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Histological Effects of Cottonseed Meal with and Without Ferrous Sulfate and Lysine in Male Broiler Rations

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Abstract: This experiment was conducted to test the hypothesis that Cottonseed Meal (CSM) with supplemental lysine and ferro sulfate improves its feeding value by detoxifying gossypol. The performance of 42-days-old straight Cobb*Cobb broiler chicks fed diets containing 5, 10, 15, 20% CSM (with and without lysine and Ferro sulfate) was compared with that of control chicks fed corn and soybean meal-based broiler rations. Four dietary treatments with Ferro sulfate and lysine, 4 dietary treatments without Ferro sulfate and Lysine. One (control), 2-5 (without Ferro sulfate and lysine) and 6-9 (with Ferro sulfate and lysine). All diets were formulated to meet minimum NRC requirements. Lysine levels were adjusted by addition of synthetic lysine at rates of 0.5% of the protein in CSM and by addition of synthetic Ferro sulfate at rates of 0.04% of the protein inCSM. In all experiments, weight gain, feed intake and Feed Conversion Ratio (FCR) of broilers at 21-42 days were significantly affected by the diets. Feeding CSM resulted in decreased body weight gain, increased feed intake and inefficient feed utilization. When, 0.5% lysine and 0.04% Ferro sulfate were added to feed, the body weight gains and feed conversion ratio of chicks were not significantly different from those fed the control diet. The FCR of chicks fed CSM plus 0.5% lysine and 0.04% Ferro sulfate at 21-42 days were significantly better than that of chicks fed CSM without lysine and Ferro sulfate. Abdominal fat pads (as a percentage of body weight) were significantly increased by the inclusion of CSM with or without the addition of lysine and Ferro sulfate ($p<0.05$). Liver and heart weights were not affected by the presence of CSM in the diet. However, this study shows that with adequate supplemental lysine and Ferro sulfate, CSM can be used in broiler diets without a reduction in performance.

Key words: Broiler, cottonseed meal, Ferro sulfate, lysine, FRC, male broiler

INTRODUCTION

Cottonseed Meal (CSM) is a byproduct of the process used to extract oil from cotton seeds arid Contains 41 or 44% crude protein, depending on the extent of hull separation prior to the oil extraction process. Although, CSM is high in protein, its utilization in poultry feed as a protein supplement is limited due to low lysine levels and by the presence of a toxic substance, gossypol. Problems related to lysine level and nutrient density are easily rectified by addition of synthetic lysine to poultry feed. However, solutions to the problems related to gossypol in CSM have been elusive. Gossypol, a naturally occurring metabolite of cotton that is concentrated in the seeds, is associated with reduced performance and increased mortality in chickens (Morgan, 2004). However, several feeding trails have shown that chick performance is not significantly affected when the dietary level of free gossypol is <250 mg kg^{-1} of feed (Gamboa *et al*., 2001). Other studies have shown that several factors, such as age, strain of chickens, dietary iron and dietary lysine may affect birds tolerance of gossypol (Adeyemo and Longe, 2007; Clawson *et al*., 1975). The quantity of CSM that can be incorporated into the diet depends largely on the amount of gossypol in the meal. In order to utilize, CSM in poultry rations great efforts have been devoted to devising practical methods to reduce and detoxify the free gossypol. Extensive feeding studies have shown that addition of ferrous sulfate to rations containing CSM reduced the adverse effects of gossypol on swine and poultry (Adeyemo and Longe, 2007; Evans *et al*., 1961). Although, the addition of Ferrous sulfate has reduced the adverse effects of gossypol, it is not widely accepted by the poultry industry. Free gossypol also binds to lysine under extreme temperatures and reduces the free gossypol available for absorption. However, extreme heat also reduces protein quality, thus, limiting the usefulness of

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this method (Husby and Kroening, 1971; Randels, 1992). Several investigators have reported the anti fertility effect of gossypol in males and famels of non-ruminant species (Adeyemo and Longe, 2007; Clawson *et al*., 1975; Gamboa *et al*., 2001). It was collectively indicated that gossypol acetic acid treatment of female rodents disrupts the normal pattern of estrous cycle through effect on pituitary and ovarian hormone secretion. It has been reported that at effective doses, gossypol causes males to be infertile because of sperm immotility and depressed sperm counts (Adeyemo and Longe, 2007).

It is well documented that gossypol binds to lysine and reduces its absorption. Therefore, lysine was added to CSM as a percentage of its protein content to improve feeding value, presumably by detoxifying gossypol. The performance of 42-days-old Cobb*Cobb broiler chicks was measured with diets containing commercial 28% protein cottonseed, Cottonseed Meal (CSM) and Feed Conversion Ratio (FCR).

MATERIALS AND METHODS

The present study was carried out at the poultry research center, Faculty of Agriculture, Zanjan University, over the period from February 2008 to May 2008. A total 336,1-days-old birds (Cobb*Cobb) was randomly divided into 24 groups of 14 birds and placed in a broiler house with wood shavings litter. CSM was included in grower diet with 5, 10, 15, 20% of diet with and without lysine and sulfate ferrous. Four dietary treatment with Ferro sulfate and lysine and another 4 dietary treatment without Ferro sulfate and Lysine. A proximate analysis was performed on CSM according to the AOAC (1989) procedures. The metabolizable energy reported by the NRC (1994) for CSM was 1.857 kcal kg^{-1} and this value was used in the formulation of the diets. One-day-old Cobb*Cobb broiler chicks were used in all experiments. Chicks were housed in Petersime battery brooders with 24 h fluorescent light. Feed and water were provided *ad libitum* for the duration of the experiment. A corn and soybean meal-based starter ration was fed to chicks from 1-21 days of age. At 21 day, chicks were weighed. The average body weights on day 21 were 30.6, 29.8, 29.6, 28.3, 29.5, 28.9, 28.8, 29.7 and 29.8 g for experiment, respectively. Body weights were measured at 21 and 42 days; residual feed was measured at 21 and 42 days and Feed Conversion Ratio (FCR) was calculated. At termination, chicks were killed by cervical dislocation. Hearts, livers, Testes and abdominal fat pads were removed and weighed. Control and experimental diets formulated and fed to chicks in experiment are shown in Table 1 and 2.

Table 1: Composition of the experimental diets

Ingredient	Broiler starter
Corn	60
Soybean meal (48% cp)	31.4
Cottonseed meal	0
Fish meal	3
Dicalcium phosphate	1.36
Limestone	1.35
Salt	0.3
Vitamin+mineral mix	0.5
Dl-Methionine	0.21
Lysine	0.08
Soybean oil	1.8
Sulfat ferrous	0
Calculated analysis	
ME (kcal kg^{-1})	2950
CP (%)	21.2
Crude fiber (%)	3.5
Lysine (%)	1.2
Free gossypol (%)	0.023
Total gossypol (%)	0.077

Vitamine mix provided the following per 100 g of diet: vitamine A, 551 IU; vitamine D3, 110 IU; vitamine E, 1.1 IU; vitamine B12, 0.001; mg; riboflavin, 0.44 mg; Niacin, 4.41 mg; D-pantothenic acid, 1.12 mg; choline, 19.13 mg; Mineral premix provided the following in mg per 100 g of diet; Mn, 6.0; Zn, 5.0; Fe, 3.0; I, 1.5 and Se 0.5

Table 2: Composition of the experimental diets (broiler grower)

Ingredient	1	2	3	4	5	6	7	8	9
Corn	5/59	95/56	29/54	51/51	82/48	69/56	54	17/51	5/48
Soybean meal (48% cp)	26/7	24/11	21/5	19	16/4	24/1	21/5	19/03	16/4
Cottonseed meal	-	5	10	15	20	5	10	15	20
Fish meal	5	5	5	5	5	5	5	5	5
Dicalcium phosphate	16/1	13/1	14/1	14/1	15/1	13/1	13/1	14/1	15/1
Limestone	26/1	28/1	27/1	27/1	26/1	28/1	28/1	27/1	26/1
Salt	3/0	3/0	3/0	3/0	3/0	3/0	3/0	3/0	3/0
Vitamin + mineral mix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dl-Methionine	22/0	0.17	0.18	0.20	0.20	0.17	0.18	0.20	0.21
Lysine	-	-	-	-	-	0.15	0.15	0.15	0.15
Soybean oil	35/5	6/5	6/2	5/9	5/6	6/4	6/1	5/8	5/5
Ferro sulfate	-	-	-	-	-	0.04	0.04	0.04	0.04
Calculated analysis									
ME (kcal kg^{-1})	2950	3232	3232	3232	3232	3232	3232	3232	3232
CP (%)	21.2	20.2	20.2	20.2	20.2	20.2	20.2	20.2	20.2
Crude fiber (%)	3.2	4.1	4.9	5.8	6.6	4.1	4.9	5.8	6.6
Lysine (%)	1.2	1.2	1.1	1.2	1.2	1.2	1.1	1.2	1.2
Free gossypol (%)	0.023	-	-	-	-	-	-	-	-
Total gossypol (%)	0.077	-	-	-	-	-	-	-	-

Statistical analysis: The experimental design was a completely random arrangement of nine treatments with tree replications. The general linear models procedure of SAS software (SAS, 1992) was used to analyze the data. Duncans means separation test was used to test for differences between means when the F-test for treat means was significant ($p<0.05$).

RESULTS AND DISCUSSION

Twenty-one days body weights ($p<0.05$) and feed conversions ($p<0.05$) were not significantly affected by dietary treatment (Table 3). Results from the 21-42 days weights indicated that the diets had significant effects on weight gain ($p<0.05$) and feed conversion ($p<0.05$) (Table 3). Chicks fed diets formulated with CSM with and without lysine and Ferro sulfate had weight gains similar to those fed the corn and soybean meal-based diet. The addition of lysine and Ferro sulfate to the CSM resulted in FCR that were statistically different from that of the without lysine, Ferro sulfate and control. The feed consumption of chicks was affected by the inclusion of CSM (without lysine and Ferro sulfate) or the addition of lysine and Ferro sulfate the increase of the cottonseed meal resulted to decrease significant difference in mortality that contrast with other authors (Boatner *et al*., 1949; Eagle, 1949). The inclusion of CSM (with and without lysine and Ferro sulfate) in the diets resulted in higher carcass fat as indicated by fat pad as a percentage of body weight (Table 4). The liver, heart, testes, Gizzard, values were not significantly affected by the use CSM (with and without lysine and Ferro sulfate) in the diets (Table 4). Several investigators have reported the anti fertility effect of gossypol in males and females of non-ruminantspecies (Adeyemo and Longe, 2007; Robinson *et al*., 2001; Wang *et al*., 1987). However, there were no differences in body weight gain of chicks fed CSM (with and without lysine and Ferro sulfate) and those fed corn and soybean meal-based control diet.

Table 3: Weekly body weights, fed consumption and feed conversion of age in broilers fed varying levels of cottonseed meal

	Body weight (g)		Feed consumption (g)		Cumulative feed to gain ratio (g g^{-1})	
Diets	Starter	Grower	Starter	Grower	Starter	Grower
1	6/30^{ab}	2/76	5/46	7/136^{a}	51/1	1/8^{ab}
2	8/29	1/69	5/46	2/137^{a}	55/1	2^{a}
3	6/29^{ab}	2/74	5/46	2/139^{a}	56/1	1/8^{ab}
4	3/28^{b}	2/67	5/46	4/120^{c}	62/1	6/1^{bc}
5	5/29^{ab}	8/68	6/46	2/129^{b}	58/1	7/1^{bc}
6	9/28^{ab}	3/76	6/46	6/122^{c}	6/1	5/1^{c}
7	8/28^{ab}	2/69	5/46	114^{d}	61/1	5/1^{c}
8	7/29^{ab}	2/71	5/46	5/128^{b}	56/1	7/1^{bc}
9	8/29^{ab}	9/67	6/46	6/119^{c}	56/1	6/1^{bc}
SEM	62/0	9/2	07/0	8/1	03/0	06/0

^{abc}The same column with different superscripts are significantly different ($p<0.05$)

Tabl 4: Organ weights at 42 days of age for broiler

Diets	Heart	Liver	Testes	Gizzard	Fats
1	0.50	1.90	0.220abc	2.00	0.95b
2	0.57	2.00	0.170c	2.10	1.80ab
3	0.63	2.20	0.250abc	2.30	2.40b
4	0.61	2.20	0.320a	2.40	1.80ab
5	0.62	2.20	0.20bc	2.10	1.80ab
6	0.57	2.00	0.31ab	2.30	2.00a
7	0.63	2.30	0.31ab	2.70	1.70ab
8	0.58	2.30	0.32ab	2.70	1.50ab
9	0.58	2.30	0.32ab	2.70	1.50ab
SEM	0.04	0.16	0.030	0.21	0.29

abc: The same column with different superscripts are significantly different ($p<0.05$)

CONCLUSION

In the present study, feed formulated with 5, 10, 15 and 20% CSM (with and without lysine and Ferro sulfate) to meet the NRC (1994) recommended level for all nutrients. The free and total gossypol, which was determined using a HPLC procedure, remained about the same in all treatments. We believe that lysine in the free form interferes with the spectrometric reading and that gossypol bound to the free amino acid is easily extracted as suggested by Tchiegang and Bourely (1997). Even with this dramatic decrease in free gossypol in the CSM, the performance of chicks fed CSM were not equivalent those fed corn and soybean meal-based diets. However, CSM with lysine and Ferro sulfate resulted in increased body weight gain and feed efficiency in a dose-dependent manner, which clearly demonstrates that in formulating diets with CSM, the amino acid levels (lysine, Threonine and Methionine) are as important as the gossypol level in CSM. Previous studies with oilseed meals (Fernandez *et al*., 1995; Zhang and Parsons, 1996) showed that overheating resulted in decreased protein solubility and chick performance.

Although, supplemental lysine and Ferro sulfate both significantly improved weight gain and feed efficiency, they were not able to prevent the increase in carcass fat associated with the use of CSM as the major protein source. The increase in carcass fat in chicks fed rations formulated with CSM as the major protein source may be attributed to the protein quality. Diets formulated with high ratios of calorie to protein resulted in high carcass fat on chickens. The results of this study suggest that diets containing CSM, even with adequate lysine, did not provide birds with the same levels of available amino acids as soybean meal. Another possible explanation for the higher carcass fat in birds fed diets with CSM is the fiber content and energy level of CSM. The CSM metabolizable energy used in diet formulation was taken from NRC (1994) publication and thus, the ME level of the diets with CSM may be greater than calculated. High energy: protein ratio increases carcass fat. Although, the use of iron to detoxify gossypol may be more economical, it does not result in improved feed utilization. The use of iron may also affect the utilization of other trace minerals and thus is not widely accepted by the poultry industry.

The results of these experiments indicate that broiler diets formulated with CSM require higher lysine levels to obtain performance comparable to soybean meal but will yield carcasses with slightly higher body fat. The higher level of lysine required is probably due primarily to the unavailability of the lysine bound to gossypol during the oil extraction and heating of CSM.

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