minerals to the fiber matrix. Minerals are found in highest concentrations in the germ and outer layers of the grains. With fermentation, the dietary fibre matrix is often altered or destroyed and more minerals would remove (Harland, 1989), so it successfully yielded Ca and P and it is easily digestible.

Conclusion

- From the analysis of Ca and P retention in soybean waste it is concluded that soybean waste fermented *T. viride* has a potential in laying hens production. Because, when used as a feed ingredient, it successfully yielded Ca and P and it is easily digestible.

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