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# The Effect of Chromium Added into Basal Diet on Serum Total Protein, Urea, Triglyceride, Cholesterol and Serum and Tissue Chromium, Zinc, Copper Levels in Rabbits[1]

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**Abstract:** This study was conducted to determine the effect of supplemantal dietary chromium on serum total protein, urea, triglycerides, cholesterol, and serum and tissue chromium, zinc, and copper contents of pregnant rabbits, their offspring and their young rabbits. Treatment groups consisted of chromium level as follows: Control Group no supplementation chromium into basal diet, Treatment I (200 ppb Group) contained 200 ppb of supplemental chromium into basal diet, and Treatment II (400 ppb Group) contained 400 ppb of supplemental chromium into the basal diet which contained 539.17 ppb chromium. For this purpose, at the begining of the study, 36 rabbits were assigned to 3 treatments. Blood and tissue were collected from pregnant rabbits before birth, from their offsprings before weaning and from weaning before mature. Serum total protein, urea, triglyceride, and cholesterol were analyzed using biochemistry analyzer. Diets, serum and tissue were analyzed for chromium, zinc, and copper using atomic absorption spectroscopy. Supplemental chromium had no effects (P>0.05) on serum total protein, urea, triglycerides, cholesterol levels in all generations. On the other hand, supplemental chromium increased (P<0.05) serum, liver, kidney, lung and muscle, zinc levels in all generations of rabbits fed diets suplemented with chromium, but decreased copper levels. The results of this investigation indicated that chromium supplementation to basal diet had synergistic effect on zinc levels but antagonist effect on copper levels.

Key Words: Chromium, protein, triglycerides, cholesterol, zinc, copper, rabbits.

## Tavşanlarda Diyete Krom İlavesinin Serum Total Protein, Üre, Trigliserid, Kolesterol ile Serum ve Organlardaki Krom, Çinko ve Bakır Düzeylerine Etkisi

**Özet:** Bu çalışmada, diyete krom ilave edilmesinin, gebe, yavru ve genç tavşanlarda, serum total protein, üre, trigliserid, kolesterol ile serum ve dokulardaki krom, çinko ve bakır düzeyleri üzerine olan etkisi araştırılmıştır. Araştırma gruplarını krom düzeyi aşağıdaki gibi oluşturmuştur. 539.17 ppb korm içeren basal diyete kom ilave edilmeyen gruplar kontrol grubunu, 200 ppb krom ilave edilen grup Deneme I (200 ppb) grubunu ve 400 ppb krom ilave edilen grup ise Deneme II (400 ppb) grubunu oluşturmuştur. Bu amaçla, araştırmanın başlangıcında 36 tavşan üç gruba ayrılmıştır. Gebeliğin son günlerindeki tavşanlardan, sütten kesimden kısa bir süre önce yavrulardan ve sütten kesilmiş erginlik döneminin sonundaki tavşanlardan kan ve doku örnekleri alınmıştır. Serum total protein, üre, trigliserid ve kolesterol düzeyleri otoanalizatörde; diyet, serum ve dokulardaki krom, çinko ve bakır düzeyleri atomik absorbsiyon spektrofotometrede belirlenmiştir. Tavşanlarda basal diyete krom ilavesinin serum total protein, üre, trigliserid ve kolesterol düzeyleri üzerine bir generasyon boyunca etkili olmadığı tespit edilmiştir (P>0.05). Öte yandan, basal diyete krom ilave edilmesiyle, serum, karaciğer, böbrek, akciğer ve kasların çinko düzeylerinde, bir generasyon boyunca artış belirlenirken (P<0.05), bakır düzeylerinde ise düşüş gözlenmiştir (P<0.05). Araştırma sonuçları, basal diyete krom ilavesinin krom ile çinko arasında sinerjetik, krom ile bakır arasında ise antagonistik bir etkiye neden olduğunu göstermiştir.

Anahtar Sözcükler: Krom, protein, trigliserid, kolesterol, çinko, bakır, tavşan.

## Introduction

Chromium is an essential element required for carbonhydrate, lipid, protein and nucleic acid metabolism (1, 2). Chromium is thought to be a Glucose Tolerance in animals (3) and humans (1). Some studies reported that chromium suplementation increased serum insulin, glucose, total protein and albumin (3–5). Şahin et al. (6) reported that dietary chromium did not affect serum insulin, cortisole and alkaline phosphatase in pregnant, offspring and young rabbits. On the other hand, supplemental chromium has resulted with accumulation of chromium in the liver (1, 7). Chromium has been shown to increase loin eye area and decrease fat tickness (8, 9) and to decrease the rate of fat deposition (10). There are contradictions in results among studies about suplemental chromium. Especially, there is no recommendation for chromium in pregnant, their offspring and young rabbits but, there are few studies in

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other animals. We have not found detailed research about the effects on serum total protein, urea N, triglycerides, cholesterol, chromium, zinc, copper and contents of chromium, zinc and copper levels in various tissues of pregnant rabbits, their offspring and young rabbits in generations of rabbits fed supplemental chromium.

The objective of this study was to investigate specifically the effect of supplementation of various amounts chromium to the basal diets on serum total protein, urea, triglycerides, cholesterol, chromium, zinc, copper and tissues contents of chromium, zinc and copper of pregnant rabbits, their offspring and young rabbits.

## Materials and Methods

### Animals

12 New Zeland White pregnant rabbits, their 6 offsprings, and 10 weaned rabbits were used in experiments for each group. Rabbits approved by the Veterinary Control and Research Institute of Elazığ. Before the experiment, rabbits were treated for internal and external parasites. Animals were assigned to three groups in a completely randomized design. For mating of animals, 3 male rabbits were kept in each group at the first week. Rabbits had unlimited access to feed and water. Rabbits were fed along generations.

### Dietary Treatments

In the experiment, various levels of chromium provided by chromium chloride ($CrCl_3.6H_2O$) were evaluated. Treatments consisted of 0 (control), 200 ppb (Treatment I) and 400 ppb (Treatment II) of supplemental chromium. The basal diet formulated using Meyer (11) guidelines and contained 539.17 ppb Cr/kg of dry matter. Ingredients composition and chemical analysis of the experimental diets are summarized in Table 1. Diets were given as pellet form.

### Sample Collection and Laboratory Analysis

Blood samples were collected at the termination of all generations and centrifuged at 3000xg for 10 min and serum was collected and stored at –20°C. Feed ingredients of diets were analyzed after grinding using AOAC (12) procedures and crude fiber was determined as described by Crampton and Maynard (13). Samples were analyzed for total protein, urea, triglyceride and cholesterol using biochemistry analyzer (Technicon RA–XT). Rabbits were slaughtered for tissue analysis and tissue were collected at the termination of all generations. Following exsanguination, samples of liver, lung, kidney, and muscle were obtained. These samples were freeze

Table 1. Composition and chemical analysis of basal diet fed to rabbits.

| Item | % |
|---|---|
| Alfalfa meal | 28.00 |
| Fish meal | 6.00 |
| Soybean meal | 21.00 |
| Wheat bran | 40.50 |
| Soybean oil | 3.00 |
| Mineral+Vitamins mix* | 0.50 |
| Salt | 0.40 |
| Limestone | 0.60 |
| Chemical analysis | |
| Dry matter | 89.95 |
| Ash | 7.60 |
| Organic matter | 82.35 |
| Crude fiber | 10.61 |
| Crude protein | 20.18 |
| Ether extract | 5.08 |
| Nitrogen free extract | 46.48 |
| DE, MJ/kg | 14.19 |
| Cr, ppb | 539.17 |
| Zn, ppm | 31.20 |
| Cu, ppm | 10.79 |

Provided: 12000000 IU Vit A, 2400000 IU Vit D3, 30000 mg Vit E, 2000 mg K3, 2000 mg B1, 6000 mg B2, 3000 mg B6, 15 mg B12, 8000 mg Cal. D. Panth. 40000 mg Nicotin amid, 800 mg folic acid, 50 mg biotin, 125000 mg Cholin cloride, 80000 mg Mn, 40000 Fe, 60000 mg Zn, 5000 mg Cu, 500 mg Co, 2000 mg I, 150 mg Se, 10000 mg antioxidant, 50 mg Zinc bacitracin/2.5 kg.

dried and ground with blender. Subsamples were stored at –4°C for later analysis. Feed ingredients and tissue were wet–digested and analyzed for chromium, zinc and copper content as described by Chang et al. (14) using graphite furnace atomic absorbtion spectroscopy (Shimadzu AA–660–GFA–4B–P/N 204–03154–02) and graphite tubes.

### Statistical Analysis

Differences among groups for blood metabolits and serum and tissue chromium, zinc, and copper data were analyzed by analysis of variance procedures and by a Duncan multiple–range test (15).

## Results

The effects of supplemental chromium on total serum protein, urea, triglyceride, and cholesterol concentrations are presented in Table 2. Total serum protein, urea, triglyceride and cholesterol concentrations were not different among treatments groups. Table 3 presents the effects of supplemental chromium on the chromium concentrations of serum and tissue. Data for zinc concentrations of serum and tissue values are presented in Table 4 and the supplemental chromium on copper concentrations of serum and tissue values are given in Table 5. Supplemental chromium affected concentrations of zinc and copper in serum and tissue.

Table 2. Effect of supplemental chromium on serum total protein, urea, total triglyceride and cholesterol.

| | Pregnant Rabbits Cr Level, ppb | | | | Offspring Cr Level, ppb | | | | Young Rabbit Cr Level, ppb | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM |
| Rabbits (n) | 6 | 6 | 6 | | 6 | 6 | 6 | | 10 | 10 | 10 | |
| Total protein (g/dl) | 6.32 | 6.35 | 6.30 | 0.42 | 6.41 | 6.39 | 6.42 | 0.32 | 6.38 | 6.30 | 6.35 | 0.34 |
| Urea (mg/dl) | 23.51 | 23.38 | 23.00 | 1.21 | 22.84 | 22.45 | 22.00 | 1.72 | 23.81 | 23.25 | 23.65 | 0.45 |
| Triglyceride (mg/dl) | 121.21 | 120.85 | 121.45 | 1.24 | 120.32 | 121.25 | 121.25 | 1.04 | 122.42 | 123.42 | 123.25 | 1.31 |
| Cholesterol (mg/dl) | 81.05 | 80.45 | 79.14 | 12.39 | 71.83 | 69.50 | 70.33 | 15.89 | 74.50 | 73.33 | 72.45 | 16.94 |

Table 3. Chromium concentrations of serum and tissue (dry matter basis) of rabbits, (mg/kg$^{-1}$, dry matter basis).

| | Pregnant Rabbits Cr Level, ppb | | | | Offspring Cr Level, ppb | | | | Young Rabbit Cr Level, ppb | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM |
| Rabbits (n) | 6 | 6 | 6 | | 6 | 6 | 6 | | 10 | 10 | 10 | |
| Serum | 0.012 | 0.024 | 0.027 | 0.080 | 0.028 | 0.032 | 0.044 | 0.53 | 0.035 | 0.042 | 0.044 | 0.49 |
| Liver | 0.18 | 0.26 | 0.31 | 0.17 | 0.24 | 0.28 | 0.35 | 0.25 | 0.26 | 0.38 | 0.40 | 0.20 |
| Lung | 0.18 | 0.22 | 0.30 | 0.22 | 0.14 | 0.18 | 0.21 | 0.25 | 0.18 | 0.19 | 0.24 | 0.15 |
| Kidney | 0.40 | 0.42 | 0.48 | 0.11 | 0.44 | 0.43 | 0.47 | 0.14 | 0.45 | 0.48 | 0.47 | 0.12 |
| Muscle | 0.20 | 0.22 | 0.24 | 0.18 | 0.32 | 0.34 | 0.37 | 0.12 | 0.33 | 0.35 | 0.39 | 0.12 |

Table 4. Zinc concentrations of serum and tissue (dry matter basis) of rabbits, (mg/kg$^{-1}$, dry matter basis).

| | Pregnant Rabbits Cr Level, ppb | | | | Offspring Cr Level, ppb | | | | Young Rabbit Cr Level, ppb | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM |
| Rabbits (n) | 6 | 6 | 6 | | 6 | 6 | 6 | | 10 | 10 | 10 | |
| Serum | 0.82$^a$ | 0.93$^b$ | 0.94$^b$ | 0.01 | 0.80$^a$ | 0.95$^b$ | 1.02$^c$ | 0.04 | 0.87$^a$ | 0.99$^b$ | 1.12$^c$ | 0.04 |
| Liver | 150$^a$ | 163$^b$ | 172$^c$ | 5.2 | 145$^a$ | 175$^b$ | 176$^b$ | 3.5 | 165$^a$ | 175$^b$ | 175$^b$ | 5.90 |
| Lung | 140$^a$ | 151$^b$ | 165$^c$ | 6.0 | 134$^a$ | 151$^b$ | 155$^b$ | 12.00 | 130$^a$ | 140$^b$ | 144$^b$ | 2.00 |
| Kidney | 85$^a$ | 89$^b$ | 90$^b$ | 2.12 | 72$^a$ | 81$^b$ | 85$^b$ | 4.30 | 75$^a$ | 100$^b$ | 92$^b$ | 10.20 |
| Muscle | 150$^a$ | 162$^b$ | 171$^c$ | 5.20 | 143$^a$ | 155$^b$ | 162$^c$ | 3.00 | 150$^a$ | 168$^b$ | 180$^c$ | 6.90 |

a, b, c: Means in the same row with different superscripts differ (P<0.05).

Table 5. Copper concentrations of serum and tissue (dry matter basis) of rabbits, ($mg/kg^{-1}$, dry matter basis).

| | Pregnant Rabbits Cr Level, ppb | | | | Offspring Cr Level, ppb | | | | Young Rabbit Cr Level, ppb | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM | 0 | 200 | 400 | SEM |
| Rabbits (n) | 6 | 6 | 6 | | 6 | 6 | 6 | | 10 | 10 | 10 | |
| Serum | $0.39^{a}$ | $0.26^{b}$ | $0.28^{b}$ | 0.05 | $0.34^{a}$ | $0.25^{b}$ | $0.20^{c}$ | 0.03 | $0.40^{a}$ | $0.28^{b}$ | $0.25^{b}$ | 0.04 |
| Liver | $160^{a}$ | $152^{b}$ | $143^{c}$ | 5.00 | $155^{a}$ | $143^{b}$ | $138^{b}$ | 12.00 | $157^{a}$ | $146^{b}$ | $139^{b}$ | 8.52 |
| Lung | $130^{a}$ | $123^{b}$ | $126^{b}$ | 2.00 | $135^{a}$ | $125^{b}$ | $128^{b}$ | 2.45 | $134^{a}$ | $126^{b}$ | $121^{c}$ | 4.00 |
| Kidney | $24.00^{a}$ | $21.3^{b}$ | $20.35^{b}$ | 1.22 | $23.07^{a}$ | $19.52^{b}$ | $21.25^{b}$ | 2.12 | $25.31^{a}$ | $22.13^{b}$ | $21.63^{b}$ | 2.34 |
| Muscle | $3.25^{a}$ | $3.00^{b}$ | $2.95^{c}$ | 0.26 | $3.40^{a}$ | $2.95^{b}$ | $2.86^{c}$ | 0.87 | $3.27^{a}$ | $3.00^{b}$ | $2.97^{c}$ | 0.78 |

a, b, c: Means in the same row with different superscripts differ ($P<0.05$).

## Discussion

Total serum protein, urea, triglyceride and cholesterol concentrations were not affected ($P>0.05$) by chromium supplementation in pregnant, their offspring and young rabbits for all groups (Table 2). The results of this study indicated that 200 or 400 ppb of Cr suplemented did not affected protein, urea, triglyceride and cholesterol concentrations of rabbits and these results are in agreement with other research conducted using other animals (8, 16, 17). Page et al. (16) reported that serum total protein, urea, tryglicerides were not effected by supplementation chromium. But, several studies have shown that chromium supplementation decreased serum triglycerides (4, 8). Chang and Mowat (4) reported chromium supplementations of calves did not affect serum cholesterol, protein, urea and tryglicerides. In studies with ruminants, total cholesterol concentrations did often not responded to suplemental chromium (4, 19). However, in nonruminants serum total cholesterol concentration was reduced (2, 17). On the other hand, Samsell (20) observed that serum total cholesterol concentrations seemed to increase in lambs. Amoikon et al. (21) reported that supplemental dietary chromium picolinate increased total blood cholesterol of pigs. It is shown that there are not agreement results about the serum metabolist in all research.

Supplemental chromium did not affect chromium content in serum and tissue of rabbits (Table 3). These results are in agreement with other research. Chang et al. (14) reported that chromium supplementation of steers did not affect chromium contents in rib lean, liver, rib fat and kidney. However, Anderson et al. (22) reported that chromium supplementation to turkeys showed that chromium concentrations of breast, liver, kidney were linearly increased with the increase in organic chromium supplementation. This difference may be due to the high supplementation chromium level (25, 100, 200 ppm). Suplementation of chromium in the diet did not result in toxic accumulation in any tissue. However, Şahin et al. (23) found that levels of chromium increased in the serum of sheep grazed and plants around Elazığ Ferrochromium Factory. Chromium concentration was 0.009, 0.092, 0.023 and 0.0125 µg/ml of animals and 0.850, 6.580, 2.730 and 1.858 ppm of plants in control groups (60–80 km from factory) I (around factory), II (4 km from factory) and II (8 km from factory) groups.

In the present study, zinc concentrations in serum, liver, lung, kidney, and muscle were increased ($P<0.05$) by chromium supplementation for all rabbits (Table 4). Copper concentrations in the tissue and serum were decreased ($P<0.05$) by Cr supplementation (Table 5). These results may be due to the supplemental chromium. This suggests that chromium affected the zinc and copper metabolism. Chang et all. (14), reported that supplemental chromium may influence zinc and copper metabolism indirectly affecting immunoglobulin production. Likewise, possibily, chromium may be an element participating in certain enzymes activities that the most common being zinc and copper (24, 25).

## Implications

The results of the current study indicated that supplemental chromium in the rabbits had affected serum and tissue zinc and copper concentrations of pregnant rabbits, their offspring, and young rabbits. But the results of the study indicated that supplemental chromium in the young rabbits did not effect serum total protein, urea, triglycerides and cholesterol of rabbits.

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