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Araştırma Makalesi
Research Article

The Effects of Iron Sulphate Supplementation to Diets Containing Cottonseed Meal on Performance and Haematological Parameters of Broilers*

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Summary : The aim of the study was to determine the effects of iron sulphate supplementation of diet containing 15 % cottonseed meal on the live weight gain, feed consumption, feed efficiency and some haematological parameters of broilers. Prior to experiment, a commercial broiler starter diet was fed to broiler chickens for two weeks. Than, 2-week old broiler chicks (n=100) were divided into control (C), and iron (FE; 0,31 % $FeSO_4\,7H_2O$) groups. The diets for two groups were adjusted to be isocaloric and isonitrogenous. At the end of the 4 weeks experiment, mean live body weights in FE group (1874.50±26.76 g) was significantly greater (p=0.03) than that of C group (1794.78±26.39 g). The values of feed conversion ratio of C and FE groups were 2.72 and 2.40, respectively. Although haematocrit and haemoglobin levels were not significant, mean corpuscular haemoglobin concentration (MCHC) were significantly different (p=0.02) between groups. Consequently, iron sulphate supplementation has a significant effect on live body weights and MCHC for diet containing cottonseed meal.

Key Words: Blood parameters, broiler, cottonseed meal, iron sulphate, performance.

Pamuk Tohumu Küspesi İçeren Yemlere Demir Sülfat Katılmasının Broylerlerde Besi Performansı ve Hematolojik Parametreler Üzerine Etkisi

Özet: Bu çalışmanın amacı % 15 oranında pamuk tohumu küspesi içeren yemlere demir sülfat katılmasının broylerlerde canlı ağırlık kazancı, yem tüketimi, yemden yararlanma ve bazı hematolojik parametreler üzerine etkisini belirlemektir. Denemenin öncesinde günlük civcivlere iki hafta boyunca ticari broyler başlangıç yemi kullanılmıştır. Daha sonra iki haftalık civcivler biri kontrol (C), diğeri demir sülfat (FE; % 0,31 $FeSO_4\,7H_2O$) olmak üzere iki gruba (n:100) ayrılmıştır. İki grubun da rasyonları izonitrojenik ve izokalorik olarak ayarlanmıştır. Dört haftalık denemenin sonunda demir grubunda (1874.50±26.76 g) ortalama canlı ağırlık kontrol grubuna (1794.78±26.39 g) göre istatistik olarak önemli derecede yüksek (p=0.03) bulunmuştur. Yemden yararlanma oranı kontrol ve demir sülfat gruplarında sırasıyla 2.72 ve 2.40 olarak hesaplanmıştır. Gruplar arasında hematokrit ve hemoglobin seviyeleri istatistik açıdan önemsiz olmalarına rağmen, ortalama korpuskular hemoglobin konsantrasyonu istatistik açıdan önemli (p=0.02) bulunmuştur. Sonuç olarak, demir sülfat katkısı, pamuk tohumu küspesi içeren yemlerle beslenen piliçlerde demir sülfat içermeyenlere göre canlı ağırlık ve ortalama korpuskular hemoglobin konsantrasyonuna olumlu etki yapmıştır.

Anahtar Kelimeler: Broyler, demir sülfat, kan parametreleri, pamuk tohumu küspesi, performans.

Introduction

The utilisation of cottonseed meal (CSM) as a dietary protein source for growing chicks becomes popular in cotton producing countries, because it is frequently cheaper than other protein concentrates. However, its utilisation in poultry diets as a protein source has been limited due to the presence of the toxic substance, gossypol, a naturally occurring metabolite of cottonseed. Gossypol is associated with depressed weight gains, increased feed intake, decreased feed efficiency, increased mortality (17) and changes in the haematological and biochemical parameters of the blood in chickens (1, 2, 12). Erythrocyte osmotic fragility has been associated with gossypol consumption and has been suggested as a tool to predict gossypol toxicity (8). The diets containing free gossypol could bind dietary iron in the small intestine and thus reduce its absorption and retention (12). Moreover, haematocrit, expressed as packed cell volume, and haemoglobin, as an indicator of dietary Fe adequacy, show the physiological status of the growing animal. It has been found that iron sulphate, calcium oxide, calcium hydroxide, calcium carbonate and sodium bicarbonate could be added to the diets containing cottonseed meal in order to eliminate the negative effect of gossypol (1, 2, 3, 20, 21). The aim of this study was to determine the effects of iron sulphate supplementation to diets containing 15 % cottonseed meal on live weight gain, feed consumption, feed efficiency, erythrocyte osmotic fragility (EOF), haemoglobin, haematocrit, and mean corpuscular haemoglobin concentration (MCHC) of broilers.

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Materials and Methods

Experimental groups and diets: Two-week old, 100 broiler chicks (Coop-500) were assigned randomly to two experimental groups. Two weeks prior to the experiment, all chicks were fed with a commercial broiler starter feed. Basal diet containing 15 % CSM was formulated for feeding four weeks experiment. The composition and chemical analyses of the basal diet is presented in Table 1. Control group (C; n=50) was fed basal diet. The diet of iron group (FE; n=50) was supplemented with iron sulphate 0,31% (2 ppm $FeSO_4$ for every ppm free gossypol in CSM).

For the preparation of the diet of iron group to obtain iso-caloric and iso-nitrogenous diets, the additive [$FeSO_4$ $7H_2O$] was first added to basal diet and the mixture was mixed with raw feed material (Table 1). Feed and water were supplemented *ad libitum* throughout the experiment. The feed consumption and body weights of the chicks were recorded weekly. Mortality was recorded as it occurred.

The nutrient components of the diets used in the experiment were determined according to the analytical methods described by AOAC (4). The metabolizable energy was calculated using the formula (19), and the free gossypol analysis was performed according to AOCS (5).

Blood collection and analysis: At the end of the experiment, feed refusals were collected and live weight measured in the morning. Experimental diets were removed same day at 9:00pm. The next morning following feed removal, 12 chicks were selected randomly from each group for blood collection. During slaughter, blood was collected in tubes containing EDTA. Blood was analysed for EOF, haemoglobin, haematocrit, and MCHC. EOF measured as a percentage of haemolysis in buffered saline solution as described by Nemi (11). Haemoglobin was determined by the method of Tietz (18). Haematocrit was calculated by using a micro haematocrit centrifuge. EOF and haemoglobin were measured using the spectrophotometer (Shimadzu UV-1601 Model).

Statistical analysis: For live weight and blood parameters, data were analysed Proc T-Test procedure of SAS (14). The values of live weight gain, feed consumption and feed conversion ratio were not analysed statistically due to group feeding.

Tablo 1. Composition and chemical analyses of basal diet.

Ingredients	%
Maize	51,40
Soybean meal	12,00
Fullfat soya	14,00
Cottonseed meal	15,00
Vegetable oil	4,00
Limestone	1,70
Salt (NaCI)	0,30
Vitamin premix[1]	0,25
Mineral premix[2]	0,15
Methionine	0,20
Dicalcium phosphate	1,00
Analyses	
Crude Protein, %	20,22
Metabolizable Energy[3], kcal/kg	3182

[1] Per 2,5 kg of Vitamin premix, 10.000.000 UI Vitamin A, 1.000.000 UI Vitamin D3, 25.000 mg Vitamin E, 3.000 mg Vitamin K3, 2.000 mg Vitamin B1, 6.000 mg vitamin B2, 4.000 mg Vitamin B6, 15 mg Vitamin B12, 20.000 mg Vitamin C, 20.000 mg Niacin, 8.000 mg CAL-D-pantotenat, 800 mg Folic acid, 300.000 mg Cholin chloride.
[2] Per kg of mineral premix, 80.000 mg Manganese, 60.000 mg iron, 60.000 mg zinc, 5.000 mg copper, 200 mg cobalt, 1000 mg Iodine, 150 mg, selenium.
[3] The metabolizable energy was calculated using the formula of Titus and Fritz (20).

Table 2. The values of live weight gain, feed consumption and feed conversion ratio for broilers during the experiment.

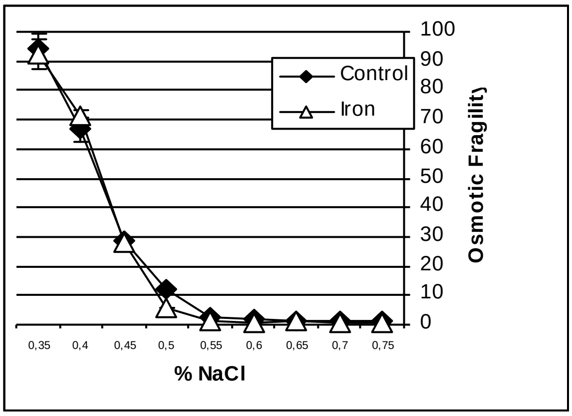
		Treatment Groups	
Week	**Measurements**	**Control**	**Iron**
3	Feed consumption, g/chick	446	455
	weight gain, g	232	257
	feed conversion*	1.93	1.77
4	Feed consumption, g/chick	771	691
	weight gain, g	376	369
	feed conversion*	2.05	1.87
5	Feed consumption, g/chick	1376	1271
	weight gain, g	428	468
	feed conversion*	3.21	2.72
6	Feed consumption, g/chick	1588	1455
	weight gain, g	501	518
	feed conversion*	3.17	2.81
3-6	Feed consumption, g/chick	4181	3871
	weight gain, g	1537	1611
	feed conversion*	2.72	2.40

* feed/gain

Table 3. The mean values of haemoglobin, haematocrit and MCHC values of broilers in different treatments at the end of the experiment.

	Treatment Groups		
	Control (n=12)	**Iron (n=12)**	
Parameters	$\bar{X} \pm Sx$	$\bar{X} \pm Sx$	***P***
Haemoglobin (g/dl)	8.12±0.20	8.69±0.20	0.06
Haematokrit (%)	32.16±1.20	30.66±1.21	NS*
MCHC (%)[1]	25.59[a]±0.72	28.45[b]±0.71	0.02

[1] Mean corpuscular haemoglobin concentration,
* NS=not significant.



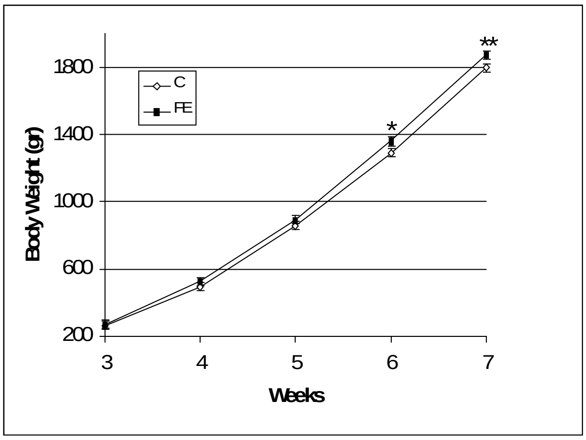


Figure 1. Mean live body weights for Broiler chicks in the experiment; *P=0.006 and **P=0.03(g) and osmotic fragility of eryhrocytes of 42 day– old broiler chicks fed different diets.

Results

Free gossypol concentration in the CSM was 852 ppm. Prior to experiment, the mean body-weights of the chicks between groups were similar (C=261 g and FE=269 g; Figure 1). At the end of the experiment chicks in FE group (1875 g) kept significantly greater (p=0.03) body weights than that of C group (1795 g) at the end of the experiment. During the experiment, broilers died 5 and 3 in C and FE groups, respectively. Feed consumption and feed conversion rates also paralleled with body-weights of the chicks. The weight gain during the experiment (for 4 weeks) was lower for C group (1537 g) than that of iron group (1611 g) (Table 2).

Haematocrit concentrations were not affected by treatment. However, MCHC differed between the groups (Table 3). The increase in EOF was lowest due to supplementation of iron in the diet (Figure 1).

Discussion

It has been reported that iron in diets forms iron-gossypol complex and can inactivate gossypol (1). The chelating effect of iron on gossypol in the diet was tested in the present study. The positive effect of iron sulphate on live weight gain and feed conversion were similar to the previous studies. El Boushy and Raterink (7) reported a positive linear effect of iron on body weight gain and on feed consumption of chicks at 6 wk of age. Rojas and Scott (13) and Waldroup and Goodner (20) also concluded that the presence of gossypol in poultry diets can be detoxified by the addition of iron salts and gossypol was inactivated by iron 1:1 molar ratio.

It has been reported that gossypol toxicosis is usually associated with microcytic-hypocheomic anaemia. On the other hand, iron sulphate addition increases the haemoglobin level of the animals consuming diets with gossypol (16). In agreement with the previous studies, at the end of the current experiment, haemoglobin and MCHC values of FE group were greater than that of C group. Free gossypol consumed by diet may interfere with normal utilisation of iron by chelating liver iron stocks, and therefore reduce the synthesis of haemoglobin. Thus, supplementation of the diet with iron may spare the liver iron concentration and this, in turn, may help improving blood haemoglobin and MCHC values. Consistent with the current study, Lindsey at al. (10) reported lower haemoglobin concentration and haematocrit percentage in animals receiving gossypol in solvent extracted CSM. Plasma albumin, urea or total protein was not affected by diet treatments (9). Gossypol is suggested to inhibit the synthesis of serum proteins that, in turn, increase serum urea and reduce blood haemoglobin and haematocrit values (16). The same study reported 24 % reduction in serum proteins by free gossypol. However, they concluded that reduction in serum proteins, due to carbonyl group of gossypol reacting to amino group of lysine in the diet, was doubtful to be significant for pigs consuming the experimental diet in the study (16). Moreover, adding lysine to rat diets containing free gossypol had little or no effect on correcting gossypol toxicity (15). As a result, the level of protein in the experimental diet may be sufficient enough not to cause a decline in serum proteins due to gossypol fed to chicks.

Osmotic fragility of erythrocytes measures ability of erythrocytes to resist osmotic stresses. In the current study, the iron supplementation in the diet affected EOF in certain NaCl concentrations (Figure 1). Similarly, researchers (6, 10) reported an increase in EOF with increasing levels of whole cottonseed or by-products in the diet. In the present study, EOF was affected by treatment suggesting alteration of membrane integrity due to iron addition to the diet which may also explain the changes in haemoglobin and MCHC values among treatments. Iron in the diet, on the other hand, was able to eliminate some of the negative effect of gossypol in cottonseed meal. FE group represented better values than the ones in C group in some parameters.

In conclusion, supplementation of iron sulphate to the diets containing cottonseed meal was found more effective than control diets. Iron in the diet seems to decrease the negative effect of gossypol on blood parameters and weight gain, and may be used to eliminate harmful consequences of gossypol in the diets of chicks.

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