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Effect of Substituting Commercial Feed with Mulberry Leaf Meal on Performance of Broiler Chickens

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Abstract
The study was conducted to evaluate the effect of substituting commercial broiler feed with mulberry leaf meal on feed intake, weight gain, feed conversion ratio and mortality. One hundred forty four Cobb 500 broiler breed chicks were randomly distributed to four treatments in completely randomized design. Treatment diets were prepared by substituting 7.5, 15 and 22.5% of commercial broiler feed with mulberry leaf meal during the starter, grower and finisher phases. The average feed intake of broilers was affected (P<0.0001) by treatment diets where substitution of mulberry leaf meal at 7.5% and above had decreased the feed intake. Analysis of variance revealed higher (P<0.0001) weight gain in control group (M0) as compared to the other treatments. Inclusion of mulberry leaf meal up to 15% has no adverse effect on the survival rate. This study has showed that mulberry leaf meal substitution at 7.5% and more seems to have good effect on broilers carcass quality and reduction of abdominal fat that can positively affect consumers' desire.
Keywords: Feed intake, Feed conversion ratio, Mortality, Weight gain

INTRODUCTION
The productivity of poultry in the tropics has been limited by scarcity and consequent high prices of the conventional chicken feed. The price of conventional protein feed resources is high and cannot permit profit maximization in poultry business. In view of this, current research interest in the poultry industry is aimed at finding alternatives to this elusive feed ingredient (Kamruzman et al 2012; Islam et al 2014). The list of possible feed alternatives includes mulberry leaves as a source of dietary protein for poultry operations. Mulberry leaves are rich in protein, minerals (Ca, P) and metabolizable energy with absence of or negligible anti-nutritional factors (Omar et al 999; Sarita et al 2006). The available information on the effect of mulberry leaf inclusion on the performance of broiler chicken is not available under Ethiopian condition. Therefore, the present experiment was undertaken with the objective of assessing the substitution of mulberry leaf meal in broiler diet on feed intake, weight gain, feed conversion ratio, carcass yield and mortality.

MATERIALS AND METHODS
Study Area
The study was conducted at Kombolcha Agriculture Technical Vocational Education (ATVET) College, South Wollo Zone, Amhara region, Ethiopia. Kombolcha is located 375 km North of Addis Ababa (Ethiopia), at 1350 m.a.s.l , 11.1^{O}N latitude & 39.4^{O}E longitudes. The annual rainfall ranges between 750 & 900 mm with 8.6 & 30.8^{0}C of minimum and maximum temperature, respectively.

Preparation of Mulberry Leaf Meal
Seventy five days old fresh mulberry leaves re-growth of S30 and K1 varieties were collected. After harvesting, leaves were separated from their stems and dried 3 to 4 days under shade. Dried leaves were ground coarsely using hand mortar and pass through sieve so as to be similar to the size of the commercial broiler ration.

Experimental chicks and their management
A total of 144 Cobb 500 day old commercial broiler chicks were purchased from Alema poultry farms, Debre Zeit, Ethiopia. The birds were reared in a deep litter housing system and chicks were allocated at random into twelve pens. Routine vaccination against Newcastle and Gumborro were given as recommended by the manufacturers on the 1st, 14th , 21st and 28th day of age.

Feed preparation and feeding
Commercial starter and grower feed used in this study were obtained from Alema Koudijs Feed PLC (a joint venture between De Heus Animal Nutrition BV and Alema Farms). Direct substitution of commercial broiler

feed with mulberry leaf meal (MLM) was used in this experiment. The MLM were prepared from the two varieties with equal amounts and then mixed thoroughly to the commercial broiler feed based on the treatment ratio. Feed and water were offered *ad libitum* during the starter (day 1-11), grower (day 12-21) and finisher (after 22 days) phases. Feed intake was measured on daily basis by taking the difference between the quantity of feed offered the previous day and the quantity of feed left the next morning.

Experimental Design and Treatments

Broilers were assigned to four treatment groups following the principles of Completely Randomized Design (CRD). Each treatment group has three replicates of twelve birds per pen. The treatments are MU0 = 100 % Commercial broiler diet (Control); MU7.5 = 92.5% Commercial broiler diet + 7.5% Mulberry leaf meal; MU15 = 85% Commercial broiler diet + 15% Mulberry leaf meal; and MU22.5 = 77.5% Commercial broiler diet + 22.5% Mulberry leaf meal

Body Weight Measurement

All chicks are weighed on group base in each pen for the first two weeks of age. After two weeks, chick weight was taken individually before morning feeding on a weekly basis.

Carcass evaluation

At the end of the experiment, four broilers were randomly picked from each replication and slaughtered after being starved overnight for about 12 hours so as to allow the emptying of the crop and excretion of the undigested feed residue. After starvation, each bird were weighed, slaughtered, dressed, eviscerated and cut into parts to record pre-slaughter weight, dressing weight, eviscerated weight, carcass weight, breast weight, thigh and drumstick weight, liver, heart, gizzard, abdominal fat and intestine. Dressing, eviscerated and carcass percentage were calculated as percent of live weight. Dressing % was calculated as % of live weight after bleeding and de-feathering. Eviscerated % were measured by removing the viscera, head, shank, trachea, lungs but with giblets (heart, liver, and gizzard) and skin and expressed as % of live weight. The carcass weight and % was taken into account without the giblet. The abdominal fat, liver, leg, drum stick, thigh, heart, gizzard, intestine and breast weight were measured individually and equated with percent of live weight.

Chemical analysis

Feed samples of both commercial feed and mulberry leaf were analyzed to determine their crude fiber(CF), ether extract(EE), nitrogen(N), dry matter (DM) & Ash contents using Proximate analysis method of the AOAC (1990). Crude Protein (CP) was calculated by multiplying the nitrogen content of the feed with 6.25. Metabolizable energy (ME) content of feed ingredients and experimental diets was determined using the method of Wiseman (1987) as follows: ME (kcal/ kg DM) = 3951 + 54.4 EE -88.7 CF - 40.80 Ash.

Statistical analysis

The model used for data analysis was;

$$Y_{ij} = \mu + T_i + e_{ij};$$

Where, Y_{ij} = is an observation (experimental unit);
μ = Overall mean;
T_i= Treatment effect (i=1-4);
e_{ij}= Random error term.

Experimental data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (2002) version 9.0. Treatment means were separated using Tukey honestly significant difference test.

RESULTS

The chemical compositions of experimental feeds and feed intake of broilers across experimental period are presented in Tables 1, 2 and 3. The crude fiber content of mulberry leaf meal (15.23% DM) was higher than the commercial feed used for starter, grower and finisher diets (5.5% DM), respectively. When the commercial feed was incrementally replaced by mulberry leaf meal, the crude fiber content of the mixture has been increased. Feed intake, body weight gain and feed conversion ratio of broilers across weeks and entire experimental period is indicated in Tables 4, 5 and 6. Substitution of mulberry leaf meal at 7.5% and above has decreased the feed intake and analysis of variance revealed higher weight gain in control group (M0) as compared to the other treatments.

Death rate was recorded throughout the experimental period (Table 7) and results revealed that inclusion of mulberry leaf meal up to 15% has no adverse effect on the survival rate. Post mortem examination has revealed presence of watery fluid both in the abdominal cavity and pericardial sac. The color of liver was whitish due to lack of oxygen, as the small heart and lung could not deliver the amount of oxygen required by muscle. With increasing level of mulberry leaf meal a decrease in weight and percentage of was observed (Table 8). Abdominal fat percentage and weight has progressively decreased as proportional mulberry supplement increases.

DISCUSSION

The crude protein content of mulberry leaf meal of this study (16.65 %) is within the range of 15.31 to 30.91% as reported by Al-Kirshi et al (2013); Guven (2012) and Iqbal et al (2012).

A decrease in feed intake as the level of mulberry leaf increased may be due to both physical characteristics (semi-powdery nature and bulkiness) and bitter taste, which may affect the appetite of the birds and reduced the feed intake. The feed intake of broilers of this study is in line with Al-kirshi et al (2010) who have reported a significant reduction of feed intake during a 10% mulberry leaf meal substitution for commercial diet. Olmo et al (2012) noted that inclusion of 10-30% mulberry foliage meal in the diets of hybrid broiler chicken significantly decreased feed intake. Udedibie and Opara (1998), Odunsi (2003) and Akande et al (2007) also reported a reduction of feed intake with increased level of mulberry leaf meal in the diets of broilers and laying hens. In contrast, Kamruzzaman et al (2012) and Lokaewmanee et al (2009) had reported no adverse effect in feed intake up to 9 % inclusion of mulberry leaf meal in the ration. From weight gain perspective, Chowdary et al (2009) has reported that highest body weight gain was observed in 10% mulberry leaf meal inclusion. On the contrary, Olmo et al (2012) and Has et al (2013) concluded that inclusion of 10 % of mulberry foliage leaf reduced body weight gain. Kamruzzaman et al (2012) has also showed that inclusion of mulberry leaf meal up to 9% has no adverse effect on body weight. The present experiment has showed that an increase in the level of mulberry leaf meal substitution at 7.5% and above has reduced weight gain accordingly. This might be due to the insoluble fiber percentage that has probably exceeded the consumable threshold for broilers leading to decreased feed intake and inability to utilize the diet as a result of poor digestion and absorption. It could be also the bitter and astringent test of mulberry leaves (Ly et al.; 2001) affecting the palatability as well the appetite of birds (Behnke, 2001). Feed conversion ratios of the present experiment was high in broilers fed mulberry leaf substituted diets (>7.5%) and is in agreement with Olmo et al (2012) who found that inclusion of 10, 20 and 30% mulberry leaf meal in the diet of broilers result in higher feed conversion ratio. On the contrary Kamruzzaman et al (2012) and Machi (2000) found no detrimental effect on feed conversion ratio after feeding up to 9% level of mulberry leaf meal. The present piece of work revealed that inclusion of mulberry leaf meal in broilers diets up to 15% has no adverse effect on their survival rate and in agreement with Simol et al (2012) who reported that there is no adverse effect on the survival rate of broilers up to 30% mulberry leaf meal substitution. From carcass characteristics perspectives our findings are in line with Olmo et al (2012) who found that substitution of mulberry leaf meal at 10, 20 and 30% progressively decreases the weight of carcass, breast, thigh, leg and abdominal fat. Ayerza et al (2002) and Martinez et al (2011) also found that inclusion of foliage mulberry leaf meal in broilers diets has decreased abdominal fat weight.

Conclusion

Based on the result of this experiment, it can be concluded that mulberry leaf meal substitution to commercial broiler diets at 7.5% and more had negative effect on growth performance and carcass characteristics, no adverse effect on the survival of the broilers, and seems to have a good effect on reduction of abdominal fat that can positively affect consumers' desire. Therefore, further study is required to understand the optimum level of substitution and know its effect on meat quality and reductions of mortality.

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Table 1. Chemical composition of commercial feed

Feed type	DM (%)	Ash (% DM)	EE (%DM)	CF (%DM)	CP (%DM)	ME (Kcal/Kg DM)
MU	89.35	21.00	2.69	15.23	16.65	1889.6
Starter diet	90.50	11.30	6.50	7.30	22.60	3196.1
Grower diet	90.40	10.70	7.20	7.10	21.40	3276.4
Finisher diet	90.20	9.30	8.00	6.90	20.00	3355.3

MU= Mulberry Leaf Meal; DM= Dry Matter; EE= Ether Extract; CF= Crude Fiber; CP= Crude Protein; ME= Metabolizable Energy;

Table 2. Chemical composition of experimental feeds for different phases of broiler growth (calculated)

Treatment	DM (%)	CP (%DM)	EE (%)	CF (%)	Ash (%DM)	ME (Kcal/Kg DM)
Starter						
MU0	90.50	22.60	6.50	7.30	11.30	3196.1
MU7.5	90.40	22.16	6.20	7.90	12.03	3098.1
MU15	90.30	21.71	5.93	8.49	12.76	3000.1
MU2.5	90.20	21.27	5.65	9.09	13.49	2902.1
Grower						
MU0	90.40	21.40	7.20	7.10	10.70	3276.4
MU7.5	90.30	21.00	6.68	7.70	11.48	3172.4
MU15	90.24	20.60	6.52	8.32	12.25	3068.4
MU22.5	90.16	20.30	6.19	8.93	13.02	2964.4
Finisher						
MU0	90.20	20.00	7.50	6.90	9.60	3355.3
MU7.5	90.12	19.75	7.14	7.52	10.46	3245.4
MU15	90.07	19.50	7.14	7.52	11.31	3135.4
MU22.5	90.00	19.25	6.42	8.78	12.17	3025.5

MU=Mulberry leaf meal; MU0 =100% commercial broiler feed; MU7.5=7.5% substitution of mulberry leaf meal; MU15=15% substitution of mulberry leaf meal; MU22.5= 22.5% substitution of mulberry leaf

Table 3. Chemical composition of commercial feeds (as manufacturer)

Feed type	CP (min.)	EE (min.)	CF (max.)	Ca (min.)	Moisture (max.)	ME (min.)
Starter	22	6.50	5.50	0.65	10	3050
Grower	19	9.00	5.50	0.65	10	3150
Finisher	18	8.00	5.50	0.75	10	3250

CP= Crude Protein; EE= Ether Extract; CF= Crude Fiber; Ca= Calcium; ME= Metabolizable Energy

Table 4. Feed intake of broilers across the experimental period

Age	MU0%	MU7.5%	MU15%	MU22.5%	SEM	P-value
Week 1	141.70[a]	137.60[b]	131.00[c]	1210.00[d]	0.54	< 0.0001
Week 2	526.60[a]	486.90[b]	452.70[c]	436.10[d]	2.58	< 0.0001
Week 3	1142.10[a]	996.50[b]	922.20[c]	909.80[d]	8.39	< 0.0001
Week 4	1920.60[a]	1753.40[b]	1654.00[c]	1628.30[c]	18.61	< 0.0001
Week 5	2852.10[a]	2604.30[b]	2539.30[bc]	2505.00[c]	29.84	< 0.0001
Week 6	3797.30[a]	3508.70[b]	3444.30[bc]	3399.10[c]	21.90	< 0.0001

MU0 =100% commercial broiler feed; MU 7.5=7.5% substitution of mulberry leaf meal; MU15=15% substitution of mulberry leaf meal; MU22.5= 22.5% substitution of mulberry leaf meal for commercial broiler diet; SEM= Standard error of the Mean.

Table 5. Average weight gain of broiler across weeks and entire experimental period

Age	MU 0%	MU 7.5%	MU 15%	MU 22.5%	SEM	P-value
Week 1	118.30[a]	85.60[b]	84.60[b]	75.10[c]	2.04	<0.0001
Week 2	389.30[a]	275.60[b]	239.20[c]	214.60[d]	4.92	<0.0001
Week 3	786.50[a]	543.10[b]	439.80[c]	397.10[d]	10.31	<0.0001
Week 4	1245.50[a]	841.70[b]	712.50[c]	624.90[d]	17.66	<0.0001
Week 5	1705.50[a]	1109.70[b]	925.80[c]	820.00[d]	23.11	<0.0001
Week 6	2195.20[a]	1568.10[b]	1291.90[c]	146.70[d]	31.91	<0.0001
45 days	2451.30[a]	1729.90[b]	1434.20[c]	1260.30[c]	58.04	<0.0001

MU0 =100% commercial broiler feed; MU7.5=7.5% substitution of mulberry leaf meal; MU15=15% substitution of mulberry leaf meal; MU22.5= 22.5% substitution of mulberry leaf meal for commercial broiler diet; SEM= Standard error of the Mean.

Table 6. Feed conversion ratio of broilers fed diet containing different substitution level of mulberry leaf meal

Age	MU 0%	MU 7.5%	MU 15%	MU 22.5%	SEM	P-Value
Week 1	1.20[b]	1.61[a]	1.56[a]	1.62[a]	0.04	0.0002
Week 2	1.35[d]	1.76[c]	1.90[b]	2.03[a]	0.02	<0.0001
Week 3	1.45[d]	1.84[c]	2.10[b]	2.29[a]	0.03	<0.0001
Week 4	1.54[d]	2.08[c]	2.32[b]	2.60[a]	0.04	<0.0001
Week 5	1.67[d]	2.35[c]	2.74[b]	3.06[a]	0.06	<0.0001
Week 6	1.73[d]	2.23[c]	2.67[b]	2.97[a]	0.04	<0.0001
45 days	1.73[d]	2.20[c]	2.57[b]	2.90[a]	0.06	<0.0001

[a,b,c,d] Means within row with different superscript letters are significantly different (P<0. 01);
MU0 =100% commercial broiler feed; MU7.5=7.5% substitution of mulberry leaf meal; MU15=15% substitution of mulberry leaf meal; MU22.5= 22.5% substitution of mulberry leaf meal for commercial broiler diet; SEM= Standard error of the Mean.

Table 7. Mortality rate of broilers fed different substitution level of mulberry leaf meal

Age	MU0%	MU7.5%	MU15%	MU22.5%
Week one	0	0	0	3
Week two	1	0	0	0
Week three	0	0	0	0
Week four	3	0	0	0
Week five	3	0	0	0
Week six	4	0	0	0
Entire period	11	0	0	0
Mortality rate	30.6%	0	0	8.3%

MU0=100%commercial feed; MU7.5=7.5% substitution mulberry leaf meal; MU15=15% substitution of mulberry leaf meal; MU22.5= 22.5% substitution of mulberry leaf meal.

Table 8. Carcass characteristics of broilers fed ration containing different substation levels of mulberry leaf meal and commercial broiler feed.

Parameter	MU 0%	MU 7.5%	MU 5%	MU22.5	SEM	P-Value
Slaughter Weight (g)	2527[a]	1788[b]	1493[c]	1327[d]	46.70	<0.0001
Dressed Weight(g)	2332[a]	1661[b]	1379[c]	1217[d]	44.10	<0.0001
Eviscerated Wt(g)	1983[a]	1387[b]	1145[c]	992[d]	38.90	<0.0001
Carcass Wt(g)	1897[a]	1319[b]	1083[c]	933[d]	38.20	<0.0001
Dressing (%)	92.30	92.80	92.40	91.80	0.44	0.44
Eviscerated (%)	78.50	77.50	76.70	74.80	0.88	0.085
Carcass (%)	75.10[a]	73.70[a]	72.60[ba]	70.40[b]	0.94	0.041
Breast Weight (g)	640.00[a]	410[b]	337.20[c]	273[c]	21.40	<0.0001
Breast (%)	25.30[a]	22.90[b]	22.60[b]	20.60[b]	0.72	0.012
Leg Weight (g)	504[a]	350[b]	307[b]	249[c]	15.30	<0.0001
T +DS (%)	19.90[a]	19.60[a]	20.50[a]	18.80[a]	0.54	0.227
Thigh (%)	10.70[ab]	11.00[a]	10.90[a]	10.00[b]	0.26	0.1077
Drumstick (%)	9.20	8.60	9.60	8.80	0.40	0.366
Liver (%)	1.50	1.62	1.64	1.71	0.06	0.3885
Abdominal fat Wt.	34.00[a]	9.30[b]	3.70[c]	1.20[d]	0.70	<0.0001
Abdominal fat (%)	1.30[a]	0.52[b]	0.24[c]	0.09[d]	0.04	<0.0001
Gizzard (%)	1.20[c]	1.57[b]	1.95[a]	2.05[a]	0.08	0.0003
Intestinal (%)	3.10[c]	4.10[b]	4.60[a]	4.80[a]	0.10	<0.0001

[a,b,c,d] Means with different superscript letters in rows are significantly different (P<0.01);
SEM = standard error of the mean; MU0=100% commercial broiler feed ; MU7.5=7.5% substitution of MLM; MU15=15% substitution of MLM; MU22.5=22.5% substitution of MLM; T=Thigh; DS= Drum Stick; AF= Abdominal Fat; Wt = Weight.