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Feeding selenium-rich fermented palm kernel cake to laying hens produces selenium-rich eggs, increases egg production and quality

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ABSTRACT

A study was conducted to determine the effect of selenium (sodium selenite) added to palm kernel cake (PKC) before fermentation on production, selenium content, and quality of eggs. The PKC was added with 0.1% sodium selenite and 1% baking yeast (Fermipan®). The mixture was added with sterile distilled water to increase the water content of the substrate. The substrates were fermented at room temperature for 5 days. The fermented products of selenium-rich fermented palm kernel cake (SRFPKC) were dried and fed to the laying hens. The experimental diets used were T-0: without SRFPKC, T-1: 0.25% SRFPKC, T-2: 0.50% SRFPKC, T-3: 0.75% SRFPKC and T-4: 1.0 % SRFPKC. The diets were given to 180 laying hens aged 22 weeks for 14 weeks. Data on egg production, feed intake and feed conversion ratio were recorded. Feed digestibilities of dry matter and protein were measured based on the method of total fecal collection. Egg qualities and egg weight loss were done using eggs stored for 1 and 30 days. Selenium and cholesterol contents of eggs were also measured. This study used a completely randomized design and was analyzed by analysis of variance. The results showed that the addition of SRFPKC increased egg production, hen day, egg weight, egg selenium, feed digestibility, improve FCR ($P<0.05$) and inhibit the process of decreasing the quality and weight of eggs stored for 30 days ($P<0.05$). In conclusion, the addition of SRFPKC increased egg production, feed digestibility, and egg selenium, improve FCR and slow down the deterioration of egg quality stored for 30 days.

Keywords: Egg production, Fermentation, Palm kernel cake, Selenium, Yeast

INTRODUCTION

Covid 19 has attacked more than 200 countries with more than 44 million deaths (WHO, 2020). The world health organization (WHO) stated that the SARS Cov-2 virus will remain for a long time, therefore a healthy lifestyle, both in terms of avoiding contact with viruses and consuming functional foods, should be sought to prevent the disease. One of the functional nutrients that can play a role in the formation of the body's immune system is the mineral selenium (Se). This trace mineral has various health functions, including preventing infection and disease caused by viruses (Beck *et al.,* 2003), as an antioxidant in the form of Glutathione peroxidase

(GSH-Px) (Cai *et al.,* 2019), can prevent cancer (Chen *et al.,* 2013), prevent heart disease (Flores -Mateo *et al.,* 2006), increase immunity (Huang *et al.,* 2012), and increase sperm fertility (Ahsan *et al.,* 2014). This mineral can also inhibit viral mutations causing viruses to become more virulent (Beck *et al.,* 2003). The emergence of several relatively virulent variants of the Covid 19 virus indicates that the SARS Cov-2 virus continues to mutate and the prevention process can be carried out by giving Se. Zhang *et al.* (2020) reported that there was a correlation between the healing of Covid-19 disease and the Se content of hair in the cases in China.

The efficacy of Se in promoting growth, production, and modulating the immune system of poultry has been well accepted. An increased egg production (Liu *et al.,* 2020), maintained egg quality during storage (Hatta *et al.,* 2020) and increased sperm fertility (Ahsan *et al.,* 2014) are some positive effects of Se for laying hens, along with its beneficial property in improving the health status of poultry (Surai, 2006). Therefore, feeding the selenium-rich feed to the laying hens can not only increase the production, egg quality, and health of chickens but at the same time can also produce selenium-rich eggs that are beneficial to humans, especially in the current Covid-19 pandemic era.

Studies on the production of selenium-rich coconut meal for broiler chicken through the bioconversion process of inorganic Se into organic have been done by a number of workers (Sundu *et al.,* 2019; Mozin *et al.,* 2019). However, data on the use of baking yeast *Saccharomyces cerevisiae* to bio-convert toxic sodium selenite into organic Se by using palm kernel cake as the solid media and its effect on egg production and the quality of 30 days of storage have not been available. A study was carried out to determine the effect of feeding selenium-rich fermented palm kernel cake on feed intake, FCR, feed digestibility, egg production, egg quality, selenium-rich egg, and egg cholesterol.

MATERIALS AND METHODS

Preparation of Substrate and Fermentation Process

Palm kernel cake (PKC) was used as a substrate and baking yeast *Saccharomyces cerevisiae* (Fermipan®) as a starter or equivalent to

Table 1. Basal diet

Feed ingredients	Concentration (%)
Soybean meal	16.2
Corn	52.0
Fish meal	8.0
Rice bran	11.0
Palm kernel cake	10.0
Calcium Carbonate	3.0
Dicalcium phosphate	1.0
Table salt	0.3
Premixes	0.3
Methionine	0.1
Lysine	0.1
Calculated nutrients	
Crude protein (%)	17.9
Metabolizable energy (kcal/kg)	2741
Calcium (%)	2.00
Phosphorus (%)	0.62
Selenium (%)	0.21
Methionine (%)	0.47
Lysine (%)	1.07

346 CFU/g. Palm kernel cake was finely ground. The fermentation method on solid media (Sundu *et al.,* 2019) was used in this study. The fine ground substrate was autoclaved for 20 minutes at a pressure of 20 psi and then cooled to room temperature. The substrate was mixed with 0.1% sodium selenite and then incubated with 1% baking yeast (Fermipan®) and added with sterile distilled water to produce 80% moisture content. The mixture was placed into a plastic that has been perforated with a needle. Substrates were stored for 5 days at room temperature. On day 5, the substrate was harvested and dried in an oven at 60^{o}C for 24 hours. The fermentation products were analyzed for proximate fractions (AOAC, 1990), selenium content (Almeida *et al.,* 2015), and cholesterol using the Liebermann Burchard method (Kleiner and Dotti, 1962).

Animals and Cages

The protocol of the study has been approved by the animal ethics committee of the Faculty of Animal Husbandry and Fisheries, Tadulako University with the approval number: 35/AEC/5/2021. The study used a total of three hundred 20-weeks-old laying hens. The laying hens were kept in the individual battery pens for 14 weeks. Prior to the data collection, vaccination against New Castle Disease was done a week after the arrival of the hens and was repeated after three months. Two weeks of adaptation period was carried out to accustom the hens to the pen and to select the hens based on two weeks of egg production was done. A total of 180 selected hens were used for research. The selection was based on the initial egg production. The feeder was located outside of the front pen. Nipple-shaped drinker was placed above the pen. The pen and the surroundings of the experimental house were cleaned and disinfected whenever necessary.

Experimental Diets

An experimental basal diet (Table 1) was formulated using the UFFF software (Pesti *et al.,* 1986). All the feed ingredients were locally purchased at a poultry shop. Diets mixture was done every two weeks to maintain the freshness of the diet. The treatment diets were arranged according to the increasing levels of Se in the diets by supplementing Se-rich fermented palm kernel cake (SRFPKC). All the treatments are presented in Table 2.

Parameters and Data Analysis

The productive performance parameters measured were: egg production (egg weight, total production, and hen day production), feed intake, feed conversion ratio (FCR), digestibilities of dry matter, and protein, excreta dry matter, egg yolk index, Haugh unit (HU), percentages of yolk, albumen and shell, the thickness of the shell, yolk color, and egg weight loss after storage of 1 and 30 days. Selenium content was analyzed by using the procedure of Almeida *et al.* (2015) and Liebermann and Burchard method was used to measure the cholesterol content of the meat (Kleiner and Dotti, 1962). The HU, the egg yolk index and egg weight loss are measured based on the following formula:

$$\text{Egg weight loss (\%)} = \frac{\text{egg weight on collection day} - \text{egg weight on measurement day}}{\text{egg weight on collection day}} \text{ X } 100$$

Table 2. Experimental diets

Treatments	Selenium (ppm)	Replication	Hens
Basal	0.21	6	6
Basal + 0.25% SRFPKC	0.87	6	6
Basal + 0.50% SRFPKC	1.53	6	6
Basal + 0.75% SRFPKC	2.20	6	6
Basal + 1.00% SRFPKC	2.86	6	6

SRFPKC: selenium-rich fermented PKC containing 265 ppm of selenium and untreated PKC contains 0.186 ppm selenium

HU= 100*Log (h+7.57) – (1.7 * W 0.37)
Where h: albumen height and W: egg wight
Egg yolk index = Yolk height / yolk diameter

This study used a completely randomized design with 5 treatments and 6 replications (Steel and Torrie, 1980). Data were analyzed by the variance analysis. Any differences detected by the analysis of variance were further tested by the Tukey test.

RESULTS AND DISCUSSION

The effect of treatment on egg production, feed consumption, FCR, dry matter (DM) of excreta, DM digestibility, protein digestibility, egg selenium, and egg cholesterol can be seen in Table 3. Effect of treatment on egg shape index, egg weight loss, percentage of yolk, albumen and eggshell, albumen and yolk profiles, and shell thickness of eggs stored for 1 day and 30 days at room temperature are shown in Tables 4 and 5.

The treatment had a significant effect ($P<0.05$) on egg production, hen day production, egg weight, FCR, dry matter digestibility, and egg selenium, but the treatment did not have a significant effect ($P>0.05$) on feed consumption, excreta dry matter and egg cholesterol content (Table 3). The effect of treatment on eggs stored for 1 day on all egg quality parameters had no significant effect ($P>0.05$). Storage of eggs for 30 days reduces egg quality, but eggs produced from hens fed a diet containing Se-rich fermented PKC (SRFPKC) can slow down the process of decreasing egg quality (albumen height, Haugh unit, yolk height, and yolk index), and decreased egg weight loss during storage ($P<0.05$). Egg index, percentage of yolk, albumen and eggshell, shell thickness, and yolk color were not affected ($P>0.05$) by the addition of SRFPKC.

Sodium selenite contains 45.7% selenium, adding 0.1% of this compound to the palm kernel cake (PKC) before fermentation, mathematically, can increase the Se concentration of the PKC to 457 ppm. The fermentation process of PKC for 5 days with *S. cerevisiae* can only produce 265 ppm selenium. This means that 58% of the Se added to the PKC before fermentation remained in the PKC or may have turned into yeast Se, while 42% of the Se was lost during the fermentation process. This loss may indicate that yeast metabolizes inorganic Se through the selenite methylation process which can produce hydrogen selenite as a waste product and gaseous product which is released freely into the air. The very strong and toxic odor smelled in this study can be an indicator of the production of hydrogen selenite during the fermentation process and this also indicates that there is a bioconversion process of inorganic Se into Se protein, either seleno-cysteine or seleno-methionine. According to Demirci *et al.* (1999), about 0.3% of Se can be converted to seleno-methionine by the yeast *S. cerevisiae*. The higher content of Se contained in the fermentation process with the addition of sodium selenite than the findings of Demirci *et al.* (1999) may indicate that some of the Se present in fermented PKC were still in inorganic form.

Studies on the effect of the addition of Se in poultry feed on egg production have been reported by many researchers (Payne *et al.,* 2005; Leeson *et al.,* 2008; Suchy *et al.,* 2014; Han *et al.,* 2017). The previous findings showed inconsistent results on the effect of Se in the diet on egg production. This inconsistency was due to a couple of factors affecting egg production, such as the concentration of Se in the diet and the source of Se (Arpasova *et al.,* 2009). A study from Payne *et al.,* (2005) showed that the addition of Se in the diet, either in the form of sodium selenite or Se yeast did not increase egg production. Leeson *et al.* (2008) found that increasing Se concentration from 0.1 ppm to 0.3 ppm in the feed increased egg production from 64.9 to 72.3%. In this present study, the addition of fermented PKC with the addition of Se (T1 to T4) increased egg production from 412 eggs (T-0) to between 430 and 438 eggs (T-1 to T-4). Henday production, egg weight, and total egg production during the study also increased with the addition of Se in the form of SRFPKC.

Table 3. Effect of diets on egg production, feed intake, FCR, dry matter excreta, feed digestibility, egg selenium, and egg cholesterol

Parameters	Treatment diets						
	T-0	T-1	T-2	T-3	T-4	P- Value	SEM
Egg production (egg)	412[b]	430[a]	431[a]	432[a]	438[a]	>0.001	1,84
Henday production (%)	81,8[b]	85,3[a]	85.6[a]	85.7[a]	86.9[a]	>0.001	0,366
Total production (g)	20423[b]	21558[a]	21640[a]	21715[a]	22008[a]	>0.001	117
Egg weight (g/egg)	49,6[b]	50.1[a]	50.2[a]	50.3[a]	50.3[a]	0.027	0,085
Feed intake (g)	50933	51779	52080	51624	52401	0.055	167
FCR	2.49[a]	2.40[ab]	2.41[ab]	2.38[b]	2.38[b]	0.008	0,012
DM excreta (%)	20.9	22.1	22.6	22.6	22.7	0.956	0,772
DM digestibility (%)	79.8[b]	81.9[a]	81.7[a]	81.7[a]	81.6[a]	>0.001	0,183
Prot. digestibility (%)	81.7[b]	83.7[a]	83.9[a]	83.7[a]	83.8[a]	>0.001	0,191
Egg selenium (mcg/100g)	53.4[c]	80.7[b]	80.6[b]	81.8[b]	90.7[a]	>0.001	2,42
Egg cholesterol (mg/100g)	773	712	842	698	630	0.076	25,2

FCR: feed conversion ratio; DM: dry matter; Prot: protein; SEM: Standard error of means

The increase in total egg production follows a logarithmic pattern with Y = 570.78ln(x) + 21404 and R^2 = 0.9446. Total egg production increased dramatically when the diet was supplemented with 0.87 ppm Se (T-1) and further addition of 2.86 ppm Se (T-4) produced only a very small increase in total egg production. The same logarithmic pattern was also shown in other egg production parameters such as hen day and egg weight. The non-significant differences in all egg production parameters between the addition of Se with a concentration of 0.87 ppm in T-1 and 2.86 ppm in T-4 (Table 2) may indicate that a concentration of 0.87 ppm is sufficient to optimize egg production, adding more than this level could not increase egg production.

Although the addition of Se at concentrations exceeding 0.87 ppm did not increase egg production, the Se content of eggs increased with increasing Se concentrations in the feed. Laying hens fed SRFPKC with a concentration of 2.86 ppm (T-4) produced eggs with the highest Se concentration. These findings indicate that the bioconversion of inorganic to organic Se occurs in this study with an indicator of Se stored in eggs. Liu *et al* (2020) found that the addition of Se in the feed can increase the Se content of eggs. The effect of additional treatment of Se concentration in the feed did not affect egg cholesterol in this present study. Even though cholesterol deposition in the egg can be affected by nutrition, particularly fatty acid content (Shahid *et al.*, 2015), the unchanged lipid content in the present experimental diet might be the reason why the cholesterol content of the eggs was unaffected due to the addition of selenium to the diet.

Table 4. Egg quality of hens stored for 1 day at room temperature

Parameters	Experimental diets						
	T-0	T-1	T-2	T-3	T-4	P- Value	SEM
Index of egg shape	0.0135	0.0128	0.0126	0.0125	0.0123	0.077	0.0037
Loss of egg weight (%)	1.353	1.284	1.255	1.252	1.229	0.989	0.0733
Egg yolk (%)	25.05	24.84	24.50	22.90	24.38	0.347	0.3360
Egg albumen (%)	63.30	62.55	63.74	66.01	64.01	0.681	1.0300
Eggshell (%)	11.65	12.61	11.76	11.09	11.61	0.064	0.1680
Height of albumen (mm)	6.64	6.59	6.68	6.79	6.88	0.796	0.0790
Height of yolk (mm)	13.43	13.23	13.50	13.21	13.65	0.742	0.1150
Eggshell thickness (mm)	0.38	0.37	0.39	0.34	0.36	0.189	0.0064
Yolk colour	8.0	8.8	8.2	8.0	9.3	0.323	0.2430
Index of yolk	0.310	0.311	0.316	0.313	0.317	0.954	0.0032
Haugh unit	83.60	83.71	83.68	83.79	83.75	1.000	0.4360

Table 5. Egg quality of hens stored for 30 days at room temperature

Parameters	Experimental diets					P- Value	SEM
	T-0	T-1	T-2	T-3	T-4		
Index of egg shape	0.787	0.772	0.815	0.778	0.768	0.672	0.0106
Loss of egg weight (%)	5.13[b]	4.22[a]	4.37[a]	4.34[a]	4.24[a]	0.039	0.111
Egg yolk (%)	26.6	28.8	25.5	25.0	24.3	0.358	0.732
Egg albumen (%)	61.6	58.3	62.3	63.3	63.2	0.208	0.757
Eggshell (%)	11.9	12.9	12.2	11.7	12.5	0.359	0.207
Height of albumen (mm)	3.09[c]	3.55[bc]	3.60[bc]	3.79[ab]	4.18[a]	>0.001	0.862
Height of yolk (mm)	7.48[b]	8.39[a]	8.67[a]	8.89[a]	9.12[a]	>0.001	0.132
Eggshell thickness (mm)	0.458	0.368	0.420	0.412	0.403	0.245	0.0124
Yolk colour	7.8	7.2	7.5	7.3	7.5	0.559	0.124
Index of yolk	0.164[b]	0.197[a]	0.196[a]	0.197[a]	0.211[a]	>0.001	0.0038
Haugh unit	51.91[b]	60.45[a]	60.47[a]	60.75[a]	62.42[a]	0.001	0.942

This finding is supported by a study by Mohiti-Asli et al (2010).

The increase in total egg production by 5.6 – 7.8% due to the addition of Se before the fermentation process in this study, was not caused by the increase in feed intake. The FCR was also affected by the addition of selenium-rich PKC. This study was not in line with previous research from Han *et al.* (2017) who found that the addition of Se in the diet did not affect the FCR. An increase in egg production without being accompanied by an increase in feed intake indicates that there might be an increase in income over feed cost which is the main target in the livestock business.

The addition of SRFPKC increased dry matter digestibility of the diets from 80% to 82% and protein digestibility from 82% to 84%. Although there was improved feed digestibility, the increased feed intake to produce more eggs led to the unimproved FCR. This might indicate that more digested nutrients in hens fed the supplemental selenium diets were not fully compensated in egg production. The increase in digestibility may be due to two mechanisms. First, fermentation on a substrate rich in fiber and mannose-based polysaccharides can produce several enzymes, such as cellulase (Hatta *et al.,* 2014) and mannanase (Bahri *et al.,* 2019). These two enzymes are responsible for the breakdown of cellulose and mannan which are the main components of fibrous substances in PKC (Sundu *et al.,* 2012). A study by Bahri *et al.* (2019) on the fermentation process of coconut meal having similar profiles in non-starch polysaccharides also indicated that fermentation of coconut meal produced a product in the form of mannose and the highest product of mannose was produced when the coconut meal was fermented for 5 days. The conversion of mannose-based polysaccharides into simple and easily digestible products in the form of mannose and the presence of enzyme products enabled the diets containing fermented PKC to have a higher digestibility than the control diet in this study. Therefore, this condition could increase the digestible dry matter intake and the digestible protein intake. Second, the addition of Se from SRFPKC can improve the health status of the digestive tract. Read-Snyder *et al.* (2009) found longer small intestinal villi in the Se-fed chickens than the intestinal villi of chickens without the addition of Se. Improving the health of the digestive tract allows the hens to be able to absorb nutrients in the intestinal villi. Although there was an increase in digestibility, the water content of the excreta was not affected by the addition of Se in this study.

Although there was an increase in egg production due to the addition of SRFPKC, the percentage of each part of the egg (yolk, albumen, and eggshell) was not affected. This finding was in agreement with the previous findings of Gjorgovska *et al.* (2012). Our results also indicated that egg quality measured one day after egg collection was statistically the same. This means that the influence of external factors such as temperature and storage time in this study was minimal or not even detected on 1 day of storage.

It is believed that albumen height and Haugh unit are affected by selenium (Pappas *et al.,* 2005) storage time, and storage temperature (Gravena *et al.,* 2011). Storage of eggs for 30 days affected the albumen height and Haugh unit of eggs fed SRFPKC diet. The Haugh unit value of eggs without SRFPKC stored for 30 days at room temperature was 51.91 and the addition of SRFPKC could maintain the Haugh unit value in the range between 60 and 62. Albumen height, yolk height, and yolk index also showed the same trend where the addition of SRFPKC could maintain the quality of albumen and yolks at 30 days of storage. This study showed that the high selenium content of eggs fed with SRFPKC plays a role in protecting the internal quality of eggs (Leeson *et al.,* 2008). The increased Se concentration in the diets increased linearly the albumen thickness of eggs stored at ambient temperature for 30 days with a linear equation of $Y = 0.3649x + 3.0822$ and $R^2 = 0.9346$. Selenium has been believed to play a role in the production and activity of glutathione peroxidase (GSH-Px). The yolk index of poultry was better fed on diets supplemented with Se (T-1, T-2, T-3, and T-4) than those of control eggs (T-0). This is probably due to the production and activity of GSH-Px as an antioxidant enzyme and this enzyme functions to maintain cell integrity and protect the eggs from yolk lipid oxidation. Measurement of thiobarbituric acid reactive substances (TBARS) to determine fatty acid peroxidation in egg yolk lipids was carried out by Gajcevic *et al.* (2009) who found that the concentration of TBARS in egg yolk lipids as an indicator of lipid peroxidation was lower in chicken eggs fed with Se.

Research on the effect of storage time on egg weight reduction has been carried out by Hatta *et al.* (2020). They found that fermentation slowed down the egg weight loss during 28 days of storage. The results of this study also showed that the effect of fermentation with the addition of sodium selenite could suppress the decrease in egg weight during 30 days of storage. The decrease in egg weight may indicate that water evaporation occurs and maximum water evaporation occurs in eggs produced by chickens fed diets without SRFPKC supplementation. Since egg weight loss is related to the number and size of the pores of the eggshell, the addition of SRFPKC might reduce the number and size of the pores of the eggshell and thus reduce the evaporation of water in the albumen and egg yolk.

Studies on the effect of Se supplementation in laying hens' diet on yolk color and egg shape index have been carried out by a number of authors (Renema and Sefton 2004; Arpasova *et al.,* 2009; Han *et al.,* 2017; Liu *et al.,* 2020). They found non-significant differences in yolk color (Han *et al.,* 2017; Arpasova *et al.,* 2009) due to dietary supplementation with Se. It has long been believed that yolk coloration is much related to the carotenoid content of the diet (Rezaei *et al.*, 2019). The similar yolk color found, in all eggs might indicate that there was not much change in the carotenoid content of the experimental diets due to the supplementation of SRFPKC. Since the egg shape index is influenced not by the diets but by genotype (Shaker *et al.*, 2017), age of hens, and ambient temperature (Nikolova and Kocevski, 2006), it is expected that the effect of supplementation of SRFPKC in the diet on egg shape index is statistically undetected in this present study. This finding is in accordance with the study of Liu *et al.* (2020).

CONCLUSION

Palm kernel cake fermentation with the addition of sodium selenite as a source of selenium can increase the selenium content of palm kernel cake, increase egg production, feed digestibility, egg selenium content, improve feed conversion, egg quality of both the yolk and albumen and slow down the egg weight loss stored for 30 days at room temperature.

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