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Original Article

Substitution effect of Soybean Meal (*glycine max*) with different level of Dried Water Velvet (*Azolla Pinnata)* Meal on Egg production and Egg Quality parameter of Potchefstroom Koekoek layer

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ABSTRACT

This study was carried out to investigate the effect of partial substitution of Soybean meal by AzollaPinnata (AZM) in the performances of layer chicken and also to determine the cost benefit analysis. One hundred eight Potchefstroom koekoek layer were randomly allocated to 4 treatment diets with 3 replications, each replicate consisted nine chickens. Experimental diets were prepared with control diet (treatment 1, T1) contained soybean meal as the major protein source without AZM and diets containing AZM at the levels of 50 g/kg (treatment 2, T2), 100 g/kg (treatment 3, T3), and 150 g/kg (treatment 4, T4) by partially substituting the soybean meal in the control diet. All production and economic data in the experiment were collected. The results obtained from the current study showed that substitution of AZM up to 150 g/kg did not significantly alter feed intake, feed conversion efficiency and egg production performance of the birds. However, higher ($P<0.05$) Hough units score, egg, albumen and yolk weight was observed at T2 and T3. Moreover, a significantly higher intense yolk color index was recorded as the level of AZM inclusion increased. The lowest marginal rate of return was recorded in control group while the highest revenue was recorded at T2 substitution of AZM. In conclusion, utilization of AZM might be cost effective protein source that can partially substitute soybean meal.

Key words: AzollaPinnata, partial substitutions, soybean meal

INTRODUCTION

Feed is the most important input for poultry production. Because feed costs account up to 70 % of total production costs, feed remain the major challenge especially in Sub-Saharan Africa where the price of the conventional feed resources increase continuously (Seyoum *et al.*, 2018). In Ethiopia Price trends of feed ingredients are average increase of 52 percent across

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five years and at an annual increase of 11 percent (Seyoumet al., 2018). Thus looking non-conventional feed ingredients like azollapinneta signify the importance of this study.

Azolla(AZM) is free floating fern which belongs to the family *Azollaceae* (Pillai *et al*., 2002). It is renowned for its rapid vegetative spread and the fastest growing aquatic macrophytes (Pillai *et al*., 2002). Moreover, *Azolla* species are abundantly available in stagnant water in tropical and subtropical regions of the world and grows in association with the blue-green algae (Collinson *et al*., 2010). Nutritionally *Azolla* has protein content 25-35%, vs 10% carbohydrates, 10 - 15% minerals vs 7-10% of amino acids on dry weight basis (Collinson *et al*., 2010), and has a considerably high feeding value (Hossiny*et al*., 2008). Also, substitution of aquatic plants at low levels in poultry diets had shown better performance, especially when they supply a part of the total protein or as a source of pigment for egg (Gangadhar*et al*., 2015).

The use of alternative high quality protein source feed is encouraged by many researchers around the globe. However, there is a scarcity of scientific work related to the use of locally grown *Azolla* in laying type chicken in Ethiopian condition. Addressing these issues will contribute to raising awareness on the importance of *Azolla* as locally available and nutritionally adequate alternative feed resources of poultry. This study was thus designed to evaluate the effects of partial substitution of soybean meal by AZM on egg production performances and egg quality characteristics of layer chicken.

MATERIALS AND METHODS

Study area

This experiment was conducted at Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) poultry farm (36^{0}50’E, 7^{0}4’N 1700 m.a.s.l.). The mean minimum and maximum temperature of the study area is 11.4℃ and 26.8℃, respectively and the mean minimum and maximum relative humidity is 39.9% and 91.4% respectively (BPEDORS, 2002).

Production and Preparation of Azolla Pinnata

AZM seedling was obtained from Sebeta Fishery and Aquatic Life Research Center and cultured and multiplied in artificially created earthen pond in the study area. AZM were manually harvested within 7 days' interval, washed, air drayed under the shade, then stored at a room temperature in airtight plastic bag until used. Then, grounded using hammer mill and sieved at size of 5 mm which hereafter is referred as air-dried AZM. Following, the experimental feeds were mixed with *AZM* by using vertical mixer for periods of 15 minutes according to the level of *AZM* added in the diets as partial substitutions of Soybean meal.

Ingredients of the experimental diets

The dietary ingredients used in this experiment were maize (Zea mays), soybean meal (Glysine max), wheat bran, Noug seed (Guizotiaabyssinica) cake, Blood and Bone meal, AZM, limestone, methionine, lysine, and salt. All ingredients except AZM were purchased from the local market. All other feed ingredients were also milled with similar sieve size and mixed at the feed processing plant located at the College of Agriculture and veterinary Medicine (Jimma University). The diets were formulated according to requirements specific for egg type chicken based on the recommendation of National research council (NRC, 1994).

Experimental design

The experiment was a completely randomized design consisting of four dietary treatments replicated three times. Nine Potchefstroom Koekoek layer chicken were randomly assigned to each of the three replicates with a total of 108 birds. The control diet (treatment 1, T1) contained soybean meal as the major protein source without AZM and diets containing AZM at the levels of 50 g/kg (treatment 2, T2), 100 g/kg (treatment 3, T3), and 150 g/kg (treatment 4, T4) by partially substituting the soybean meal in the control diet. The trial lasts for 13 weeks in addition to one week of adaptation.

Management of experimental birds

The study was carried out in accordance with regulations of the Jimma university college of Agriculture and veterinary medicine research and Ethical review board. Chicks were raised in a deep-litter housing system with concrete floor covered by wood shaving at a depth of 5 cm. The birds were fed twice a day, water provided ad libitum and the residual feeds were removed and weighed to calculate feed intake. Clean water was provided ad libitum throughout the experimental period and appropriate ventilation was maintained in all pens. All layers were kept in 16hour lighting. All necessary bio-security measures were put in place.

Chemical analysis

Representative sample of the feedstuffs were ground to pass through 1mm screen then analyzed for dry matter (DM), ash, CP, ether extract (EE) and crude fiber (CF). Proximate nutrients were performed as outlined by (AOAC, 1995). Samples of AZM and feeds offered were analyzed for DM (method 950.46), ether extract (EE, method 920.39), CF (method 962.09), and ash (method 942.05). The CP was assessed using Kjeldahl procedure (method 954.01) and the nitrogen content was multiplied by 6.25 to obtain the crude protein. Calcium was determined by atomic absorption spectrophotometer and phosphorus by colorimetrically methods. The metabolizable energy (ME) of diets was estimated using the equation of (Wiseman, 1987). (ME = 3.951+54.40 fat – 88.70 CF – 40.80 ash). All the samples were analyzed in duplicates at Animal Nutrition Laboratory of Jimma University.

Data Collection

Feed intake and egg production

Feed intake and egg production Chickens were fed on replicate basis and each day a measured amount of feed was offered in the morning (between 7.00 and 8.00 a.m.) and late afternoon (between 4.00 and 5.00 p.m.) and refusals were always collected and weighed in the morning of the following day before feed is offered. Feed intake on group basis was then computed by subtracting the feed refusal from that of feed offered. Eggs were collected daily and egg production rate was calculated on hen-housed basis by considering the number of hens that were housed initially.

Egg weight was determined on weekly basis and the average egg weight was calculated. Total egg mass was computed by multiplying the average egg weight with total number of eggs produced. Daily egg mass per hens was computed through dividing the total egg mass by the number of hens that were initially housed and total number of days in which the hens were in lay. Feed conversion ratio was calculated as grams of feed: grams of egg mass output. Body weight was taken at the beginning and end of the experimental period and then total weight gain was calculated by subtracting the initial body weight from that of the final.

Egg quality

A total of 96 eggs (24 eggs per treatment) were collected after eight weeks of the commencement of the experiment. The temperature and humidity in the egg storage room were kept at an optimum level 14°C to 20°C and 75% to slow down the loss in quality. Data were taken from the stored eggs on the 2ndday after collection. In the determination of the external and internal egg quality measurements were obtained by carefully making an opening around the sharp end of the egg, large enough to allow passage of both the albumen and the yolk through it without mixing their contents together. The yolk was carefully separated from the albumen and placed in a petri dish for weighing. Simultaneously, the associated albumen was placed on another petri dish and weighed. After each weighing, the petri dishes were washed in clean tap water and wiped with dry cotton cloth before next weighing (Doyon *et al* 1986). The egg weight, albumen weight and yolk weight measurement was determined by sensitive electric balance. The shell thickness was measured at three regions (large, middle and small end) using a micrometer gauge and the averages were used. Albumen height and yolk height were measured by tripod micrometer unit. The egg shape index was calculated from egg width and length with the formula SI=W/L *100 (Anderson, 2004). The yolk index was calculated from the width and heights of the yolk by formula YI= YH/YD *100 (Anderson, 2004) and the yolk color was measured with the use of Roche color fan (Haugh, 1937). The breaking strength was also measured with the use of Egg Force Reader (06-UM-001 Version D) apparatus.

Partial budget analysis

Partial budget analysis was conducted to determine the profitability of the partial replacements of Soybean meal by *Azolla Pinnata*on the basis of cost of feeds and labor at the time of experiments and the cost analysis was estimated according to Knott *et al.*, (2003).

Data analysis

All data were subjected to analysis of variance using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS, 2010). The comparison of means was performed at 5% level of significance with the following CRD model $Y_{ij} = \mu + T_i + e_{ij}$. Where: Y_{ij} = represents the jth observation (experimental unit) taken under treatment i. μ = over all means T_i = ith treatment effect e_{ij} = is a random error component that incorporates all other sources of variability in the experiment.

RESULTS AND DISCUSSIONS

Nutrient contents of AZM and experimental diet

As shown in Table 1, AZMcontains considerable amounts of CP but lower calcium and phosphorous values. The CP content in AZM found in the present study is consistent with previous finding of (Alalade *et al.*, 2007) who's reported the CP content of AZM was 25.33%, which is in good agreement with the present findings. In this study it is reported that AZM contained ether extract 3.01, crude fibre 11.06, total ash 23.59, Ca 1.70 and P 1.05 percent. There is a variation in the nutrient composition of Azolla meal in different studieswhich could be attributed to differences in the response of Azolla strains to environmental conditions such as temperature, light intensity and soil nutrients which consequently affect their growth morphology and composition (Sanginga *et al.*, 1989)

The CF content was found to be quite low when compared to other leaves of unconventional feed resources and is consistent with previous reports (Balaji*et al.*, 2009). It has been reported that AZM are of a good source of calcium (Ca) but relatively low in phosphorous (P) content indicating the need for P supplementation of diets containing AZM in order to maintain the recommended proportion between Ca and P for the different classes and production stages of poultry (Alaladeand Iyayi, 2006).

Nutrient compositions of the treatment diets are presented in Table 2. The CP contents of all experimental diets were similar although it was slightly lower in the T4 than the rest of the diets. The ash content increases with increased level of AZM in the experimental diets. The contents of CF, Ca and P were also comparable across all treatments diets. The calculated ME diets was higher in T1 than other treatment diets showing a decreasing tendency as the level of AZM increases. As the inclusion rate of AZM in the diet increased there was a proportional increase in CF, EE, ash and Ca contents in the diets while the content of ME (kcal/kg DM) was slightly decreased and this in accordance with the previous result (Dev *et al.*, 2015).

Table1: Analyzed values of AZM

Nutrients	Value (%Dry Matter)
Ash (%)	18.79
Cru2de protein (%)	24.89
Ether extract (%)	3.74
Crude fiber (%)	14.21
Calcium (%)	1.65
Phosphorus (%)	0.35

Table 2: Proportion of feed ingredients and nutrient compositions of the experimental diets

Feed ingredients (%)	Treatment diets			
	T1	T2	T3	T4
Maize	50	50	50	50
Soybean Meal	25	20	15	10
wheat bran	7	7	7	7
Limestone	6.95	6.95	6.95	6.95
Noug-seed Cake	6	6	6	6
Blood and Bone Meal	4	4	4	4
Layer vitamin & mineral Premix	0.69	0.69	0.69	0.69
Salt	0.25	0.25	0.25	0.25
L-lysine	0.09	0.09	0.09	0.09
DL-methionine	0.02	0.02	0.02	0.02
Azolla Meal	0	5	10	15
Total (%)	100	100	100	100
Analyzed (%, DM)				
%Ash	12.5	12.7	13.1	13.5
% Crude protein	16.25	16.14	16.00	15.88
% Ether extract	3.22	3.33	3.78	3.91
% Crude fiber	8.88	8.92	9.05	9.06
%Calcium	3.81	3.86	3.90	3.96
%Phosphorus	0.92	0.93	0.95	0.98
Calculated (%, DM)				
ME (kcal/kg DM)	2828.5	2822.8	2819.4	2809.3

DM: dry matter; ME: Metabolizable energy.

Body weight and feed intake

Initial, final body weight and average Feed Consumption of the bird (AFC) have been presented in table 3. In the current study there was no significance difference in the aforementioned parameters. The average feed consumption of the bird per day (AFC) indicated that the daily feed intake of the bird was not significantly affected by substitution of *AZM*. These results are consistent with the results of (Sanginga, 1989) who reported that for partial replacement of soybean by *AZM* to 15 % in the layer diet showed none significant feed consumption. In contrast, the current result of AFC was lower than the report of (Dev *et al*., 2015). *This* might be the age and breed/strain deference of the experimental unit.

The present study showed a decreased feed intake with increased FCR in chickens reared in T3 and T4 diets which can be explained by the combined effects of increasing CF content of diets with increasing inclusion levels of AZM. The decrease in feed intake and increased FCR may be further explained by the low concentration of ME in AZM and is consistent with previous findings (Sanginga, 1989). Similar observations pertaining to inclusions of high levels of leaf meals on reduced feed intake have also been reported for inclusion of 10% and 15% Lablab purpureus leaf meal for layer hens (Odunsi, 2003).

Birds fed on AZM included diet had similar final body weight gain performances to that of control diets and these results are contrary with the results of (Sushree*et al*., 2017) who reported that inclusion of dried dietary incorporation of AZM at either 5 or 10% level reduced the body weight of laying hens. However, these findings are also in good agreement with those of (Melesse *et al*., 2013) who reported increased growth performances in Koekoek chickens fed with graded levels of Moringastenopetala leaf meals.

Production performance

Initial and final body weight of the experimental chicks, Average feed consumption in gram per day per bird, Hen Housed Egg (HHE) and Feed Efficiency for Egg Mass (FEEM) are presented in table 3. The results showed non-significant differences for all egg production performance traits, Hen Housed Egg (HHE) and Feed Efficiency for Egg Mass (FEEM)] among all treatments. The average feed intake g/d/bird in the current study was lower than the reports of (Alalade *et al*., 2007) who reported 125 ± 3.46g/d/b, 125 ± 3.46g/d/b, 125 ± 3.46g/d/b and 125 ± 3.46g/d/b for 0%, 5%, 10% and 15% respectively.

Table 3 Least square means (±SEM) of initial & final body weight, Average feed consumption in gram per day per bird, Hen housed egg production, Feed efficiency for egg mass and blood lipid profilelayer chicken fed with diets containing various levels of AZM

	Treatment groups				
Parameters	T1	T2	T3	T4	**P value**
Initial Body Weight (kg)	1.74 ± 0.08	1.80 ± 0.08	1.69 ± 0.08	1.72 ± 0.08	0.83
Final Body Weight (kg)	2.01 ± 0.1	2.29 ± 0.1	2.33 ± 0.1	2.22 ± 0.1	0.19
AFC/g/d/b	104.8 ± 3.7	102.5 ± 3.7	101.1 ± 3.7	100.3 ± 3.7	0.29
HHE (%)	74± 0.5	78 ± 0.4	78 ± 0.4	74± 0.4	0.31
FEEM(g)	2.15 ± 0.08	1.93 ± 0.08	1.89 ± 0.08	1.95± .08	0.14

AFC/g/d/b: Average feed consumption in gram per day per bird; HHE: Hen housed egg production; FEEM: Feed efficiency for egg mass

Egg quality

Egg quality measurements, after 8 weeks of feeding, are summarized in Table 4. External egg quality traits (Egg weight, egg widths, egg lengths, egg shape index, egg shell weights, egg shell strengths and egg shell thicknesses) were compared with the control group. Our results indicated that diets with AZM had no effect on external egg quality parameters, except for egg weight. There is a significant ($p < 0.01$) eggs weight differences among treatments was observed. Accordingly, the lowest egg weight was recorded in T1 as compared to T2 andT3. Egg weight significantly increased up to 10 % substitution levels of AZM. this result is in line with the finding of (Sanginga, 1989).

The results of internal egg quality characteristics (Albumen Heights, Albumen Index, Albumen Weights, Albumen Ratio, Yolk Heights, Yolk Index, Yolk Weights, Yolk Ratios, Yolk Color and Haugh Units) have been shown in table 4. The present study showed significant ($P< 0.05$) differences in Albumen Weights, Yolk Weights, Yolk Color and Haugh Units.The albumin and yolk weight was significantly highest at 5% and 10 % level of substitution as compared to the control group. Whereas as it decreased with increasing levels of substitution compared to control group. The present result possibly indicated that utilization of *AzollaPinnata*in chicken diets have positive effect at 5 and 10 % partial substitution of Soybean meal with improvements in albumen weights. The albumen weights of current finding reported was slightly in agreement with the finding of (Khatun, 2008) for 0%, 5%, 10% and 15% respectively for *Azolla* incorporated diets. The variation of albumen weight may possibly be attributed to 5 -10% level of substitution by *AzollaPinnata*. However, the variation of albumen weight may attribute to difference in egg weight, breed and age of the bird when compared with reports of authors relating to albumen weights. The values of yolk weights in the current finding was comparable with report of (Khatun, 2008)) who reported yolk weight of 17.05 ± 0.56g, 17.35 ± 0.17g and 18.12 ± 0.66g for 0%, 5%, and 10% respectively. However, the current finding was lower than the reports of (Alalade *et al.,* 2007) who reported yolk weights of 12.94 ± 0.17g, 12.41 ± 0.17g, 12.18 ± 0.17g and 12.73 ± 0.17g for 0%, 5%, 10% and 15% respectively with dissimilarity among treatment groups. This difference might be able to variation of egg weight and age.

The color scores of egg yolks of hens that were fed a diet containing AZM were higher than those that were fed a control diet the higher mean values were observed at 10% and 15% partial substitutions of Soya meal by *AZM*. This revealed that, partial substitutions of *AZM* in poultry diet improved yolk color level with increasing level at 10and 15%. This may possibly be due to contribution to its content of carotene substance in the plant. Furthermore, yolk color improvements will alleviate the less consumption preference of the consumer in intensive poultry production system. In line with our finding, (Sanginga, 1989; Sushree *et al*., 2017) reported improvement of yolk score color. Generally, the feeding of marine plants has been reported to have a significant increase in yolk color score (Melesse *et al*., 2013) and a dose dependent increase in the egg yolk color score as a result of addition of *Spirulina platensis* algae in layer diets reflects the deposition of carotenoids in yolk and resulting increased egg yolk pigmentation (Sakaida and Takashi, 2003). The degree of yolk color preferred by consumers varies widely throughout the world, though deeper hues bring significant premiums in most markets. Baking operations and the food processing industry prefer darker colored yolks over adding artificial coloring agents (Selim *et al*., 2018). The highest calculated Haugh units were observed at 10% level of partial substitution whereas it showed gradual decrease with increased level of partial substitution from 10 to 15% of partial substitution. The variations Haugh unit value might be due to difference of albumen heights, egg weights and feed compositions (Selim *et al*., 2018).

Table 4: Least square means (±SEM) of internal and external egg quality characteristics layer chicken fed with diets containing various levels of AZM

Parameters	Treatments group				SEM	p value
	T1	T2	T3	T4		
External egg quality						
Egg Widths(cm)	4.06	4.14	4.12	4.04	0.04	0.30
Egg Lengths(cm)	5.40	5.58	5.55	5.43	0.09	0.45
Egg Shape Index (%)	74.21	75.19	74.19	74.44	0.76	0.78
Egg Shell Strengths(kg/cm2)	2.83	3.09	3.06	2.82	0.07	0.24
Egg Weights(g)	48.70^{b}	53.20^{a}	53.40^{a}	51.30^{ab}	2.00	0.01
Internal egg quality						
Egg Shell Weights(g)	4.65	4.90	5.33	5.11	0.18	0.12
Egg Shell Thickness (mm)	0.50	0.54	0.52	0.52	0.01	0.17
Albumen Heights (mm)	5.22	5.94	5.92	5.55	0.20	0.10
Albumen Index (%)	14.20	14.56	11.67	12.11	0.81	0.08
Albumen Weights(g)	27.90^{b}	31.87^{a}	31.62^{a}	30.34^{ab}	0.86	0.04
Albumen Ratio (%)	57.01	59.69	59.05	58.88	1.22	0.49
Yolk Heights(mm)	17.50^{a}	17.71^{a}	16.74^{ab}	16.45^{b}	0.15	0.02
Yolk Index (%)	43.38	41.56	52.43	44.17	4.66	0.41
Yolk Weights(g)	15.24^{b}	16.47^{a}	16.45^{a}	15.92^{ab}	0.26	0.03
Yolk Ratios (%)	31.43	31.07	30.93	31.15	0.33	0.76
Yolk Color(№ 1-15)	2.70^{b}	3.87^{ab}	5.17^{a}	5.17^{a}	0.47	0.02
Haugh Units (%)	74.19^{b}	79.41^{a}	77.27^{a}	75.62^{ab}	0.87	0.01

SEM: standard error of the mean

a-b Row means with different superscript letters are significant at $p<0.05$.

Table 5: Partial budget analysis of the experiments

Variables	Treatments (N=108), (Periods = 90days)				
	Unit	T1	T2	T3	T4
TFI	Kg	233.89	252.46	230.93	212.73
TFC	ETB	1988.06	2145.91	1962.91	1808.215
TAC	Kg	0 .00	2.05	4.08	6.14
CAP	ETB	0 .00	14.62	29.1	43.78
TVC	ETB	1988.06	2160.53	1992.01	1851.99
TE	Number	1706	1942	1909	1752
TESP	ETB	5971	6797	6639.5	6132
TR	ETB	5971	6797	6639.5	6132
NR	ETB	3982.94	4636.47	4647.49	4280.02
CTR	ETB	0.00	826	668.5	-507.5
CTVC	ETB	0.00	172.47	3.945	-140.02
CNR	ETB	0.00	653.53	11.025	-367.48
MRR		0.00	3.79	2.79	2.62

Note: TFI (Total feed intake), TFC (Total feed cost), TAC (Total Azolla consumed), CAP (Cost of Azolla production), TVC (Total Variable cost), TE (Total eggs), TESP (Total egg sell price), TR (Total Revenue), NR (Net revenue), CTR (Change in total return), CNR (Change in net return) and MRR (Marginal rate of return)

ETB (Ethiopian birr)

Partial budget analysis

The partial budget analysis of the present study has been presented in table 5. The economic efficiency of substitution of Soybean meal by *Azolla Pinnata* on the basis of total variable cost (cost feed and Azolla) within experimental periods showed that positive effect across the treatments over the control groups.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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