ORIGINAL ARTICLE

Effect of Partial Substitution of Soybean Meal (Glycine Max) by Mosquito Fern (*Azolla pinnata*) on Growth Performance and Carcass Characteristic of Cobb500 Broiler Chickens

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ABSTRACT

This study was designed to assess the effects of partial substitution of soybean meal with *Azolla pinnata meal* (AZM) on growth performance and carcass components of broiler chickens. The experiment was conducted in Jimma University, consisting of four dietary treatments replicated three times with ten chicks per replicate using a completely randomized design. The control diet (T1) contained soybean meal as the major protein source without AZM and treatment diets containing AZM at the levels of 25 g/kg (T2), 50 g/kg (T3), and 75 g/kg (T4) by partially substituting the soybean meal in the experimental diet. The final body weight and total weight gain of the individual bird was ($p<0.01$) higher in chickens fed with T3 than those reared on T2, and T4 diets. The feed conversion ratio of chickens fed on T3 was ($p<0.03$) lower than those reared in T2 and T4. Chickens fed with T3 diet had higher values of slaughter, dressed carcass and breast yield than those reared in T2 and T4 diets. The results of economic analysis revealed that the highest net return was calculated from chicken fed on T3. Moreover, no significant differences were observed in major commercial carcass components among treatment diets. Thus, partial substitution of soybean meal by AZM might be considered as viable option in smallholder poultry production practices taking into account the high cost of soybean meal.

Keywords: *Azolla pinnata*, Broiler Chickens, Carcass, Soybean meal

INTRODUCTION

Feed is the most important input for poultry production. Since feed cost accounts 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). Although both cereal grains and legumes are locally produced, they are staple food for human. In Ethiopia price trends of feed ingredients are average increase of 52 % across five years and at an annual increase of 11 % (Seyoum et al., 2018). Thus looking non-conventional feed ingredients like *Azolla pinneta* signify the importance of this study. Of aquatic plants, *Azolla pinnata* is free floating fern which belongs to the family *Azolceae* (Pillai et al., 2002) is renowned for its rapid vegetative spread and the fastest growing aquatic macro-phytes (Collinson et al., 2010; Vinu and Nampoothiri, 2017). *Azolla* makes possibility for cheaper production in poultry sector and have been known as the cheapest and most abundant potential protein sources (Alalade and Iyayi, 2008). The same authors indicated the *Azolla pinnata* contain the gross energy value of 2039 Kcal/kg, almost all essential amino acids and minerals (iron, calcium, magnesium, potassium, phosphorus and manganese). Also, (Pillai *et al*., 2002) reported that an appreciable quantities of vitamin A precursor, beta-carotene and vitamin B_{12}. However, with various recommendations of different scholars to utilize shuck like non-conventional type of feed, there is a lack of scientific works to use *Azolla pinnata* in meat type chicken in Ethiopian condition. This study was thus designed to evaluate the effects of partial substitution of soybean meal by *Azolla pinnata* on growth performance and carcass components of broiler chickens.

MATERIALS AND METHODS

Description of the Study area

This experiment was conducted at Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) poultry farm. The college is located between $36^0 50'$ east longitude and $7^0\ 4'$ north latitude and at an elevation of 1700 meter above sea level. 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, 2000).

Experimental diets and their preparation

Azolla seedling was obtained from Sebeta Fishery and Aquatic Life Research Center and cultured and multiplied in artificially created earthen pond in the study area. Azolla were manually harvested within 7 days interval, washed, air dried under shade, then stored at a room temperature in airtight plastic bag until used. Then the dried Azolla leaf was grounded in a hammer mill in sieve size of 5 mm which hereafter is referred to as 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.

The dietary ingredients used in this experiment were maize (*Zea mays*), soybean meal (*Glysine max*), wheat bran, Noug seed cake (*Guizotia abyssinica*), 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 meat type chicken based on the recommendation of National research council (NRC, 1994).

Experimental design

The experiment was employed using a completely randomized design consisting of four dietary treatments replicated three times. Ten Cobb-500 broiler chicks were randomly assigned to each of the three replicates with a total of 120 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 25 g/kg (treatment 2, T2), 50 g/kg (treatment 3, T3), and 75g/kg (treatment 4, T4), by partially substituting the soybean meal in the control diet.

Management of experimental birds

One hundred twenty Cobb-500 day old broiler chicks were purchased from Alama Poultry Farm PLC (Bishoftu, Ethiopia) and used for the experiment. The chickens were vaccinated against major poultry viral and bacterial diseases including Marek's disease, Newcastle and infectious bursal disease (Gumboro) according to the schedule provided by the company where the chicks were purchased. The vaccines were obtained from National Veterinary Institute (Bishoftu, Ethiopia). The chicks were reared under the brooder house for two weeks at the experimental site during which they were provided with starter rations. Then after, the experimental chicks were randomly transferred into experimental pens. Each pen had a dimension of 1.5×2 m which was designed to accommodate 10 chickens along with feeders and drinkers. Wood shavings were used as litter at a depth of about 5 cm. The experimental pens, drinkers and feeders were first cleaned and disinfected and aerated. Similar management conditions (light, temperature, ventilation and relative humidity) were provided to all birds. Clean and fresh water was provided *ad libitum* throughout the experimental periods.

Data on feed intake and body weight

During the experimental period, chicks were fed on replicate basis *ad libitum* and each day a known amount of feed was offered between 08:00 and 18:00 hours. The leftover feed was always collected in the next morning before feed is offered and weighed. Feed intake was then determined by subtracting the leftover from the offered feed. Body weight was taken at the beginning of the experiment (considered as initial weight) and then on weekly basis between 7:00 and 8:00am before feeding. The body weight taken at the end of the experiment was considered as final body weight. Total body weight gain was then computed by subtracting the initial body weight from the final. Feed conversion ratio (FCR) was calculated based on total feed intake and total body weight gain.

Data on carcass components

At the end of the experimental period, two chickens (1 male and 1 female) per replicate whose body weight was closest to the mean of their respective groups were selected and kept in a separate pen without feed. After overnight fasting of the selected birds, each of them was weighed (considered as slaughter weight) and eviscerated manually. The dressed carcass weight was taken after defeathering and removal of feet, head and the viscera while the skin is included. The dressed carcass, breast, thighs and drumsticks were weighed with bones. The wings were removed by a cut through the shoulder joint at the proximal end of humerus. The breast portion was obtained as described by (Hudspeth et.al., 1973). The thigh and drumstick portions were obtained by cutting through the joint between the femur and ilium bone of the pelvic girdle. The drumstick was separated from the thigh by a cut through the joint formed by the femur, fibula and tibia. The dressing percentage was calculated from dressed carcass weight as a percentage of the slaughter weight.

Chemical analysis

Proximate nutrients analyses were performed as outlined by AOC (1995) procedure. Samples of AZM and feeds offered per treatments 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. All the samples were analyzed in duplicates. The metabolizable energy (ME) content of feed ingredients was estimated using the following formula; ME (kcal/kg DM) = 3,951+54.40 fat – 88.70 CF – 40.80 ash (Wiseman, 1987).

Economics of production

The partial budget analysis took into considerations are the cost of feeds and labor at the time of experiment were used. The analysis involved calculation of the variable cost and profits of the experimental diet. Net income (NI)) of the experimental group were calculated as the amount of money left when total variable costs (TVC) are subtracted from the total returns (TR) as follows: NI = TR – TVC (Knott et al, 2003). The change in net income (ΔNI) or Net return (ΔNR) were calculated by the difference between change in total return (ΔTR) and the change in total variable costs (ΔTVC) as follows (Knott et al., 2003):ΔNI (= ΔTR – ΔTVC). The marginal rate of return (MRR) measures increases in net income (ΔNI) associated with each additional unit of expenditure (ΔTVC) and it was calculated as MRR= (ΔNI/ΔTVC) where: NR: net return, TR: total return and TVC: total variable costs (Knott et al., 2003).

Statistical analysis

The data were subjected to analysis of variance using the general linear model procedures of analysis system JMP (SAS, 2009). Treatment means were compared using Tukey's HSD test. The following statistical models were used to analyze the data: $Y_{ij} = \mu + A_i + e_{ij}$; where: Y_{ij} = individual values of the dependent variables (feed intake, body weight and weight gain, carcass traits); μ = overall mean of the response variable; A_i = the effect of the i^{th} AZM level (i = 1, 2, 3, 4,) on the dependent variable; and e_{ij} = random variation in the response of individual bird.

RESULTS AND DISCUSSION

Nutrient contents of *Azolla pinnata Meal* and experimental diets

AZM contains considerable amounts of CP but lower calcium and phosphorous values (Table 1). In the current study the CP content of *AZM* was 24.9 %. This result was comparable to crude protein level reported by Balaji et al., (2009) which is 24.5% and consistent with Sreemannaryana et al., (1993). However, Anand Titus and Geeta Pereira (2007) reported that the CP% might vary from 20-25 %. In this study the AZM contained ether extracts 3.01 %. Similar result was reported by Subudhi & Singh (1977) and Sreemannaryana et al., (1993) with respect to current finding. But variation in ether extract value was reported as low as Ali and Lesson (1995), 1.60% and as high as 3.7% by Balaji et al., (2009). Crude fiber level in *AZM* meal was 14.21 %. The crude fibre content was close agreement with the values obtained by Anitha et al., (2016); Balaji et al., (2009) and Cherryl et al., (2014), respectively. On the contrary, Singh & Subudhi (1978) reported less value and it ranged between 9.1 to 13.1 % while ash content of *AZM* was 18.8 %. The result was higher than the reports of Basak et al., (2002) who reported 15.7 % of ash in AZM. Generally, there is a variation in the nutrient composition of AZM in different studies which could be attributed to differences in the response of *AZM* strains to environmental conditions such as temperature, light intensity and soil nutrients which consequently affect their composition.

Nutrient compositions of the treatment diets are presented in Table 2. The CP content of all experimental diets was 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 treatment diets. The calculated ME content of the diets were 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 Ash while the content of EE and ME is lower than soybean meal The increasing trend of EE with an increase of AZM proportions in diets may be explained by the replacement of soybean meal with AZM as the fat contents in soybean are higher than that of AZM.

The calculated and analyzed contents of all treatment diets for CP were comparable and within the recommended levels for grower and finisher broilers (Scanes et al., 2004). However, the CP values are slightly above the minimum dietary CP level recommended for growing chickens of dual purpose breeds and reared under similar agro-ecological conditions (Melesse et al., 2017). As the levels of AZM in the diet increased there was a proportional increase in CF, ash and Ca contents in the diets while the content of EE was slightly decreased and this in accordance with the previous results (Melesse et al., 2017). The decreasing trend of EE with an increase of AZM proportions in diets may be explained by the replacement of soybean meal with AZM as the fat contents in soybean are higher than that of AZM.

Feed intake and body weight

Chickens in T1 consumed (p=0.009) more feed than those reared in the other diets. Broiler chickens fed on T2 diets had a higher feed intake (kg/bird) than those of T4. The FCR (kg feed/kg gain) in broilers reared in T3 was (p=0.002) lower than those of T1, T2 and T4. The results in Table 3 indicate that the final body weight and total weight gain of the individual bird was (<0.001) higher in chickens fed with T1 and T3 than those reared on T2 and T4 diets. The chickens reared in T2 and T4diets had intermediate values with no significant difference between them. No significance differences were observed in FCR among chickens fed onT1, T2 and T4 diets.

Table 1. Chemical composition of *Azolla Pinnata*

Nutrients	Value %Dry Matter)
Ash (%)	18.79
Crude protein (%)	24.89
Ether extract (%)	3.74
Crude fiber (%)	14.21
Calcium (%)	1.65
Phosphorus (%)	0.35
Calculated (%, DM)	
ME (kcal/kg DM)	2127.4

Table 2. Proportion of feed ingredients and nutrient compositions of the experimental diet

Feed ingredients (%)	Treatment diets			
	T1	**T2**	**T3**	**T4**
Maize	50	50	50	50
Soybean meal	28	25.5	23	20.5
wheat bran	7.33	7.33	7.33	7.33
Blood & Bone meal	6	6	6	6
Wheat Meal	5	5	5	5
Azolla Pinnata	0	2.5	5	7.5
Limestone	2	2	2	2
Vitamin Premix	1	1	1	1
Salt	0.5	0.5	0.5	0.5
Lysine	0.09	0.09	0.09	0.09
Methionine	0.08	0.08	0.08	0.08
Analyzed (DM%)				
Ash%	9.17	9.67	9.81	10.1
Crude protein %	24	23.79	23.54	23.1
Ether extract%	9.6	9.4	9.3	9.1
Crude fiber %	7.01	8.03	8.12	8.31
Calcium %	0.40	0.41	0.43	0.44
Phosphorus %	0.15	0.16	0.17	0.18
Calculated (%, DM)				
ME (kcal/kg DM)	3477.32	3355.56	3336.43	3296.86

DM, dry matter; ME, Metabolizable energy

The present study showed a decreased feed intake 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. As the levels of AZM in the diet increased there was a proportional increase in CF this result a decrement of voluntary feed intake of chicken. The decrease in feed intake and increased FCR in T4 may be further explained by the low concentration of ME in *AZM* meal as compared to soybean meal. The present results are in contrast with the previous observations of Basak et al. (2002); Ali and Leeson (1995); Who reported that at higher levels of inclusion (up to 15%) of *AZM* meal resulted in none significance feed intake in chickens. Balaji et al., (2009) reported that the cumulative feed intake of birds up to sixth week of age was 3677, 3655, 3659 and 3722g for 0, 1.5, 3.0 and 4.5 % *AZM* based diets, respectively revealing no significant difference among the dietary groups. Dhumal et al., (2009) observed non-significant differences in weekly feed consumption between control, 2.5 and 5 % *AZM* fed groups.

In the present study, chickens reared in the T3 diet had significantly lower FCR than those fed on T1, T2 and T4 diets. These observations can be explained by the fact that T3 diet contains appropriate proportions of soybean meal and *AZM* as the main CP source with better amino acid profile, which might have contributed to a better FCR. Moreover, the poor growth performance and higher FCR values of chickens in T2 and T4 diets might be due to reduced feed intake. Basak et al (2002) reported that poorest feed conversion ratio was obtained in treatment group which received 10 and 15 percent AZM.

Broilers fed on T3 diet had similar final and total body weight gain performances to that of control diets and these results are comparable with the results of Dhumal et al., (2009) who reported that the partial replacement of SBM at 2.5 and 5 % levels by *AZM* meal has no adverse effect on weekly body weights of broilers. However, in third, fourth and fifth weeks, the body weights were higher than that of control. These findings are also in good agreement with those of Basaket al., (2002) who reported significant ($P<0.01$) improvement in live weight of broiler chicks than that of control diet fed birds (1579g) when they were fed with 5 %*AZM* meal replacing sesame meal (1637g) at 6 weeks of age, while lower live weights were recorded in 10 and 15 % *AZM* incorporated diets. Similarly, the current study has indicated that AZM can be used as alternative cheap feed resource by providing useful nutrients to broiler chickens in smallholder settings.

Carcass components

As presented in Table 4, significantly higher values of slaughter, dressed carcass was obtained in chickens fed with T1and T3 diet. However, Basak et al., (2002) reported that the highest dressing percentage was observed in the birds fed with 5 % level of *AZM* (72.16) when compared with 0, 10 and 15 % groups. Similarly, Parthasarathy et al., (2002) reported significantly ($P<0.01$) higher dressing percentage (69.66%) at 8 weeks of age in broiler birds fed 5%diet compared to control (67.79), 10 (65.76), 15 (65.38) and 20 per cent (65.19) AZM fed groups.

Table 3. Least square means (±SEM) of growth performance traits and feed conversion ratio for broiler chickens fed diets with different levels of *Azolla Pinnata* meal

Parameters	Treatment diets				SEM	P value
	T1 (n = 6)	T2 (n = 6)	T3 (n = 6)	T4 (n = 6)		
Initial body weight (kg)	0.214	0.213	0.223	0.242	0.01	0.1039
Final body weight (kg)	2.108 ^{a}	2.014^{b}	2.096 ^{a}	1.961 ^{b}	13.20	<0.001
Total weight gain (kg)	1.894 ^{a}	1.801^{b}	1.873 ^{a}	1.719^{b}	13.21	<0.001
Feed intake (kg/bird)	4.470^{a}	4.227^{b}	4.204^{bc}	4.116^{c}	15.09	0.0090
FCR (kg feed/kg gain)	2.36^{a}	2.35^{a}	2.24 ^{b}	2.39 ^{a}	0.01	0.002

SEM, standard error of the mean; ANOVA, analysis of variance, ^{abc} Row means with different superscript letters are significant.

The lowest slaughter weight and dressed carcass was observed at higher levels of AZM inclusion (T4), this might be attributed to the dilution effects of nutrients and poor digestion/metabolism in mono-gastric animals fed with high levels of *AZM Pinnata* that might be related to anti-nutritional effects. The poor nutrient digestion/metabolism in higher AZM inclusion levels could probably occur due to the presence of anti-nutritional factors like invertase and protease inhibitors (Fasakin and Balogun, 2001) which might impair the bioavailability of the nutrients.

Non-significant effects of AZM substitution for soybean meal were observed in all studied carcass and giblets components. The non-significant differences in gizzard and heart weight yields suggest that AZM based diets did not have any detrimental effect on the normal growth of these organs; the observations are in contrary with previous findings of Basaket al., (2002) who reported that the mean giblet percentage was significantly higher in birds receiving 15 percent AZM (6.44) compared to other treatment groups. Similarly Parthasarathyet al., (2002) reported that there was a significant difference (P<0.01) in the weights of organs viz., heart, gizzard and giblets in birds fed 15 and 20 per cent AZM diets.

Table 4. Least square means (±SEM) of weights (g) and yields (%) of main carcass and organ components of broilers fed with diets containing various levels of *Azolla Pinnata* meal

Parameters (kg/bird)	Treatment diets				SEM	P value
	T1(n = 6)	T2(n = 6)	T3(n = 6)	T4(n = 6)		
Slaughter weight (g)	1674.5 ^{a}	1479.5 ^{b}	1671.1 ^{a}	1395.8 ^{c}	8.5	<0.001
Dressed carcass (g)	1145.8 ^{a}	966.3 ^{b}	1151.8 ^{a}	915.4 ^{c}	4.4	<0.001
Dressing (%)	68.4 ^{a}	65.3^{b}	68.9 ^{a}	65.6 ^{b}	0.4	0.0003
Yield of main carcass parts (%)						
Breast meat	18.1	18.1	18.1	18.2	0.04	0.1282
Thighs	9.5	9.7	9.5	9.5	0.06	0.1128
Drumsticks	7.5	7.5	7.5	7.9	0.08	0.1128
Wings	4.4	4.6	4.6	4.7	0.15	0.7526
Breast meat	18.1	18.1	18.1	18.2	0.04	0.1282
Giblets						
Gizzard	2.3	2.5	2.5	2.3	0.12	0.5221
Liver	2.2	2.3	2.2	2.3	0.08	0.5790
Heart	0.8	0.9	0.9	0.9	0.02	0.0637

SEM, standard error of the mean; ANOVA, analysis of variance; *Yield for each carcass was calculated as a percentage of slaughter weight, ^{abc} Row means with different superscript letters are significant

Economics of production

Except feed cost, other cost was constant and feed cost was only factor that differ the total production cost of broiler. The total cost per broiler was highest in T1 and lowest in T4 dietary treatments and the difference was significant between treatments (Table 5). As the AZM is an unconventional feed and the price per kg was lower than soybean meal without AZM meal and that is why the feed cost and total production cost per in AZM meal may be responsible for highest in the T1 group and the cost gradually lower in the other treatment groups for the same reason. The Total dressed meat produced in dietary treatments T2 and T3 were low (Table 5). So, the total costs of production per kg dressed meat were increased which reduced profit. Total cost per broiler was highest in T1 but the profit per broiler highest in T3, which was statistically similar with T1. The main cause of highest profit in broiler in T3 and groups were body weight of broilers. As the body weight was higher in T1 and T3 increase the profit for the same.

Table 5: Partial budget analysis of dietary treatment substituted with different level of *Azolla Pinnata* meal (AZM) for broiler chickens

Variable cost	Treatments			
	T1	T2	T3	T4
Total Feed consumed kg/treatment	4.5	4.2	4.2	4.1
Total AZM g/treatment	0.0	105.7	210.2	308.7
Feed cost /treatment (birr)	62.6	59.2	58.9	57.6
Total AZM cost/ treatment	0.0	0.7	1.5	2.2
Total feed cost/ treatment (birr)	62.6	59.9	60.3	59.8
Total dressed meat produced trt/kg	1.15	0.97	1.15	0.92
Total return(birr)	137.5	116.0	138.2	109.8
Net return(birr)	74.9	56.0	77.9	50.1
Change in total return	0.0	-21.5	0.7	-27.6
Change in total variable cost	0.0	-2.7	-2.3	-2.8
Change net return	0.0	-18.9	3.0	-24.9
Marginal rate of return	0.0	7.1	-1.3	8.9

T1 (0g/kg of AZM), T2 (25g/kg of AZM), T3 (50g/kg of AZM) T4 (75g/kg of AZM), meat sale=120 birr/kg. Feed price; 14birr /kg

CONCLUSION

The inclusion of azolla meal at 10% was either superior or at par with control diet (T1) with respect to overall performance and net economic returns. From the results it can be concluded that broilers could be raised successfully without compromising performance while substituting soybean meal up to 10% with azolla meal.

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