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Organic Egg Production, Egg Quality, Calcium Utilization, and Digestibility in Laying Hens Fed with Mushroom (*Flammulina velutipes*) Stem Waste

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ABSTRACT

The possibility of using mushroom (*Flammulina velutipes*) stem waste (FVW) in layer diets was explored in this study. A total of 180, 40-week-old ISA Brown laying hens were randomly assigned to 5 groups, with 6 replications of 6 birds for each group. Layers were fed a standard basal diet as control; antibiotic (0.05% flavomycin); 2% FVW; 4%FVW; and 6% FVW. No significant differences (p>0.05) were observed on hen daily egg production, egg weight, egg mass, unmarketable eggs, feed intake, feed conversion ratio, and live weight among experimental groups. Haugh unit was found higher (p<0.05) in FVW fed groups than in antibiotic fed groups. Shape index, shell weight, shell thickness, yolk weight, yolk index, yolk weight and albumen weight were not affected (p>0.05) by feeding FVW in this study. Yolk color was found higher (p<0.05) in FVW fed groups than in control and antibiotic groups. The shell color was higher (p<0.05) both in 4% FVW and 6% FVW fed groups than in control and antibiotic groups. Calcium retention and calcium in egg shell deposition were higher (p<0.05) both in 4% FVW and 6% FVW fed groups than in control and antibiotic groups respectively. Apparent nutrient retention for dry matter, crude protein, crude fat, and organic matter were higher (p<0.05) in FVW fed groups than in control and antibiotic groups. FVW at 6% level in layer ration can be used for organic eggs production which would be safe and sound for human consumption.

INTRODUCTION

Commercial poultry production is ranked among the highest source of animal protein in the world. The citizens of developing countries are not interested in the egg quality but only the quantity so the knowledge regarding the quality of commercial eggs are limited. Egg quality can be ensured by supplementing additives or nutrients in the diets of laying hen. Dietary optimum nutrient retention and utilization, especially calcium retention is very important for profitable layer farm. Lower deposition of calcium may lead to poor egg shell, creates huge broken eggs or unmarketable eggs which ultimately decreases the profit level of farmers. Thus, the layer farmers fall into huge economic losses. Antibiotic feed additives have long been used as growth promoters in poultry nutrition (Mahanta *et al.,* 2017). The extensive use of antibiotics in poultry industry with the purpose of increasing production performance has led to human health hazards. Poultry feeds containing natural herbs increase being considered as feed supplement in poultry diets as well as strong substitutes to antibiotics (Park *et al.,* 2014; Mahanta *et al.*, 2017). Since the last few years animal researchers are searching for herbal feed resources which have no health hazard on improving poultry production (Dhama *et al.,* 2015; Laudadio *et al.*, 2015). Higher consumer awareness on poultry products that are free from chemical residues such as antibiotics has resulted in global impact (Wolfenden *et al.,* 2010).

In this connection it is important to produce organic eggs for public health safety. Modern farmers are constantly looking for organic feeds to produce organic eggs by more economical means to better supply the market demands, as organic eggs have a high demand in markets for public health concerns. Mushroom has long been reported as good dietary elements for humans. Tang et *al.*(2016) reported that *Flammulina velutipesis* one of the main edible mushrooms in the market, it_has long been recognized for its nutritional, and medicinal values. *F. velutipes* is an excellent source of vitamins, minerals, and many medicinal properties such as immunomodulatory effect via induction of cytokines, antifungal, antibacterial, antiviral, antioxidant, antiprotozoal, anti-inflammatory, antitumor, and cholesterol-lowering activities (Wu *et al.,* 2010). Due to recent ban on using antibiotics as growth promoters in poultry diet, mushroom wastes could be a proper substitute for antibiotic (Hong-Gu *et al.,* 2014). Furthermore, Al-Shammari *et al.* (2017) reported that medicinal plants can be used as growth promoters, and as an alternative to antibiotics in chicken diets. Till this date, there have been limited scientific investigations involving *F. velutipes* mushroom on health promoting benefit in farm animals study.

Therefore, the aim of this study was to examine the efficiency of *Flammulina velutipes* stem waste (FVW) inclusion in laying hens ration on egg production performance, egg quality, calcium utilization and digestibility.

MATERIALS AND METHODS

The experiment was carried out at the animal shed building under the college of Chinese Medicine Materials, Jilin Agricultural University. A total of 180 ISA Brown laying hens of 40 weeks of age_were randomly assigned to 5 equal treatment groups, with 6 replications of 6 birds for each treatment. Feed and water were offered twice daily *ad libitum*. Hens were exposed to a 17-hour light and 7 h dark period throughout the whole experiment from40 weeks to 47 weeks. Birds were given a standard basal diet considered the control group; antibiotic (0.05% flavomycin) group; 2%*F.velutipes* mushroom stem waste (FVW) fed group; 4%FVW fed group; and 6%FVW fed group respectively. The *F. velutipes* mushroom stem was collected from a domestic mushroom farm in Changchun. The collected mushroom stem was sun dried and transferred to a feed mill for further uses. Mushroom and antibiotics were mixed with diet formulated by the National Research Council (NRC, 1994) specification using a feed mixer in a Feed Mill (Jilin Hanghong Animal Husbandry Co. Ltd, China) to treat all the diets equally. All the representative samples in triplicate were obtained and analyzed for proximate components according to the procedures of AOAC, (2004). Nitrogen was determined using an FP-528 nitrogen determinator (LECO Corporation, USA). The amount of amino acids was measured using a Hitachi L-8800 automatic amino acid analyzer (Hitachi, Japan). The analyzed nutritional composition of the experimental diet and FVW were presented in Table 1.

Egg production percentage was calculated by dividing the total number of eggs by hen per day and was expressed in percentage. Egg weight was presented as the average egg weight per hen divided by the number of days during the experimental period and egg mass was calculated as laying percentage multiplied by the egg weight. Total feed intake was determined as the difference between feed offered and residual feed in trough feeders and feed conversion ratio (FCR) was then calculated as feed intake divided by egg mass respectively. Unmarketable eggs were considered based on curled eggs (broken eggs, abnormal shape etc).

The Shape Index was measured by following the ratio of width and length of selected eggs by digital caliper (0.01 mm, model 1116-150 Insize Co, Suzhou, China). Individual eggs were weighed and were broken to separate the yolk, albumen and egg shell. The yolks were then carefully scraped with paper napkins to remove adhesive albumin and finally placed in a pre-weighed definite Petri dishes. The difference in the weight of each Petri dish after and before the introduction of the yolk was considered as the weight of the yolk. The eggshell (including shell membrane) was also socked by paper napkins and weighed by electronic weighing scale. The albumin weight was calculated from the whole egg weight by subtracting the yolk and egg shell weight. The yolk and albumen heights were determined using a tripod micrometer. The relative weight of the egg shell, yolk and albumin were expressed in percentage of the whole egg weight. Eggshell thickness was measured at three different locations (top, middle and bottom of the egg) using a micrometer. Yolk index was calculated as a ratio of yolk width and yolk length. Haugh unit were measured from egg weight and egg albumen height by following formula as described by Eisen *et al.* (1962).

Haugh unit = 100 log [albumen height (mm) + 7.57 - 1.7 egg weight$^{0.37}$ (g)]

Table 1 – Experimental diets for layer (g/kg)

Ingredients	Control	Antibiotic	2%FVW	4%FVW	6%FVW
Maize corn	579.0	578.5	561.0	547.0	534.0
Soyabean meal	262.0	262.0	260.0	257.0	253.0
Soyabean Oil	30.0	30.0	30.0	27.0	24.0
FVW[a]	-	-	20.0	40.0	60.0
Lysine	1.0	1.0	1.0	1.0	1.0
Methionine	2.0	2.0	2.0	2.0	2.0
Dicalcium	30.0	30.0	30.0	30.0	30.0
Limestone	92.0	92.0	92.0	92.0	92.0
Common salt	2.0	2.0	2.0	2.0	2.0
Vit -mineral premix[b]	2.0	2.0	2.0	2.0	2.0
Antibiotics	-	0.5	-	-	-
Total	1000	1000	1000	1000	1000
Chemical analysis[c]					
DM, (g/kg)	932.2	932.2	933.3	933.55	935.50
CP (g/kg)	169.5	169.3	170.2	169.90	170.1
Ca, (g/kg)	41.0	40.80	41.0	41.15	41.0
P, (g/kg)	7.20	7.20	7.30	7.25	7.30
EE, (g/kg)	51.2	52.1	50.70	52.10	50.20
CF, (g/kg)	25.9	25.2	29.80	33.1	37.30
Calculated analysis					
ME (MJ/kg)	11.50	11.50	11.52	11.48	11.50
Lysine,(g/kg)	10.5	10.5	10.40	10.4	10.4
Methionine, (g/kg)	5.0	5.0	5.0	4.9	4.9
Cystine (g/kg)	2.7	2.7	2.8	2.7	2.8

[a]FVW-*F. velutipes*mushroom stem waste at 2%, 4% and 6% . FVW= Analyzed composition of *F. velutipes* mushroom stem waste (g/kg) DM 887.0 ±0.85; CP 13.15±0.49; CF 20.05±0.106; EE 2.7±0.014; Ash 10.5±0.085; Ca 1.8±0.1; P 6.2±0.28; Lysine 12.5±0.04; Leucine 7.8±0.02; Isoleucine 4.4±0.01; Alanine 11.1± 0.07; Therionine 8.1±0.07; Valine 6.2±0.05; Arginine 3.2 ±0.02; Cystine 6.1±0.01; Methionine 3.67±0.05; value are expressed as mean ± standard deviation (n=6).

[b]Provided per kg of the complete diet: retinyl acetate, 4500 IU; cholecalciferol, 1200 IU; DL-α-tocopheryl acetate, 25000 IU; thiamin, 5000 mg; riboflavin, 20000 mg; phylloquinone,10000 mg; niacin, 45000 mg; pantothenic acid, 35000 mg; biotin, 1500 mg; folic acid, 3000 mg; cyanocobalamin, 40 mg; zinc, 45 mg; manganese 50 mg; iron, 30 mg; copper, 4 mg; cobalt, 120 µg; iodine, 1 mg; selenium, 120 µg.

[c]DM= dry matter; CP= crude protein; Ca=calcium; P= phosphorus; EE=crude fat; CF=crude fiber; ME= metabolisable energy.

The sensory test of selected table eggs was evaluated by giving score in terms of appearance and overall acceptability of eggs on a 5 points scale (very poor to very good; 1 to 5). Egg shell color and yolk color were considered comparing with egg shell color fan (Robotmation Co. Ltd, Japan) and yolk color chart fan (Robotmation Co. Ltd, Japan) ranged 1-15 (Very light -pale to very dark brown; 1 to 15).

For calcium utilization, samples of feed, excreta, and egg shell from each replicated group were collected every 24 hour during the last three consecutive days of the experiment. Samples were dried in an oven at 105°C for 24 hours, ground and then ashed in a muffle furnace at 650°C for 6 hours. The calcium retention was calculated by deduction of calcium found in excreta from calcium intake in feed. The calcium balance was estimated by the deduction of calcium in egg shell from calcium retention. Apparent calcium retention and mass calcium balance were determined according to the procedure described by Chowdhury & Smith, (2002).

Six hens per treatment were transferred into individual cages to facilitate the collection of excreta samples for determination of apparent nutrient retention. Diets contained Cr_2O_3 at 2 g/kg were used as indigestible marker. Feed intake and total excreta output were measured over 5 consecutive days, at the end of the experiment. The Cr_2O_3 content was determined by the method described by Fenton & Fenton (1979). The apparent nutrient retention (ANR) was calculated using the following formula, on basic of quantity of feed and feces nutrients with the appropriate corrections for differences in dry matter content.

ANR =100 – 100 x [(Cr_2O_3 in Diet x Nutrient in Excreta) / (Cr_2O_3 in Excreta x Nutrient in Diet)]

Data were subjected to one-way analysis of variance using a software (SPSS, 2006). Significant effects of dietary treatments on experimental groups were evaluated with Duncan's test (Duncan, 1955). Statements of statistical significance are based on a probability of $p<0.05$.

RESULTS AND DISCUSSION

Laying hen performance

There were no significant ($p>0.05$) differences on hen day egg production, egg weight, egg mass, unmarketable eggs, feed intake, feed conversion efficiency (FCR), and live weight among experimental groups during the entire 8 weeks study period (Table 2), which ensured the fact that feeding mushroom stem waste (FVW) in layer had no adverse effects on optimum laying performance. Similar findings were reported by Na *et al.* (2005) who observed that inclusion of *F. velutipes* media at 5 to 10% in layer diets had no significant effects on egg production, egg weight, egg mass, and FCR. Hong-Gu *et al.* (2014) found that fermented *F. velutipes* mycelium had no significant effects on egg production in laying hens. In addition, Daneshmand *et al.* (2012) reported that dietary inclusion of oyster mushroom, garlic, and propolis extract had no effects on broiler bird's body weight gain. In contrast by the author Hong-Gu *et al.* (2014) found that *F. velutipes* mycelium could improve egg size, egg mass, and FCR. Results associated with other laying performance parameters in the current study differ from the past study must be correlated with mushroom types, different level of inclusion in diet and bird stain.

Table 2 – Effect of *F. velutipes* mushroom stem waste (FVW) on performance in layer[1]

Parameters	Control	Antibiotic	2%FVW	4%FVW	6%FVW	SEM	*p*-value
Hen day egg production (%)	86.81	85.58	87.24	87.19	86.36	0.725	0.957
Egg weight(g/egg)	65.02	64.61	64.12	64.43	65.22	0.135	0.057
Egg mass(g/d)	56.45	55.31	55.92	56.17	56.32	0.424	0.937
Feed intake (g/d)	116.21	115.96	115.89	116.17	116.08	0.101	0.837
FCR(g/g)	2.07	2.11	2.08	2.08	2.07	0.017	0.956
Unmarketable eggs (%)	0.41	0.98	0.46	0.51	0.41	0.080	0.183
Initial live weight (gm)	1850.17	1856.39	1852.04	1848.57	1847.86	11.710	1.000
Final live weight (gm)	1931.17	1934.10	1939.81	1935.53	1940.96	11.581	0.999

[1]data represent the mean value of 6 replicates with 6 hens each treatment.

SEM- pooled standard error of the means.

Egg quality parameters

Egg quality parameters including, egg shape index, shell weight, shell thickness, yolk weight, yolk index and albumen weight were not differs ($p>0.05$) among experimental groups which ensured the fact that mushroom stem waste had no adverse effects on egg quality parameters. Haugh unit were found higher ($p<0.05$) in all the levels of FVW fed groups than antibiotic fed groups (Table 3). Metin *et al*. (2017) stated that dietary supplementation of herbal extract powder could improve the haugh unit of eggs in laying hens. Similar report by Hong-Gu *et al*. (2014) who stated that dietary supplementation of 4% *F. velutipes* mycelium could improve the haugh unit in laying hens. The appearance value for the table eggs were higher ($p<0.05$) in 6% FVW fed group than control and antibiotics fed groups. Shell color were found higher ($p<0.05$) both in 4% FVW and 6% FVW fed groups compared with control and antibiotic fed groups. Yolk color were found higher ($p<0.05$) in all the levels of FVW fed groups than control and antibiotic fed groups. Overall, acceptability were higher ($p<0.05$) both in 4% FVW and 6% FVW fed groups compared with control and antibiotic fed

Table 3 – Effect of *F. velutipes* mushroom stem waste (FVW) on egg quality[1]

Parameters	Control	Antibiotic	2%FVW	4%FVW	6%FVW	SEM	*p*-value
Shape index	78.08	78.10	79.9	78.61	78.66	0.306	0.503
Shell weight (%)	11.05	11.08	11.18	11.19	10.93	0.089	0.898
Shell thickness (mm)	0.38	0.38	0.38	0.38	0.38	0.001	0.168
Yolk weight (%)	23.56	24.65	23.55	23.38	24.23	0.196	0.278
Yolk index	33.22	32.19	32.82	33.36	33.26	0.249	0.532
Albumen weight (%)	65.39	64.27	65.26	64.43	64.84	0.201	0.310
Haugh unit	79.21^{ab}	75.63^{b}	80.25^{a}	79.62^{a}	80.90^{a}	0.597	0.045

[1]data represent the mean value of 6 replicates with 5 eggs each treatment (n=30).

a, b - means in the same row with different letters are significantly different at $p<0.05$.

SEM-pooled standard error of the means.

groups (Table 4). This study found the similarity with the past study by Hwang *et al*. (2012) who stated that dietary supplementation of mushroom could improve the acceptability of table eggs in laying hen on sensory evaluation. Yolk color in laying hens is primarily dependent upon the content and profile of carotenoid pigments present in the feed and can be easily altered by manipulation of ingredients to match consumer preference (Islam *et al.,* 2017). This study speculated that the higher yolk color might be associated with mushroom supplementation in experimental diets. *F. velutipes* has long been popular for its color, test and aroma (Miles & Chang, 2004).

Calcium utilization in laying hen

Calcium (Ca) retention was higher ($p<0.05$) both in 4%FVW and 6%FVW fed groups than in control and antibiotic groups although there were no differences in total Ca intake among the treatment groups. Similarly, calcium excretion in excreta was lower ($p<0.05$) both in 4%FVW and 6%FVW fed groups than in control and antibiotic groups. The calcium in egg shell deposition were found higher ($p<0.05$) both in 4% FVW and 6% FVW fed groups than control and antibiotic groups. However, there was no significant ($p>0.05$) difference in Ca balance among experimental groups (Table 5). Lower calcium retention in control and antibiotic group were because of higher fecal calcium excretion in the experimental hens in this study. Abdelqader *et al*. (2013) stated that enhancing calcium availability and absorption in gut of hen could be potential strategy for improving egg shell quality as well as reducing unmarketable eggs from aged hen. The higher concentration of calcium in FVW might have a positive impact on increasing calcium retention and calcium deposition in egg shell.

Table 4 – Effect of *F. velutipes* mushroom stem waste (FVW) on sensory evaluation of table eggs[1]

Parameters	Control	Antibiotic	2%FVW	4%FVW	6%FVW	SEM	*p*-value
Appearance	3.59^{b}	3.53^{b}	3.73^{b}	3.77^{b}	4.01^{a}	0.060	0.001
Shell color	10.43^{c}	10.52^{bc}	10.80^{ab}	10.90^{a}	11.11^{a}	0.059	0.001
Yolk color	10.11^{b}	10.01^{b}	10.46^{a}	10.69^{a}	10.73^{a}	0.059	0.031
Acceptability	3.94^{c}	3.96^{c}	4.11^{bc}	4.29^{ab}	4.36^{a}	0.040	0.019

[1]data represent the mean value of 6 replicates with 3 eggs each treatment (n=18).
a, b,c- means in the same row with different letters are significantly different at $p<0.05$.
SEM-pooled standard error of the means.

Apparent nutrient retention

Feeding experimental hens on diets with FVW resulted in a significant higher ($p<0.05$) DM, CP, and EE retention comparison to those fed on the control group and antibiotic groups. However, OM retention was found highest ($p<0.05$) in 2% FVW fed group among the experimental groups (Table 6). Addition of FVW in experimental diets could improve nutrient retention, which was associated to keep layer's body weight optimum in this study. Dry matter (DM) and ether extract (EE) digestibility were reported to be higher with dietary supplementation of mushroom in broiler study (Shang *et al*., 2016). In addition, feed additives originated from plants can stimulate the digestive functions and therefore could be used in poultry rations as feed additives (Mahanta *et al.*, 2017). In contrast with Daneshmand *et al*. (2012) reported that inclusion of oyster mushroom and antibiotic treatments could not affect protein and organic matter retention in broiler. These differences might be associated with mushroom type, mushroom parts, bird's strain, and mushroom inclusion levels in the experimental diets compared with the current study.

Table 5 – Effect of *F. velutipes* mushroom stem waste (FVW) on calcium utilization in layer[1]

Parameters	Control	Antibiotic	2%FVW	4%FVW	6%FVW	SEM	*p*-value
Ca intake in feed (g/d)	4.74	4.73	4.74	4.75	4.74	0.010	0.978
Ca in excreta (g/d)	2.73^{a}	2.70^{a}	2.66^{a}	2.23^{b}	2.25^{b}	0.043	0.001
Ca retention (g/d)	2.01^{b}	2.02^{b}	2.08^{b}	2.52^{a}	2.49^{a}	0.045	0.001
Ca in egg shell (g)	1.90^{b}	1.92^{b}	1.95^{b}	2.32^{a}	2.30^{a}	0.035	0.001
Ca balance (g/d)	0.10	0.11	0.12	0.20	0.19	0.045	0.213

[1]data represent the mean value of 6 replicates with 3 sample each treatment (n=18).Ca=calcium.
a, b- means in the same row with different letters are significantly different at $p<0.05$.
SEM- pooled standard error of the means.

Table 6 – Effect of *F. velutipes* mushroom stem waste (FVW) on apparent nutrient retention (g/kg) in layer[1,2]

Parameters	Control	Antibiotic	2%FVW	4%FVW	6%FVW	SEM	*p-value*
DM	833.75[d]	839.02[c]	841.61[b]	849.89[a]	850.07[a]	1.308	0.001
CP	675.89[d]	693.74[c]	721.49[b]	722.26[b]	743.49[a]	4.864	0.001
EE	794.62[e]	796.53[d]	809.30[c]	851.11[b]	866.16[a]	6.022	0.001
OM	839.97[d]	845.79[c]	860.86[a]	859.84[ab]	858.82[b]	1.753	0.001

[1]data represented the mean value of 6 pens of one bird each treatment (n=6).

[2]DM= dry matter; CP=crude protein; EE=crude fat; OM=organic matter.

a,b,c,d,e- means in the same row with different letters are significantly different at $p<0.05$. SEM-pooled standard error of the means.

CONCLUSION

Focusing on organic egg production for human health benefits, mushroom (*Flammunila velutipes*) stem waste can be used in layer ration for effective utilization of natural resources as well as substitute to antibiotics in commercial layer production. In this study we propose to add mushroom stem waste at 6% level in layer diets without any adverse effects on egg production.

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CONFLICTS OF INTEREST

There is no conflict of interest relevant to this publication.

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