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## RESEARCH ARTICLE

# High blood iron and magnesium concentrations in broilers fed with different plants in free-range systems

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# Serbest sistem yetiştiriciliğinde farklı bitkilerle beslenen broylerlerde yüksek kan demir ve magnezyum düzeyleri

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**Öz**

**Amaç:** Serbest sistem etlik piliç yetiştiriciliğinde farklı bitkiler ile beslemenin bazı biyokimyasal parametreler üzerine etkileri araştırıldı.

**Gereç ve Yöntem:** Çalışmada Hubbard Isa Red-JA etlik piliç civcivleri kullanıldı: 1. grup (kontrol) kapalı kafeslerde konsantre yem ile ad libitum beslendi. 2. grup yonca, 3. grup kılçıksız brom ve 4. grup % 50 yonca + % 50 kılçıksız brom ekili alanlarda mobil kafesler aracılığıyla beslendi. Tüm gruplar arasına kekik ekildi. 42. ve 84. günlerde her ana gruptaki civcivlerin 8'inden (toplam 64 hayvan) kan örnekleri alındı. Serum demir, magnezyum, total lipid, üre, ürik asit, kreatinin, total bilirubin, direkt bilirubin konsantrasyonları ile gama glutamil transferaz, laktat dehidrogenaz ve kreatin kinaz aktiviteleri belirlendi.

**Bulgular:** Demir konsantrasyonları 42. günde 2. ve 3. gruplarda, 84. günde 2. ve 4. gruplarda kontrol grubuna kıyasla arttı ($p < 0.05$). Magnezyum konsantrasyonları 42. günde 2., 3. ve 4. gruplarda kontrol grubuna kıyasla daha yüksekti ($p < 0.05$). Total lipid konsantrasyonları kontrol grubuna kıyasla 42. günde 3. ve 4. grupta daha yüksek iken, 84. günde 2. grupta daha düşük olarak tespit edildi ($p < 0.05$). Ürik asit konsantrasyonları 84. günde 2., 3. ve 4. gruplarda kontrol grubuna kıyasla azaldı ($p < 0.05$).

**Öneri:** Sonuç olarak etlik piliçlerin serbest sistemde yonca, kılçıksız brom ve kekik ile beslenmesi önerilmektedir.

**Anahtar kelimeler:** Serbest sistem yetiştiriciği, etlik piliçler, demir, magnezyum, kan biyokimyası

**Abstract**

**Aim:** The effects of feeding with different plants on some biochemical parameters in free-range broiler farming were investigated.

**Materials and Methods:** Hubbard Isa Red-JA broiler chicks were used: The 1st group (control) was fed ad libitum with consantrated feed in closed cages. The 2nd group was fed with *Medicago sativa* L, the 3rd group with *Bromus inermis* Leyss and the 4th group with 50% alfalfa+50% bromegrass cultivated areas, by means of mobile cages. *Thymus vulgaris* L. was cultivated among all groups. On the 42nd and 84th days, blood samples were taken from 8 of the chicks in each main group (totally 64 animals). Serum iron, magnesium, total lipid, urea, uric acid, creatinine, total bilirubin, direct bilirubin concentrations, and gamma glutamyl transferase, lactate dehydrogenase and creatin kinase activities were determined.

**Results:** Iron concentrations increased in the 2nd and 3rd groups on the 42th day and in the 2nd and 4th groups on the 84th day, compared to control ($p < 0.05$). Magnesium concentrations were higher in the 2nd, 3rd, and 4th groups, compared to control, on the 42nd day ($p < 0.05$). Total lipid concentrations were higher in the 3rd and 4th groups on the 42nd day, while lower in the 2nd group on the 84th day, compared to control ($p < 0.05$). Uric acid concentrations decreased on the 84th day in the 2nd, 3rd and 4th groups, compared to control ($p < 0.05$).

**Conclusion:** It is suggested to feed broilers in free-range system with *Medicago sativa* L, *Bromus inermis* Leyss and *Thymus vulgaris* L.

**Keywords:** Free range system, broilers, iron, magnesium, blood biochemistry

## Introduction

As a result of the sensitivity of poultry consumers to healthy food, studies on alternative production systems have increased in recent years. As an alternative to closed traditional poultry systems, free-range, organic, semi-intensive and extensive systems have been developed in which the animals can be released outside and thus arising the animal welfare (Sarıca et al 2010). Organic and free-range poultry systems are becoming increasingly common in developed countries, especially in Europe; in 2012, France and Britain were in the first place in organic poultry production among the European Union countries (EUROSTAT 2013). Instead of lean cultivation of forage plants, priority is given to mixed cultivation of species that generally adapt to each other, thus more efficient, balanced and long-term feed can be produced in mixed cultivation. It is recommended that the mixtures of cultivated forage plants have to contain at least one legume and one cereal; with the cultivation in the form of mixtures, the forage will be richer in terms of nutrients compared to the single cultivation (Gökkuş 2014). Alfalfla (*Medicago sativa* L) is an important perennial forage legume in animal husbandry. It is the basic component of feed of dairy cattle as well as of horses, sheep, birds and other species of livestock (Radovic et al 2009). Bromegrass (*Bromus inermis* Leyss.), a cereal, is considered and grown as a mix or pure stand for both grazing and haymaking (Açıkgöz 1991). Thyme was suggested to have antioxidant, antibacterial and antifungal activities, as well improving the general health and perfomance of broilers (Cross et al 2007, Abdel-Ghaney et al 2017).

Blood biochemistry is reported to be changed in the studies regarding feeding and breeding style of poultry; the addition of organic alfalfa flour in different concentrations (5% and 10%) to the mixed feed of broilers grown in a system in which organic production was made, has no significant effect on serum total cholesterol, LDL- and HDL- cholesterol and total lipid parameters ($p > 0.05$), whilst it increased the level of triglycerides ($p < 0.05$) (Tan and Kırkpınar 2015). In the system where intensive breeding was carried out, while the alanin amino transferase (ALT) and lactate dehydrogenase (LDH) activities of turkeys fed with the addition of alfalfa (1.5%) were not significantly affected, uric acid and total cholesterol concentrations decreased significantly compared to the control group (Krauze and Grela 2010). While there were no significant differences in the concentrations of total cholesterol, total protein, phosphorus, sodium, potasium levels, aspartate amino transferase (AST) and ALT activities between the caged system and free-range system in chickens, glucose and calcium concentrations raised in the chickens feed in free-range system compared to that in the caged system (Güneş et al 2002).

It is possible to obtain poultry products with high iron (Fe) content by addition of Fe to the ration, but also the dose and type of Fe (organic or inorganic) applied is important; Fe-methionine chelate (organic) and $FeSO_4$ (inorganic) were added to the rations in order to increase Fe concentrations in chicken muscle, eggs, and liver (Seo et al 2008, Abbasi et al 2015, Taschetto et al 2017).

With this study, it was aimed to investigate the changes in serum iron (Fe), magnesium (Mg), total lipid, urea, uric acid, creatinine, total bilirubin, direct bilirubin concentrations, and gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatin kinase (CK) activities in broiler chickens grown in free-range breeding system during 42 and 84 days, in cultivation based on different forage plants including; 1- *Medicago sativa* L. (alfalfa) + *Thymus vulgaris* L. (thyme), 2- *Bromus inermis* Leyss (bromegrass) + thyme and 3- 50% alfalfa + 50% bromegrass+thyme.

## Material and Methods

### *Pasture management and mobile broiler house*

The experiment was held at four decares field in Research and Application Farm of Selçuk University, Faculty of Veterinary Medicine The field was allocated to 3 equal part for planting; alfalfa was planted in the first part, bromegrass was planted in the second part and alfalfa (50%) + bromegrass (50%) were planted in the third part and also thyme was planted in all 3 parts. When the alfalfa and bromegrass were 10 cm, broilers were released into the pasture. Two mobile broiler houses (4 m long x 2.25 m width) were placed in each part for roam freely in the pasture. When the broilers were released into the pasture, cage wires (9 depth x 9 length x 1.5 height) were used to prevent the broilers in one group from passing into the other groups. Pasture was covered with cage wires to prevent wild birds getting inside from outside.

### *Experimental design and animals*

One-day-old broiler chicks (Hubbard Isa Red-JA; n = 480) were randomly allocated to 4 experimental groups with 16 replications in 8 mobile broiler houses (30 birds per replication, giving 15 chicks per square meter). Chicks in the control group were kept only in mobile broiler houses. The chicks were fed and housed in mobile broiler houses at 1-28 d for all experimental groups. During 1-28 d, the broiler chicks were fed with a complete diet that was specially formulated to meet their requirements, which comprised 2900 kcal/kg metabolizable energy and 230 g/kg crude protein, after the 28 d, the broiler chicks were fed with complete diet, which comprised 3200 kcal/kg metabolizable energy and 200 g/kg crude protein (NRC 1994) (Table 1). After 28 d, chicks in the second group were released into the alfalfa planted part, chicks in the third group were released into the bromegrass planted part, chicks in the fourth group were released into the alfalfa (50%) + bromegrass (50%) planted part, from

Table 1. Components of the diet (g/kg mixture)

| Parameters | Diets | |
|---|---|---|
| | 1-28 d | 29 -84 d |
| Corn | 559.9 | 551.3 |
| Soybean meal | 261.0 | 136.7 |
| Full-fat soybean | - | 98.0 |
| Fish meal | 11.0 | 11.0 |
| Corn gluten | 61.0 | 61.0 |
| Sunflower soapstock | 11.0 | 40.0 |
| Sunflower meal | 57.5 | 58.5 |
| Dicalcium phosphate | 19.0 | 20.0 |
| Limestone | 13.0 | 13.0 |
| Salt | 2.5 | 2.5 |
| Vitamin and mineral premix | 3.5 | 3.5 |
| Methionine | - | 2.0 |
| Lysine | 0.1 | 0.2 |
| Anticoccidials (lasalocid sodium) | 0.5 | 0.5 |

mobile broiler houses. Broiler chicks were released from mobile broiler houses between 07:00 AM and 07:00 PM except control group. Feed and water were provided ad libitum in mobile broiler houses and planted parts. The experimental period was done in June, July, August and ended at the 84th day.

### *Diets and pasture analyses*

Diets, alfalfa and bromegrass were analyzed for dry matter, ash, crude protein, crude fat, and crude fiber (Table 2).

### *Laboratory analysis*

At 42 and 84 d of age, 2 birds were randomly selected from each replication (giving 8 chicks per experimental group), and killed by cervical dislocation and blood samples were taken. Serum samples were obtained by centrifugation at 4000 rpm for 15 min and then stored at -80° C until the analysis.

Total serum total lipid concentrations were determined by

Table 2. Chemical composition of the diets and pasture

| Chemical analysis | 1-28 d | 29 -84 d | Alfalfa | Bromegrass |
|---|---|---|---|---|
| Calculated metabolisable energy (kcal/kg) | 2910 | 3190 | - | - |
| Dry matter (g/kg) | 912.9 | 918.4 | 170.0 | 203.4 |
| Ash (g/kg) | 56.7 | 53.8 | 132.0 | 118.1 |
| Crude protein (g/kg) | 230.6 | 230.6 | 241.6 | 164.1 |
| Crude Fat (g/kg) | 82.7 | 97.6 | 19.9 | 29.4 |
| Crude fiber (g/kg) | 55.4 | 58.0 | 213.8 | 291.8 |

FAR Diagnostics (Italy), Fe, Mg, urea, uric acid, creatinine, total bilirubin, direct bilirubin concentrations, and GGT, LDH, CK activities were determined by Abbott Architect (Germany) commercial kits in Architect ci8200 auto analyzer. Two analysis were performed in each samples for all parameters.

### *Statistical analysis*

Data were presented as mean ± SE and evaluated with ANOVA and Duncan as posthoc using SPSS program (for Windows, release 23.0, IBM Corp., USA). The level of $p < 0.05$ was considered as statistically significant.

## Results

Biochemical parameters on the 42nd and 84th day of the study is presented in Tables 3 and 4. Iron concentrations of the studied groups (2nd, 3rd and 4th group) increased significantly ($p < 0.05$) compared to the group fed with concentrated feed (control group) in the animals slaughtered on the 42nd and 84th days except the 4th group on day 42nd and group 3 on day 84th ($p > 0.05$).

When the concentrations of Mg were examined, the concentrations of Mg of all the animals (2nd, 3rd and 4th group) studied on the 42nd day were significantly higher than in the control group ($p < 0.05$), while there was no difference between the groups on the 84th day.

In animals that were slaughtered on the 42nd day, the total lipid concentrations were higher ($p < 0.05$) in the group which was given bromegrass (3rd group) and alfalfa+bromegrass (4th group), whereas there was no difference in alfalfa (2nd group) group, compared to that in the control group (1st group). When the animals slaughtered on the 84th day were examined, total lipid concentrations in the alfalfa group (2nd group) were found to be lower compared to that in the control group ($p < 0.05$).

When urea concentrations were examined, no significant change was observed in the animals slaughtered on the 42nd day, whereas it was found to be significantly higher in the alfalfa group (2nd group) on the 84th day compared to that in the control group ($p < 0.05$).

As regards uric acid concentrations, no significant change was observed in the animals slaughtered on the 42nd day, whereas the uric acid concentrations of the 2nd, 3rd and 4th groups decreased significantly compared to that in the control group, on the 84th day ($p < 0.05$).

When the creatinine concentration was examined, there was no difference between the creatinine concentrations of the animals slaughtered on the 84th day, whereas they were elevated in the bromegrass group (3rd group) on the 42nd day, compared to that in the control group ($p < 0.05$).

Direct bilirubin concentrations were increased in the 2nd group on the 42nd day, and 3rd group in on the 84th day compared to that in the control groups ($p < 0.05$).

In terms of GGT and CK enzyme activities and total bilirubin concentrations, there was no difference in both animals of 42nd and 84th days compared to the control groups. In animals that were slaughtered on the 42nd day, LDH enzyme activity was found to be lower in the alfalfa (2nd group) and bromegrass groups (3rd group) compared to that in the control group ($p < 0.05$).

Table 3. Some biochemical parameters on the 42nd day of the study (Mean ± SE, n = 8)

| Parameters | 1<br>(Control) | 2<br>(Alfalfa + Thyme) | 3<br>(Bromegrass + Thyme) | 4<br>(%50 Alfalfa + %50 Bromegrass + Thyme) | References |
|---|---|---|---|---|---|
| Fe (µg/dl) | 76.25±5.27$^{b*}$ | 92.13±5.08$^{a}$ | 95.88±3.64$^{a}$ | 85.38±3.55$^{ab}$ | 63.38-102.62 [1] |
| Mg (mg/dl) | 1.72±0.05$^{b}$ | 1.97±0.07$^{a}$ | 1.99±0.06$^{a}$ | 2.12±0.08$^{a}$ | 1.7-3.75 [2] |
| Total lipid (mg/dl) | 225.0±33.2$^{b}$ | 276.3±20.2$^{ab}$ | 320.0±25.6$^{a}$ | 321.3±25.4$^{a}$ | 1370 [3]<br>586-793 [4] |
| Urea (mg/dl) | 1.88±0.14 | 1.83±0.15 | 1.91±0.13 | 1.94±0.08 | 0.95-2.27 [5] |
| Uric acid (mg/dl) | 3.29±0.35 | 3.13±0.34 | 3.59±0.37 | 3.80±0.74 | 2-10 [6]<br>3.17-6.57 [7] |
| Creatinine (mg/dl) | 0.262±0.001$^{b}$ | 0.250±0.007$^{b}$ | 0.282±0.006$^{a}$ | 0.258±0.007$^{b}$ | 0.25-0.37 [8] |
| Total bilirubin (mg/dl) | 0.092±0.012 | 0.077±0.008 | 0.112±0.003 | 0.104±0.017 | 0.0006-0.0041 [9]<br>0.29-0.53 [7] |
| Direct bilirubin (mg/dl) | 0.030±0.005$^{b}$ | 0.050±0.005$^{a}$ | 0.036±0.005$^{ab}$ | 0.037±0.007$^{ab}$ | 0.09-0.23 [7] |
| GGT (IU/L) | 22.75±0.73 | 22.25±0.96 | 23.13±1.34 | 24.50±1.87 | 11.78-30.78 [7] |
| LDH (IU/L) | 1050.25±105.27$^{a}$ | 772.38±106.38$^{b}$ | 702.63±17.35$^{b}$ | 1100.50±112.07$^{a}$ | 700-1250 [10] |
| CK (IU/L) | 3282.00±442.60 | 2798.75±181.76 | 3280.13±197.82 | 3082.25±155.71 | 2485-131205 [7] |

*Different letters on the same line are statistically significant ($p<0,05$), 1: Betini et al 2011, 2: Karagül et al 1999, 3: Nadia et al 2008, 4: Alkhalf et al 2010, 5: Garg et al 2004, 6: Sakas 2002, 7: Silva et al 2007, 8: Piotrowska et al 2011, 9: Sese et al 2013, 10: Imaeda et al 1999.

Table 4. Some biochemical parameters on the 84th day of the study (Mean ± SE, n = 8)

| Parameters | 1<br>(Control) | 2<br>(Alfalfa + Thyme) | 3<br>(Bromegrass + Thyme) | 4<br>(%50 Alfalfa + %50 Bromegrass + Thyme) |
|---|---|---|---|---|
| Fe (µg/dl) | 86.88±3.36$^{b*}$ | 104.50±4.85$^{a}$ | 95.75±4.72$^{ab}$ | 101.25±3.85$^{a}$ |
| Mg (mg/dl) | 2.04±0.05 | 2.19±0.06 | 2.18±0.09 | 2.25±0.09 |
| Total lipid (mg/dl) | 353.8±18.1$^{a}$ | 245.0±10.4$^{b}$ | 351.3±24.5$^{a}$ | 293.8±20.3 $^{ab}$ |
| Urea (mg/dl) | 1.91±0.14$^{b}$ | 3.06±0.43$^{a}$ | 2.02±0.13$^{b}$ | 2.61±0.25$^{ab}$ |
| Uric acid (mg/dl) | 8.78±0.82$^{a}$ | 6.39±0.63$^{b}$ | 5.70±0.47$^{b}$ | 5.81±0.99b |
| Creatinine (mg/dl) | 0.326±0.013 | 0.296±0.012 | 0.300±0.006 | 0.320±0.007 |
| Total bilirubin (mg/dl) | 0.132±0.010 | 0.107±0.010 | 0.118±0.017 | 0.128±0.011 |
| Direct bilirubin (mg/dl) | 0.030±0.004$^{b}$ | 0.028±0.004$^{b}$ | 0.050±0.009$^{a}$ | 0.025±0.007$^{b}$ |
| GGT (IU/L) | 21.38±2.01 | 25.75±0.10 | 23.00±0.85 | 25.00±1.54 |
| LDH (IU/L) | 1196.75±113.31 | 988.88±115.88 | 1037.75±43.95 | 884.74±162.58 |
| CK (IU/L) | 5236.00±577.77 | 4946.13±630.65 | 4548.63±331.61 | 5228.00±381.87 |

*Different letters on the same line are statistically significant ($p<0.05$)

## Discussion

In this study, the effects of feeding on the biochemical parameters in broilers fed with different ration (in the lands consisting of alfalfa+thyme, bromegrass+thyme and 50% alfalfa+50% bromegrass+thyme) in free-range system were investigated in a feeding trial consisting of 42 and 84 days. At 42nd day, serum Fe concentrations of broilers fed with fresh alfalfa + thyme (92.13 µg/dl), were found to be 20.82% higher than in the broilers fed with concentrated feed (control) (76.25 µg/dl) and serum Fe concentrations of broilers fed with brome grass + thyme (95.88 µg/dl) were found to be 25.74% higher than in the broilers fed with concentrated feed ($p < 0.05$). It was also found that serum Fe concentrations increased ($p < 0.05$) by 21.00% in fresh alfalfa + thyme group (104.50 µg/dl) compared to control (86.88 µg/dl) and 16,54 % in 50% alfalfa + 50% brome grass group (101.25 µg/dl) compared to control group at 84th day. Taschetto et al (2017) reported that $FeSO_4$ administration into broiler breed hens at different doses (24.6, 48.6, 74.3, 99.6, 125.6, and 148.2 ppm Fe), increased egg yolk Fe content and serum haemoglobin concentrations in a dose dependant manner; egg yolk Fe concentrations increased by 22.87% in 148,2 ppm Fe supplemented group compared to Fe deficient group (24.6 ppm). It is also reported (Abbasi et al 2015) that serum Fe concentrations increased from 496 ppm to 619 ppm (24.79% increase) after 82 ppm $FeSO_4$ administration compared to 37 ppm $FeSO_4$ administration, which 82 ppm was the highest dose. They also reported significantly higher Fe concentrations in liver (17.19 % increase), bone marrow (22.05% increase), spleen (17.19 % increase) and egg yolk (14.62 % increase) after 82 ppm $FeSO_4$ administration compared to 37 ppm. It can be clearly suggested that Fe supplementation results in higher Fe concentrations both in serum and tissues of chickens. In this study, we think that it is important to increase the blood serum Fe concentrations by 20-25% without any Fe supplementation to the diet. Vitamin C is known to increase the absorption of Fe from intestinal mucosal cells. It is thought that vitamin C accomplishes this effect by reducing ferric ($Fe^{+3}$) form to ferro ($Fe^{+2}$) form and by inhibiting the formation of insoluble and non-absorpable Fe complexes (Teucher et al 2004). In a study of broilers (Çınar et al 2014), vitamin C added to the basal diet was reported to increase serum Fe concentrations significantly. Vitamin C content of clover is reported to be 22.11 mg/100 g wet weight (Kim et al 2017) whereas in thyme, it was reported to be 16.3 mg/100 g wet weight in the first year harvest (Baranauskine et al 2003). In this study, broilers fed with green leafy plants (alfalfa, brome grass and thyme) are thought to have taken vitamin C to increase Fe absorption. In addition, a positive correlation has been reported between sunlight and vitamin C synthesis in plants: vitamin C ratio of the Trifolium incarnatum (a legume-like clover) grown in a bright environment was higher (20.5% higher) than grown in a dark environment (Arın et al 2014). It is reported that Vitamin C concentrations in plants decrease with the aging of the plant, while it is high in fresh plants (Bartoli et al 2000). Considering that the green plants used in this study were fresh and in bright environment, it is thought that broilers, grown in the free-range system, take more Vitamin C with fresh green plants compared to control group (group fed with concentrated feed), leading to increased Fe absorption and serum Fe concentrations. Iron deficiency, one of the most common deficiencies in the world, is one of the leading risk factors for some diseases and deaths and is thought to affect about 2 billion people in the world, especially women (Abbaspour et al 2014). Iron deficiency is largely caused by poor Fe absorption from dietary sources. Total Fe concentration in chicken meat is approximately 0.59 mg/100 g whereas it is 2.09 mg/100 g in beef (Lombardi-Boccia et al 2002). Because the calorie, fat and cholesterol content of beef is higher than chicken meat, chicken meat is considered as low fat and protein-rich meat (Demirulus and Aydın 1995). Therefore, increasing the Fe content of chicken meat will make it more advantageous than beef. It is possible to obtain poultry products with high Fe content by the addition of Fe to the ration, but also the dose and type of Fe (organic or inorganic) applied is important. Fe-methionine chelate (organic) and $Fe\text{-}SO_4$ (inorganic) were added to the rations in order to increase Fe concentrations in chicken muscle, eggs, and liver. It was stated that Fe concentrations increased significantly in the muscle, egg, and liver with Fe supplementation where Fe-methionine chelate was the most productive Fe preparation in terms of bioavailability. As supplementation with Fe over 200 pmm did not result in enrichment of tissue Fe content, it is suggested not to add Fe into the diet higher than 200 ppm (Seo et al 2008). Also, The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP 2013) limits the maximum Fe level to 450 mg Fe/kg feed in order to avoid the harmful effects of Fe, which can be added to poultry feed. In this study, obtaining high blood Fe concentrations in all trial groups can be considered as an advantage over free-range system compared to closed system. Therefore, it is a fact that Fe- rich poultry products will lead to a very important preference reason for consumers. This study suggest that it would be possible to obtain Fe-rich meat, liver, spleen and egg products from poultry grown with fresh alfalfa, bromegrass and thyme, in the free range system.

On the 42nd day of the study, serum Mg concentrations in the 2nd, 3rd and 4th groups were determined higher than in the control group. In the literature searches, there was no study related to the relationship between vegetative nutrition and serum Mg concentrations. In a study (Gümüş et al 2017), it was reported that plasma Mg concentrations increased significantly with the addition of thyme oil to the feed in quail. Magnesium contributes to the structural development of bone and is necessary for the synthesis of DNA, RNA and antioxidant glutathione. Magnesium also plays an important role in the active transport of calcium and potassium ions from cell membranes leading to nerve impulse conduction and muscle contraction. Hypomagnesemia is seen in 2% of the general population and 10-20% of hospitalized patients (WHO 2018). In Mg deficiency, drowsiness, chills, and tetany are observed due to frequent accompanying hypokalemia and hypocalcemia (Shills 1969). As in Fe, broilers that are grown in free range system with high blood Mg concentrations will cause more Mg in their products. Therefore, it is a fact that the poultry products rich in Mg will lead to a very important preference reason for consumers.

In a intensive breeding system, it has been reported that turkeys fed with the clover addititition (1.5 %) had decreased blood triglyceride and total cholesterol concentrations compared to the group given the basal diet (Poultry feeding standard) (Krauze and Grela 2010). Also Ohshima et al (1996) reported that alfalfa reduced trigliceryde concentrations in chicks. Given that the total lipid concentrations constitutes triglyceride and total cholesterol values, decreased total lipid concentrations in 2nd group of 84th day in this study, which alfalfa was dominant, seems similar with the literature (Ohshima et al 1996, Krauze and Grela 2010). On the other hand, this similarity was not observed at 42nd day groups which increased total lipid concentrations were determined. This may be due to the age of the broilers. When these results are evaluated, it can be suggested that feeding with alfalfa decrease total lipid concentrations by decreasing total cholesterol and triglyceride concentrations.

Urea is the excretion product of ammonia through kidneys, which is the result of the breakdown of the proteins into amino acids in liver. Normally, urea is found in very low concentrations in chicken plasma, which the concentrations increase in dehydration conditions, and it was emphasized that urea may be important in diagnosis as a marker of kidney diseases in pigeons (Lumeij and Kantor 1987). In this study, there was no change in the urea concentrations of the 42th day trial groups, whereas a significant increase was detected in the alfalfa group (2nd group) only at the 84th day trial.

Uric acid is the final product of nitrogen catabolism in poultry (Sakas 2002). In a study (Miles and Featherson 1976) in chicks, they examined uric acid excretion as an indicator of protein quality in the diet and itwas found that chicks, which fed with rations containing high quality protein supplemented with methionine, lysine, and arginine, grew faster and with better performance and had less uric acid excretion (with less uric acid production). The data also reveal that the organism's quality protein digestion and uric acid excretion are two important parameters for the evaluation of protein quality; as protein efficiency increases, the level of excreted uric acid decreases (Hevia and Clifford, 1977). In a system with intensive breeding, uric acid concentrations of turkeys fed with alfalfa (1.5% and 3.0%) addition to the basal diet (Poultry feeding standart) were reported to significantly decrease ($p < 0.05$) compared to the control group (Krauze and Grela 2010). In the presented study, decreased uric acid concentrations of the 2nd, 3rd and 4th groups in animals slaughtered on the 84th day showed the importance of free range breeding with different grassland plants in terms of protein quality.

The increase in blood LDH and CK activities in animals can give information about damage to liver, heart and muscles (Sakas 2002). Serum LDH and CK activity are related to liver and muscle tissue, and their activities can vary significantly in liver diseases, muscle disorders and stress-induced muscle destructions (Hocking et al 2001, Sakas 2002, Tang et al 2013, Özhan and Şimşek 2015). In the study, except decrease of LDH in 2 group (2nd and 3rd groups on 42nd day), there was no change regarding LDH and CK activities. It is reported that (Sandercock et al 2001) muscle damage during acute heat stress caused an increase in plasma CK concentrations in 35 and 63 day-old poultry. The trial was carried out in June-July-August. Considering that in both trials, broilers were walking in the free range system between 07.00-19.00 in the daytime, unchanged LDH and CK activities may show that at the time of the study, broilers were not exposed to heat stress in the free range system.

## Conclusion

In the experiment, obtaining high blood Fe and Mg concentrations due to feeding with different ration (alfalfa+thyme, bromegrass+thyme, alfalfa+bromegrass+thyme), it can be suggested that broilers fed with green plants in the free system can produce winged products with high Fe and Mg content. It is also suggested that free-range system has advantages over the closed system in terms of animal welfare.

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## Conflict of Interest

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evalu-

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## Ethical Approval

This study was approved by the Selcuk University Veterinary Faculty Laboratory Animal Production and Research Center Ethic Committee (No: 29.03.2016-2016/33)

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