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# Comparative Evaluation of Biochemical Components of Blood Serum and Toxicological Parameters of Kivircik Lambs Fed on Conventional and Organic Fodder

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**Abstract.-** Seral biochemical components of 40 Kivircik lambs fed on conventional and organic fodder were analyzed and compared. It was found that there were no significant differences in serum urea, uric acid, total cholesterol, albumin, sodium, potassium, chloride and β-globulin fraction, with the exception of glucose ($p<0.001$), triglyceride ($p<0.05$), total protein ($p<0.01$), globulin ($p<0.001$), albumin ($p<0.001$), $\alpha_1$-globulin ($p<0.01$), $\alpha_2$-globulin ($p<0.01$), and γ-globulin ($p<0.001$) fractions between conventionally raised and organically raised groups. The analysis of muscle, liver, feed and drinking water for lead, cadmium, aflatoxin and ochratoxin A showed no significant differences between organic and conventional groups, however ochratoxin A level in clover may cause adverse effects on lambs' health.

**Keywords:** Organic animal, protein fractions, cadmium, aflatoxin, pesticides.

## INTRODUCTION

Organic farming is a form of agriculture that relies on crop rotation, green manure, compost, biological pest control, and mechanical cultivation to maintain soil productivity and control pests, excluding or strictly limiting the use of synthetic fertilizers and synthetic pesticides, plant growth regulators, livestock feed additives, and genetically modified organisms. Organic breeding is to choose plant and animal species that are resistant to disease and adapted to local conditions. Organic livestock feed should contribute to the health and well-being of the individual animal. For this reason, organic farmers provide their livestock with organically grown feed that not only helps their animals grow and produce, but also helps to improve the animal's health and welfare. Because the quality and composition of organic livestock feed is so important to the production of organic meat and other animal products, these factors are strictly regulated (Vaarst *et al.,* 2004; Gibson *et al.,* 2007)

Relative and total amounts of serum biochemical changes and protein fractions are affected by nutritional, physiological status, infections and inflammation, and some toxicological parameters are therefore important health indicators. The albumin and globulin components of serum protein are composed of separate albumin, $\alpha_1$-, $\alpha_2$-, β- and γ-globulin fractions. Usually, decreased albumin precedes the development of generalized hypoproteinemia in dietary protein deficiencies (Bell and Freeman, 1971; Kaneko, 1989; Wedler *et al.,* 1998; Werner and Reavill, 1999).

Lately, organic farming and breeding are arising in the world. Organic breeding can supply more natural and better meat quality, and improve animal's health and welfare. Thus, the objective of this study was to compare some biochemical and toxicological parameters between organically and conventionally bred Kivircik lambs.

## MATERIALS AND METHODS

*Animals and feeding*

This study was performed using 40 Kivircik lambs, which were obtained from Marmara Animal Research Institute. The specimens were grouped in two. Group one had 10 male and 10 female Kivircik lambs that were the offspring of Kivircik sheep grown conventionally. Group two consisted of 10 male and 10 female lambs that were the offspring of organically grown Kivircik sheep.

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All animals were weaned at 90 days of age. The study was ended when the animals reached a live weight of 35 kg. All the lambs went through adaptation period for 2 weeks before the study. The lambs in the conventional breeding group were given a mix of conventionally grown barley, sunflower meal, salt, limestone, a vitamin-mineral mixture and, 15.67 % crude protein and 2432.75 kcal/kg ME. They were also given 100 grams of dry clover (14.60 % crude protein and 1410.03 kcal/kg ME) to prevent digestion problems. The lambs in the organic breeding group were fed in the natural pastures of the organic sheep unit. They were fed organically grown barley (11.40% crude protein and 1735.66 kcal/kg ME) in addition to intensive food during periods when the pasture was insufficient. As intensive feed, a mix of certificated barley (Ecocert SA F-32600), which is produced organically, and a lamb feed made from sunflower meal, limestone, salt, vitamin-mineral mixture, all of which are produced conventionally, was used. Feed and drinking water were distributed *ad libitum.* Feed consumption and weight gain of lambs were monitored by a controlled weigh-in performed in 14 day periods. The nutritional contents of the feedstuff used are shown in Table I.

**Table I.- The composition and nutrient matters of feedstuff used in the feeding of Kivircik Lambs.**

| Feeds (%) | Conventional (Group I) | Organic (Group II) |
|---|---|---|
| Organic barley | - | 78.0 |
| Barley | 78.0 | - |
| Sunflower meal | 20.0 | 20.0 |
| Limestone | 1.4 | 1.4 |
| Salt | 0.5 | 0.5 |
| Vitamin-mineral mixture* | 0.1 | 0.1 |
| **Nutrient matters** | | |
| Dry matter (%) | 87.51 | 87.46 |
| Crude ash (%) | 3.33 | 3.30 |
| Organic matters (%) | 84.18 | 84.16 |
| Crude protein (%) | 15.67 | 14.37 |
| Ether extract (%) | 1.86 | 2.18 |
| Crude fiber (%) | 11.10 | 10.16 |
| Nitrogen free extra matters (%) | 55.55 | 57.44 |
| Metabolic energy (kcal/kg) | 2432.75 | 2475.39 |

*Concentration per kg of Vitamin-Mineral Mixture: Vit. A, 15.000.000 mg; Vit. D, 3.000.000 mg; Vit. E, 30.000 mg; Mn, 50.000 mg; Fe, 50.000 mg; Zn, 50.000 mg; Cu, 10.000 mg; Co, 200 mg; I, 800 mg and Se, 500 mg.

*Housing and pasture*

The conventional feeding part of the study was performed in the Research and Practice Sheep Fold of Marmara Research Institute. Male and female lambs were sheltered in different divisions that had semi-automatic feed-boxes and water cups. The organic feeding was carried out in the Organic Sheep Unit. Male and female lambs were held separately in the pasture field during the day and kept separated in different divisions in the sheep-fold at night. Wooden feed-boxes and concrete water cups were used for organically raised lambs. The shelter and pasture plans in the organic sheep unit were created taking into consideration the organic agriculture regulations (Anonim, 2005). The natural pasture in the organic sheep unit was used for organic feeding. Table II shows the nutritional contents and ME levels of the crude nutrient matters (AOAC, 1990) of feed samples taken from the pasture during different periods.

**Table II.- The content and metabolic energy levels of the crude nutrient matters of feed samples taken in the different periods from the pasture.**

| The crude nutrient matters | 24 May to 05 July | 05 July to 16 August | 16 August to 24 Sept. |
|---|---|---|---|
| Dry matter (%) | 49.41 | 90.92 | 93.02 |
| Crude ash (%) | 11.61 | 9.41 | 10.32 |
| Organic matters (%) | 88.39 | 90.59 | 89.68 |
| Crude protein (%) | 8.52 | 7.81 | 6.26 |
| Ether extract (%) | 2.15 | 0.80 | 0.85 |
| Crude fiber (%) | 30.72 | 38.79 | 42.14 |
| Nitrogen free extra matters (%) | 46.99 | 43.19 | 40.43 |
| Metabolic energy (kcal/kg) | 1755.73 | 1450.96 | 1280.93 |

*Collection of sample and laboratory analyses*

At the end of the study, blood samples were taken from vena jugularis externa using vacutainer blood collection tubes and, transported on ice chest to the laboratory for analysis. Blood samples were centrifuged at 3000 rpm for 5 min, and serum

separated was stored at -20˚C for later analysis. Serum albumin, $\alpha_1$-globulin, $\alpha_2$-globulin, β-globulin and γ-globulin fractions were determined by Biotec Fischer SPE Agarose Gel Electrophoresis Cit (Code No: 370501) (BIOTEC-FISCHER GmbH, Daimlerstrasse 6, Reiskirshen, Germany). Densitometry scan operations were performed by using Biotec Fischer TurboScan 1.6.2 program. Serum glucose (Integra 2172682), total protein (Integra 2053039), total cholesterol (Integra 2055643), triglyceride (Integra 2144620), blood urea nitrogen (Integra 2055660), uric acid (Integra 2054728), albumin (20737461), globulin levels were measured by using Roche Cobas Integra 400 Plus auto analyzer. Sodium, potassium and chloride levels were determined by using Ion Selective Electrode (ISE).

A total of 38 muscle (taken from Musculus semitendinosus) and liver samples were taken from 19 organically raised lambs (9 male and 10 female) and 19 conventionally raised lambs (10 male and 9 female). In addition, concentrated feed, other feed (pasture, barley and clover) and drinking water samples were taken. Muscle, liver, feed and drinking water samples were analysed for lead and cadmium by graphite furnace atomic absorption spectrometer (GF-AAS), (Perkin Elmer Analyst 700, USA) according to the procedure described by AOAC method (AOAC, 2000). Aflatoxins ($B_1$ and total) and ochratoxin A analysis in feed and feed materials were performed by High Performance Liquid Chromatography (HPLC) system (Hewlett Packard, 1100) by using R-Biopharm Rhone Ltd (2003) and AOAC method. Pesticide analyses in feed were done by Gas Chromatography system (Agilent 6890 GC, USA; GC-ECD, GC-FPD, GC-MS, GC-NPD) by using the AOAC method.

*Statistical analysis*

The statistical analysis to estimate serum protein fractions, some biochemical changes and toxicological parameters in organically bred Kivircik was performed by the statistical software program SPSS running "Analyze of One-Way Anova" test. Sex (male/female) and groups (conventional/organic) were used as fixed factors. The model also included all main effect and their two-way interactions (SPSS for Windows Release 10.0.1., 1989-1999).

## RESULTS

Biochemical parameters (glucose, urea, uric acid, total cholesterol, triglyceride, total protein, albumin, globulin, sodium, potassium, chloride) and the serum protein fractions (albumin, $\alpha_1$-globulin, $\alpha_2$-globulin, β-globulin and γ-globulin) are shown in Table III. It was found that, there are no significant differences in serum urea, uric acid, total cholesterol, albumin, sodium, potassium, chloride and β-globulin fraction, with the exception of the glucose ($p<0.001$), triglyceride ($p<0.05$), total protein ($p<0.01$), globulin ($p<0.001$), albumin fraction ($p<0.001$), $\alpha_1$-globulin ($p<0.01$), $\alpha_2$-globulin ($p<0.01$), γ-globulin ($p<0.001$) fractions between organic breeding and conventional breeding groups.

While serum urea, uric acid, total cholesterol levels, and all protein fractions were not significantly different for male and female (sex) specimen in both groups, serum glucose ($p<0.01$), triglyceride ($p<0.001$), total protein ($p<0.01$), albumin ($p<0.05$), globulin ($p<0.01$), sodium ($p<0.01$), potassium ($p<0.01$) and chloride ($p<0.01$) levels were found to be statistically different. Group X Sex interaction between serum biochemical parameters [urea ($p<0.05$), triglyceride ($p<0.05$), globulin ($p<0.01$), and albumin fraction ($p<0.001$), γ-globulin ($p<0.001$)] of organically grown Kivircik showed significant difference, except for the serum glucose, uric acid, total cholesterol, total protein, albumin, sodium, potassium, chloride, and $\alpha_1$-globulin, $\alpha_2$-globulin and β-globulin fractions.

In toxicological investigations, lead was detected in 47% of muscle samples, while cadmium was not detected in any of the samples. Lead was found in all liver samples, whereas, cadmium was found in only 36% of the liver samples. Lead and cadmium results from a total of 38 muscle and liver samples, feed and drinking water samples are presented in Table IV. Aflatoxins and pesticides (including 160 types) were not found in concentrated feeds, pasture herb, barley, clover, but ochratoxin A was found as 8.7 μg/kg in pasture herb and as 165.42 μg/kg in clover.

**Table III.- Serum biochemical parameters and protein fractions in Kivircik Lambs feeding and breeding for organic production.**

| Biochemical parameters | Conventional feed | | | Organic feed | | |
|---|---|---|---|---|---|---|
| | Male | Female | Mean | Male | Female | Mean |
| Glucose (mg/dl) | 67.70±3.97 | 61.60±16.18 | 64.65±11.89[a**] | 99.50±13.60 | 79.20±14.97 | 89.35±17.38[b] |
| Urea (mg/dl) | 20.77±2.01 | 17.78±4.61 | 19.27±3.78 | 18.15±4.01 | 19.75±2.74 | 18.95±3.44 |
| Uric Acid (mg/dl) | 0.82±0.06 | 0.80±0.03 | 0.81±0.44 | 0.85±0.06 | 0.82±0.05 | 0.82±0.61 |
| T. cholesterol (mg/dl) | 46.60±7.69 | 43.00±11.37 | 44.80±9.62 | 48.30±7.33 | 43.20±8.12 | 45.75±7.97 |
| Triglyceride (mg/dl) | 14.67±3.75 | 13.73±2.87 | 13.20±3.58 | 18.91±2.44 | 16.86±3.34 | 15.38±4.60 |
| T. protein (g/dl) | 6.82±0.37 | 6.33±0.86 | 6.57±0.67[a*] | 7.56±0.45 | 6.82±0.80 | 7.19±0.74[b] |
| Albumin (g/dl) | 4.08±0.20 | 3.61±0.54 | 3.84±0.46 | 3.79±0.11 | 3.76±0.46 | 3.77±0.33 |
| Globulin (g/dl) | 2.74±0.20 | 2.72±0.34 | 2.73±0.27[a**] | 3.77±0.43 | 3.07±0.51 | 3.42±0.58[b] |
| Sodium (mEq/l) | 148.10±3.72 | 128.70±31.88 | 138.40±24.23 | 145.70±3.83 | 133.60±16.58 | 139.65±13.25 |
| Potassium (mEq/l) | 4.29±0.33 | 3.86±1.01 | 4.07±0.76 | 4.76±0.35 | 3.90±0.60 | 4.33±0.65 |
| Chloride (mEq/l) | 108.00±3.68 | 94.20±23.54 | 101.10±17.86 | 106.30±2.00 | 96.80±12.07 | 101.32±14.20 |
| **Protein Fractions (%)** | | | | | | |
| Albumin | 59.63±3.32 | 55.69±3.89 | 57.66±4.06[a**] | 51.00±3.43 | 55.22±2.87 | 53.11±3.76[b] |
| Globulin $\alpha_{1}$- | 6.66±1.59 | 7.66±1.55 | 7.16±1.61[a*] | 5.75±1.47 | 5.97±0.84 | 5.86±1.17[b] |
| Globulin $\alpha_2$ | 11.81±0.88 | 11.92±1.46 | 11.86±1.18[a**] | 10.50±1.87 | 10.72±1.22 | 10.61±1.54[b] |
| Globulin β- | 3.65±0.86 | 3.91±0.22 | 3.78±0.63 | 3.78±0.71 | 3.32±0.77 | 3.55±0.76 |
| Globulin γ- | 18.25±2.58 | 20.82±2.53 | 19.53±2.82[a**] | 28.97±5.13 | 24.77±3.38 | 26.87±4.75[b] |

[Male (n=10); Female (n=10)], Mean ± SEM and range for each value.
[a,b] Means within a row with the different letters are significantly different, ;*p<0.01, **:p<0.001

## DISCUSSION

The scientific information about the nutrient requirements and serum protein fractions of organic sheep are not sufficient. Changes in the constituents of the individual protein fractions and changes in the relative amounts of each fraction in serum can be observed in many diseases, nutrition-related problems (Wedler *et al.,* 1998). On the end of the experimental periods, the albumin fractions between conventional and organic groups were found as 57.66 and 53.11% in this study, respectively (Table III). This parameter was higher in the conventional group. Serum albumin fraction was reported as 51% by Kessabi and Lamnaour (1981) in Timahdite sheep, 45% by Copland (1986) and 38% by Keay and Doxey (1984) in Scottish Blackface X Cheviot Gimmers.

The $\alpha_1$-globulin fractions in the present study were found lower in organic group than conventional group. The data were identical when compared with results obtained from a study reported by Keay and Doxey (1984) in 1.5-2 years old sheep. The values of the mean serum $\alpha_2$-globulin fractions (11.86 and 10.61% in conventional and organic groups, respectively) were identical with reported (11.7%) by Kessabi and Lamnaour (1981). The α-globulin fraction is the most rapidly migrating of all the globulins, and in most species, except in ruminants, it migrates as $\alpha_1$ (fast) and $\alpha_2$ (slow) fraction. In general, the $\alpha_1$-globulins are smaller than the $\alpha_2$, but there appears to be no functional separation between the two fractions (Kaneko, 1989). Serum γ-globulin fraction values were determined as 19.53 (organic group) and 26.87% (conventional group). The data in present study were significantly higher ($p<0.001$) for organic group compared to conventional group. This parameter was found as 36.93% by Copland (1986). Deficiencies in gamma globulin fraction can occur with immunodeficiency states, overwhelming infection, protein-losing GI diseases and severe inanition. Gross hemolysis may show a severely restricted (usually gamma) spike composed of hemoproteins that can be misinterpreted as being clinically important.

**Table IV.- Pb and Cd levels in muscle, liver, feeds and drinking water of organic and conventional groups.**

| Groups | n | Mean±SEM (mg/kg) | | Minimum (mg/kg) | | Maximum (mg/kg) | |
|---|---|---|---|---|---|---|---|
| | | Pb | Cd | Pb | Cd | Pb | Cd |
| **Muscle** | | | | | | | |
| Organic (Male) | 9 | 0.003±0.003 | - | 0.031 | - | 0.031 | - |
| Organic (Female) | 10 | 0.012±0.003 | - | 0.010 | - | 0.027 | - |
| Total organic (Male + Female) | 19 | 0.008±0.003 | - | 0.010 | - | 0.031 | - |
| Conventional (Male) | 10 | 0.004±0.002 | - | 0.003 | - | 0.021 | - |
| Conventional (Female) | 9 | 0.009±0.004 | - | 0.008 | - | 0.036 | - |
| Total conventional (Male + Female) | 19 | 0.007±0.002 | - | 0.003 | - | 0.036 | - |
| **Liver** | | | | | | | |
| Organic (Male) | 9 | 0.029±0.007 | - | 0.002 | - | 0.064 | - |
| Organic (Female) | 10 | 0.033±0.006 | 0.005±0.002 | 0.017 | 0.001 | 0.075 | 0.010 |
| Total organic (Male + Female) | 19 | 0.031±0.004 | 0.003±0.001 | 0.002 | 0.001 | 0.075 | 0.010 |
| Conventional (Male) | 10 | 0.016±0.005 | 0.004±0.002 | 0.001 | 0.002 | 0.049 | 0.014 |
| Conventional (Female) | 9 | 0.034±0.007 | 0.001±0.001 | 0.008 | 0.008 | 0.054 | 0.008 |
| Total conventional (Male + Female) | 19 | 0.025±0.005 | 0.003±0.001 | 0.001 | 0.002 | 0.054 | 0.014 |
| **Feed** | | | | | | | |
| Concentrated feed (Organic) | 1 | 0.050 | - | | | | |
| Pasture herb (Organic) | 1 | 0.173 | - | | | | |
| Barley (Organic) | 1 | - | 0.022 | | | | |
| Total organic (feed + pasture + barley) | 3 | 0.074 | 0.007 | | | | |
| Concentrated feed (Conventional) | 1 | 0.119 | 0.036 | | | | |
| Clover (Conventional) | 1 | - | 0.022 | | | | |
| Total conventional (feed + cloverl) | 2 | 0.064 | 0.022 | | | | |
| **Drinking water** | | | | | | | |
| Water (Organic) | 1 | 6.7µg/L | <1µg/L | | | | |
| Water (Conventional) | 1 | 3.5µg/L | <1µg/L | | | | |

- Non detectable.

The variability observed in sheep could have several causes, such as age, sex, time of the last feeding. Mean serum glucose concentrations in organic group (89.35±17.38 mmol/l) were higher than in conventional group (64.65±11.89 mmol/l). Glucose level in organic group is statistically ($p<0.001$) different than normal range for reported glucose (50-80 md/dl) by Karagul *et al.* (1999). Total protein concentrations in our study were determined as 6.57±0.67 g/dl (conventional group) and 7.19±0.74 g/dl (organic group). Although changes in serum total protein concentrations between the groups were small, the concentration in conventional group was lower than in organic group. The values recorded in this study are similar to those reported by Karagul *et al.* (1999) and Mert (1996). The protein concentration will increase when protein intake exceeds the requirement for maintenance and growth. Organic group, compared to conventional group, show significantly ($p<0.001$) higher levels of serum globulin in the present study.

Organic food production has gained a tremendous momentum in recent years since organic nutrition might play an important role in human health. Consumers prefer organic foods due to safety of these food product types. In this study, lead and cadmium levels in feed, drinking water, muscle and liver, and aflatoxins, ochratoxin A and pesticide levels in feed were investigated. According to our findings (Table IV), mean lead concentration of organically raised lamb group (0.008±0.003 mg/kg) was slightly higher than

conventionally raised lamb group (0.007±0.002 mg/kg), however this difference was found to be insignificant ($p>0.05$). Statistical analysis for cadmium in muscle samples could not be done because cadmium was not detected in muscle samples. Differences between organically raised lamb group and conventionally raised lamb group in the liver samples for lead and cadmium concentrations were not significant ($p>0.05$).

Maximum acceptable lead and cadmium levels in European Union (2006) and Turkey (2002) for muscle (excluding offal) were 0.1 mg/kg and 0.05 mg/kg (wet weight), respectively, and acceptable cadmium level (no limit for lead) for liver was 0.5 mg/kg (Commission Regulation, 2006; Turkish Food Codex, 2002). Detected lead and cadmium levels in muscle and liver samples (Table IV) were mucher low than the acceptable lead and cadmium levels. Therefore these samples posed no risk to human health from these aspects. Maximum acceptable lead concentrations (no limit for cadmium) in Turkey (Keay and Doxey, 1984; Turkish Food Codex, 2003) for feed and feedstuffs (as dry weight) were 5 mg/kg and 10 mg/kg, respectively. Our feed and drinking water lead levels were dramatically lower (Table IV) than the indicated limits. Probably, lead and cadmium concentrations in our muscle and liver samples were low, as well.

Although aflatoxin $B_1$ and total aflatoxin were not detected in feed samples, ochratoxin A level was high (165.42 µg/kg) in clover which was used in feed for conventionally raised lamb group. In European Union and Turkey, there is no indicated limit for ochratoxin A levels in feed, but for foods, this level is 5 µg/kg for raw cereal grains and 3 µg/kg for all products derived from cereals (Turkish Food Codex, 2003; Commission Regulation, 2005). Although aflatoxin levels are not risky for animal healthy, the ochratoxin A level may be harmful.

In conclusion, because the differences in the levels of such serum protein fractions and biochemical parameters between the two groups are not biochemically significant, further study needs to be performed to determine the impact of the production parameters and the body condition scores of the animals. Toxicologically, the differences are not significant between organically raised and conventionally raised lamb groups. The parameters investigated in this organic breeding posed no risk to human health. On the other hand ochratoxin A level in clover may cause adverse effects in lambs' health.

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