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IMPACT OF DIETARY CHROMIUM, ASCORBIC ACID AND THEIR COMBINATION ON PRODUCTIVE PERFORMANCE AND IMMUNE RESPONSE IN LAYING HENS

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ABSTRACT

This study was conducted to investigate the effect of increasing dietary levels of chromium, vitamin C and their combination on the productive performance and immune response of laying hens under summer season of Egypt (31.5°C). One hundred and twenty Hy-line hens were evenly distributed to four groups of 30 hens each. The first group (control) was fed a basal diet without any supplementation. The second group was fed the basal diet containing 80 mg Cr/kg and third group was fed 205 mg vitamin C/ kg diet. Finally, the fourth group was fed a basal diet containing 80 mg Cr/kg and 250 mg vitamin C/kg. When the chicks were 36 weeks of age, a delayed-type hypersensitivity test was performed. Also, blood samples were collected at 40 weeks of age for the determination of plasma calcium, plasma phosphorus and plasma cholesterol. It could be noticed that the chromium, vitamin C and their combination had no effect on body weight. The laying hens fed a basal diet containing 80 mg chromium significantly consumed less feed compared to control group. Opposite trend was noticed in the other fed diet containing 250 mg vitamin C. The higher levels of chromium, vitamin C and their combination significantly increased shell thickness, plasma calcium and plasma phosphorus compared to control fed-group. Conversely, the plasma cholesterol and egg yolk cholesterol were significantly reduced when laying hens fed a diet added higher level of chromium, compared to the control-fed group. Cell-mediated immune function, as measured by an *in viva* hypersensitivity response to an intradermally injection of a T lymphocyte-dependent mitogen (phytohemagglutinin-P), as significantly increased in the laying hens fed a diet containing chromium, vitamin C and their combination compared to the control groups. Finally, it could be noticed that the chromium and ascorbic acid supplementation at higher levels is beneficial for laying hens during heat stress to improve the performance and immunity.
Keywords: chromium, ascorbic acid, laying hens, immunity, egg quality, cholesterol

INTRODUCTION

Poultry require supplementary dietary vitamins since common feed ingredients used in poultry production do not provide adequate quantities to meet minimum requirements (Leeson and Summers, 2001). There is increasing evidence that the concentrations of trace elements required for healthy animals are often below what is required for animals experiencing an immunological challenge. In the past few years there has been a great deal of research published showing the key role of trace minerals in maximizing immunological function. Although chromium (Cr) is not currently considered an essential trace element for poultry, this micronutrient may play a nutritional and physiological role. Moreover, the National Research Council (NRC) has recommended 300 mg Cr /kg diet for laboratory animals (NRC, 1995). Currently there are no NRC recommendations for chromium (Cr) in poultry diets (NRC, 1994). Supplementation with an organic source of Cr such as chromium picolinate (Kim *et al*., 1996), if it has greater biological availability, may prove to be beneficial to birds under heat stress because birds may obtain more chromium (Cr) despite lowered feed consumption. Chromium is generally accepted as the active component in the glucose tolerance factor, which increases the sensitivity of tissue receptors to insulin, resulting in increased glucose uptake by cells. Research suggests Cr involvement in carbohydrate metabolism including glucose uptake, glucose utilization for lipogenisis, and glycogen formation (Anderson *et al*., 1991). It was hypothesized that increased glucose uptake should increase oxidation of glucose which would be otherwise converted to fatty acids and stored as triglycerides in adipose tissues. In accordance to vitamin C, poultry have the ability to synthesize ascorbic acid, or vitamin C, in their body (Chang and Mowat, 1992); hence, no recommended requirement is established by the NRC (1994). However, environmental and pathological stressors are known to alter vitamin C use or synthesis or both in the fowl (Pardue and Thaxton, 1986). Although synthesis in the neonatal chick is apparently limited (Horning and Frigg, 1979), it is generally assumed that the endogenous synthesis is adequate to meet biological demands in poultry. During certain conditions vitamin C supplementation provides benefit to poultry (Pardue and Thaxton, 1986). Several researchers observed significant improvement in growth of chicks by the addition of vitamin C under high temperature. Broilers fed diets containing vitamin C were less stressed due to having reduced body temperature and respiratory rates (Pardue and Thaxton, 1986) and showed higher feed intake (Kutlu and Forbes, 1993; Mckee and Harrison, 1995) than control birds. Substantial reports are available that show under field conditions feeding vitamin C enhanced productivity, immune response, disease resistance, and survivability under stressful conditions (Gross, 1988; Pardue *et al.*, 1985; Zulkifli *et al*., 1996). The present study was designed to evaluate the impact of chromium, vitamin C and their combination on the productive parameters and immunocompetence of laying hens under summer season of Egypt.

MATERIALS AND METHODS

This investigation was carried out at Poultry Breeding Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. One hundred and twenty 30-wk-old, Hy-line White strain were randomly assigned to four groups of 30 hens each. The hens were individually housed in individual cages, on a 16-hour light schedule. The first group (control) was fed a basal diet without any supplementation. The second group was fed the basal diet containing 80 mg Cr/kg and third group was fed 205 mg vitamin C/ kg diet. Finally the fourth group was fed a basal diet containing 80 mg Cr/kg and 250 mg vitamin C/kg. Chicks in all groups were reared under the same environmental, managerial and hygienic conditions. Feed and water were supplied *ad libitum*. The hens received a typical layer diet containing 2800 ME kcal/kg and 18% CP to meet or slightly exceed the nutrient requirement by NRC (1994). The average high and low ambient temperatures, recorded during the experimental period were 31.5 and 28.7°C, respectively.

Productive parameters: Egg number and egg weight were recorded daily from the 32 to 40 weeks of age. Internal and eggshell quality was determined at 40 weeks of age. The breaking strength was measured according to Fathi and El-Sahar (1995) which assessed the resistance of the egg to crushing. Shell thickness was measured by a micrometer. Albumen height (H) was measured by a tripod micrometer. The cholesterol content of the egg yolk was determined by using the methods of Hammad *et al*. (1996) and Berrio and Hebert (1990). The eggs were hard-boiled for 15 min, cooled immediately, stored at 2-8 C and analysed within a week for cholesterol content. The yolks were separated and sample of 0.1 g of yolks, pooled from two eggs belonging to same hen, were weighed accurately. Yolk lipids were extracted with isopropanol (4ml/ 0.1 g of yolk, Rieden-de Haen) then vortex mixed and centrifuged at 3000 rpm for 10 min. The yolk cholesterol concentration was determined in the filtered samples by UV spectrophotometer using a commercial kit. The cholesterol concentration of the egg yolk (mg cholesterol/g of egg yolk) was calculated. Plasma calcium, phosphorus and cholesterol were determined by commercial kits.

***In vivo* cell-mediated immunity:** A phytohemagglutinin-P (PHA) injection assay (Cheng and Lamont 1988) was used to evaluate *in vivo* T-cell-mediated immune response of Hy-line laying hens. Birds were injected intradermally in the toe-web with 0.5 mg of PHA (Sigma Chemical Co., St. Louis, Missouri) in 0.1 ml of phosphate buffered saline (PBS) after marking the injection site. The thickness of toe-web was measured (to nearest 0.01mm) at 0, 24, 48 and 72 hrs poat PHA-P injection. Toe-web swelling was calculated as the difference between the thickness of the wing web prior to and after (24 hrs) injection of PHA.

Statistical analysis: Data were subjected to a one-way analysis of variance with genetic group effect using the General Linear Model (GLM) procedure of SAS User's Guide, 2001. When significant differences among means were found, means were separated using Duncan's multiple range tests. Correlation coefficients were calculated to analyze the relationship between some traits.

RESULTS AND DISCUSSION

Productive parameters: Data presented in Table (1) show that the supplementation of chromium, vitamin C and their combination had no effects on body weight of laying hens. Similar results were obtained by Cupo and Donaldson (1987) with broiler chicks and Þahin *et al.* (2001) with rabbits. Conversely, Lien *et al.* (1999) found that chromium markedly enhanced weight gain due to increased feed consumption in broilers. Steele and Rosebrough (1979) reported that 20 ppm Cr supplementation improved growth rate. The laying hens fed diet with chromium, vitamin C and their combination produced significantly better egg mass and egg number, compared to the control-fed group. There was no significant difference among treated groups for egg weight, however, the egg weight of vitamin C fed group was heavier than those of other groups. Lien *et al*. (1996) found that the egg production and egg weight were not affected by chromium supplementation. The conflicting results could be attributed to the different chromium levels in both studies. With respect to feed consumption, it could be speculated that the laying hens fed higher level of chromium had significantly consumed less feed compared to control group. Supplementation with an organic source of Cr such as chromium picolinate (Kim *et al*., 1996), if it has greater biological availability, may prove to be beneficial to birds under heat stress because birds may obtain more Cr despite lowered feed consumption. Inversely, the laying hens fed diet containing higher level of vitamin C significantly consumed more feed compared to control group. The combination between chromium and vitamin C was intermediated. Uyanik *et al*. (2002) indicated that chromium supplementation to the diet of layers may be of practical value due to the reduction in feed consumption and improvement in efficiency of feed utilization.

Table 1: Productive Parameters of laying hens as affected by chromium, vitamin C and their combination.

	Treatments					
Time (hr)	**Control**	**Chromium**	**Vitamin C**	**Chromium + vitamin C**	**Pooled SE**	**Prob.**
		Body weight (g)				
30	1382	1390	1351	1368	28.70	NS
40	1429	1439	1392	1397	30.54	NS
		Egg mass (g)				
32-40	2572^{b}	2961^{a}	2975^{a}	3010^{a}	16.70	0.01
		Egg number (no.)				
32-40	42.8^{b}	48.8^{a}	48.8^{a}	49.5^{a}	0.17	0.01
		Egg weight (g)				
32-40	60.6	60.8	61.2	60.9	0.14	NS
		Feed consumption (g)				
32-40	6097^{b}	5896^{c}	6247^{a}	6229^{a}	21.13	0.05
		Feed conversion ratio				
32-40	2.39^{a}	2.00^{b}	2.11^{b}	2.08^{b}	0.02	0.01

^{a,b}Means within each row with the same letters did not significantly differ at 0.05 level of probability.

Interior and eggshell quality: Effect of higher levels of chromium, vitamin C and their combination on interior and egg shell quality is presented in Table (2). There were no significant differences among treated groups for percentages of albumen, yolk and shell. Inversely, the higher level of chromium, vitamin C and their combination significantly increased haugh unit, compared to the control-fed group. Also, the shell thickness and shell breaking strength were significantly improved by added higher levels of chromium, vitamin C and their combination.

Table 2: Interior and eggshell quality measurements of laying hens as affected by chromium, vitamin C and their combination

Item	Treatment				Pooled SE	Prob.
	Control	Chromium	Vitamin C	Chromium + vitamin C		
Egg weight, g	60.74[b]	63.30[a]	62.40[a]	63.03[a]	2.15	0.01
Albumen, %	60.61	60.45	60.51	60.16	3.59	NS
Yolk, %	30.10	30.26	30.14	30.15	0.63	NS
Haugh unit	81.56[b]	84.12[a]	84.17[a]	85.63[a]	2.14	0.01
Shell, %	9.29	9.29	9.35	9.69	0.17	NS
Shape index	77.21	77.38	77.54	78.05	1.14	NS
Shell thickness, mm	0.331[b]	0.360[a]	0.354[a]	0.355[a]	0.01	0.01
Breaking strength, kg	2.96[b]	3.16[a]	3.25[a]	3.67[a]	0.12	0.01

[a,b] **Means within each row with the same letters did not significantly differ at 0.05 level of probability.**

Blood constituents: Data presented in Table (3) show that the higher chromium and vitamin C levels significantly increased both calcium and phosphorus in plasma, compared to the control-fed group. However, the higher chromium level significantly reduced plasma cholesterol level, compared to the control-fed group. Studies in human and various animal species on the influence of Cr supplementation on lipid parameters are conflicting. Amoikon *et al*. (1995) reported that fasting plasma cholesterol concentrations in pigs were increased by chromium picolinate. Different forms of Cr reduced total cholesterol in humans (Press *et al*., 1990), pigs (Page *et al*., 1993), lambs (Kitchalong *et al*., 1995) and layers (Lien *et al*., 1996). In accordance of vitamin C, it could be noticed that the higher level of vitamin C slightly reduced plasma cholesterol, compared to the control-fed group. Moreover, the combination between chromium and vitamin C significantly reduced plasma cholesterol compared to other groups.

Egg yolk cholesterol: Egg yolk cholesterol levels of domestic fowl are known to be influenced by genetic and environmental factors (Sheridan *et al*., 1982). The dietary intake of a hen has been shown to influence the hepatic lipogenesis (Leveille *et al*., 1975), and egg cholesterol content (Beyer and Jensen, 1992). Data listed in Figure (1) show that the laying hens fed higher level of chromium or chromium and vitamin C combination had significantly lower egg yolk cholesterol, compared to the control-fed group. Lower yolk cholesterol content in the eggs collected from chromium supplemented diet fed hens than that of the controls is in agreement with the results of Lien *et al*. (1996), who found a dose-dependent reduction in the yolk cholesterol of hens fed chromium picolinate supplemented diet. Also, reduced egg yolk cholesterol concentrations may result from the reduced feed intake due to chromium supplementation (Table 1). Many studies have been done to reduce yolk cholesterol by supplementing diet with different substances (Elkin and Rogler, 1990; Mohan *et al*., 1995). There was no significant difference between control and vitamin C groups for egg yolk cholesterol. Uyanik *et al*. (2002) indicated that chromium supplementation to the diet of layers may be of practical value due to the reduced egg cholesterol content without any adverse effect on egg quality.

Table 3: Some blood constituents of laying hens as affected by chromium, vitamin C and their combination.

Item	Treatment				Pooled SE	Prob.
	Control	Chromium	Vitamin C	Chromium + vitamin C		
Plasma calcium, (mg/dl)	22.17 ^{b}	27.51 ^{a}	25.16 ^{a}	27.81 ^{a}	1.09	0.01
Plasma phosphorus (mg/dl)	7.21 ^{b}	8.94 ^{a}	7.95 ^{a}	8.66 ^{a}	0.30	0.01
Total cholesterol (mg/dl)	167.51 ^{a}	158.92 ^{b}	161.17 ^{b}	157.60 ^{b}	3.54	0.01

^{a,b} Means within each row with the same letters did not significantly differ

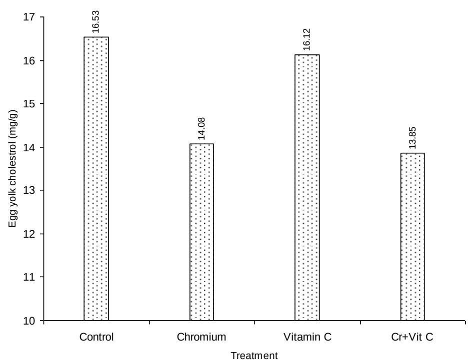

Figure 1: Egg yolk cholesterol of laying hens as affected by chromium, vitamin C and their interaction

***In vivo* cell-mediated immunity:** The PHA intradermally reaction, a T-lymphocyte-dependent response, has been well researched and has been shown to be a reliable indicator of *in vivo* cellular immunity in poultry (Goto *et al*. 1978, McCorkle *et al*. 1980). The skin response reflects a complex series of physiological events such as mitogen-receptor and lymphocyte-macrophage interactions, release of chemical mediators, cellular proliferation, and changes in vascularity (Chandra and Newberne 1977). Histologically, PHA is strongly mitogenic to T-lymphocytesa, and intradermali injections elicit macrophage infiltration and dense perivascular accumulations of lymphocytes 24 hrs postinjection in chickens (Goto *et al*. 1978, McCorkle *et al*. 1980). The increased infiltration by basophilsa and eosinophils 24 hrs post-injection has been described as a cutaneous basophil hypersensitivity response (Stadecker *et al*. 1977). *In vivo* cell-mediated immune response as measured by PHA stimulation (toe-web) is presented in Figure (2) and Table (4). There was significant difference in the mean toe-web between treatments. It could be noticed that the hens fed a diet added Cr had significantly hyper responder to PHA-P injection, compared to the control-fed group. In dairy cows chromium caused increased antiovalbumin response but did not affect the immune response to the red blood cells. These data suggest that chromium supplementation may enhance resistance to mastitis in dairy cows (Burton *et al*., 1993). Chromium reduced serum cortisol levels. Glucocorticods, which include cortisol, are known to suppress the immune system. With respect to vitamin C, it could be speculated that the vitamin C significantly increased the toe-web swelling, compared to the control-fed group. Vitamin C increases the phagocytic activity of macrophages. Huge amount of peroral intake of vitamin C (2 g/day) increased the ascorbic acid concentration of leukocytes, granulocytes and blood plasma in human (Evans, 1982). Higher lymphocyte proliferation, measured by 3H-thymidine incorporation in DNA of human lymphocytes over 18 hours after previous incubation for 2 days with concanavalin A at varying ascorbic acid concentration, proved the dose-related immunostimulant effect of ascorbic acid.

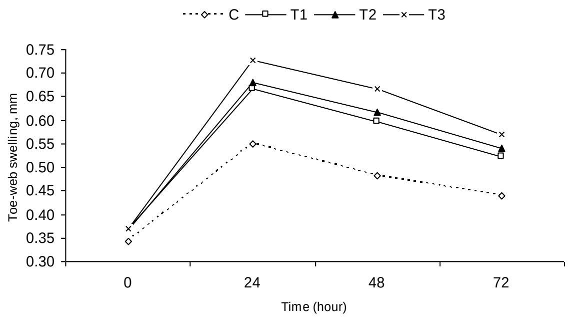

Figure 2: Toe-web swelling (mm) of laying hens as affected by chromium, vitamin C and their combination.

Table 4: Toe-web swelling (difference) of laying hens as affected by chromium, vitamin C and their combination

Time (hr)	Control	Chromium	Vitamin C	Chromium + vitamin C	Pooled SE	Prob.
	Treatment					
D1	0.21^{b}	0.30^{a}	0.31^{a}	0.36^{a}	0.02	0.01
D2	0.14^{b}	0.23^{a}	0.25^{a}	0.30^{a}	0.03	0.01
D3	0.10^{b}	0.15^{a}	0.17^{a}	0.20^{a}	0.02	0.01
		Treatment effect (%)				
		Chromium	Vitamin C	Chromium + vitamin C		
D1		+43.6	+51.6	+72.58		
D2		+61.9	+78.6	+111.9		
D3		+58.6	+79.3	+106.9		

D1, D2 and D3: toe-web swelling difference at 24, 48 and 72hrs after PHA-P injection.
Treatment effect was calculated as follows; (treated-control)/control*100

Correlations: Phenotypic correlations between eggshell quality, some blood parameters and egg yolk cholesterol are presented in Table (5). Moderate relationship between eggshell thickness and eggshell breaking strength was observed in all treatments. That is meant the eggshell thickness did not accurate indicator for breaking strength in most cases, but there are other factors affecting eggshell breaking strength such as ultrastructure eggshell. There was positive relationship between eggshell thickness and both plasma calcium and phosphorus in the control-fed group. Similar relationships, but statistically significant, were observed in other groups. The eggshell braking strength was significantly positively correlated with plasma calcium in all groups.

The same trend, but low, was observed between plasma phosphorus and eggshell breaking strength. Most of the cholesterol found in egg yolk is derived from circulating plasma lipoproteins, which are synthesized in the liver in response to hormonal changes with the onset of lay (Annison, 1971). The present result found that the egg yolk cholesterol significantly positive correlated with plasma cholesterol in control and vitamin C groups. Inverse relationship was noticed in remaining groups, whereas there was significantly negative relationship between plasma cholesterol and egg yolk cholesterol in chromium and chromium-vitamin C combination groups. Marks and Washburn (1991) found a negative correlation between yolk and plasma cholesterol. However, no correlation was found between yolk and plasma cholesterol in quail studies by Hammad *et al*. (1996).

Table 5: Phenotypic correlation coefficients among eggshell quality, some blood parameters and egg yolk cholesterol.

	Shell breaking strength	Plasma calcium	Plasma phosphorus	Plasma cholesterol	Treatment
Shell thick ness	0.37	0.48	0.35		Control
	0.42	0.75**	0.68**		Chromium
	0.35	0.62*	0.56*		Vitamin C
	0.49	0.77**	0.61*		Cr + VC
Breaking strength		0.63*	0.28		Control
		0.72**	0.31		Chromium
		0.75**	0.29		Vitamin C
		0.68**	0.35		Cr + VC
Egg yolk cholesterol				0.51*	Control
				-0.72**	Chromium
				0.53*	Vitamin C
				-0.68**	Cr + VC

*** and ** : Significant correlation at P< 0.05 and P < 0.01, respectively.**

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تـأثير إضـافة كروميـوم العليقـة وفيتـامين سـى والتوليفـة بينـهم علـى الأداء الإنتـاجي والاستجابة المناعية للدجاج البياض

علاء الدين محمد عبد المتعال

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هدفت هذه الدراسة إلى بحث تأثير زيادة مستوى عنصر الكروميوم وفيتامين سي وخليطهما على الأداء الإنتاجي والاستجابة المناعية في الدجاج البياض إثناء فصل الصيف فى مصر (31.5 درجة مئوية). استخدم عدد 120 دجاجة بياض من سلالة الناي لاين الأبيض، قسمت إلى أربعة مجاميع (30 دجاجة/مجموعة). غذين المجموعة الأولى (الكنترول) على عليقة بياض إنتاجي بدون أي إضافات. غذيت المجموعة الثانية على عليقة بياض إنتاجية + 80 مللجم كروميوم/كجم عليقة، بينما غذيت المجموعة الثالثة على عليقة تحتوى على 250 مللجم فيتامين سي/كجم، إما المجموعة الرابعة فقد غذيت على عليقة تحتوى على كل من الكروميوم وفيتامين سي. عند 36 أسبوع من العمر، تم تقدير المناعة الخلوية باستخدام مادة PHA-P. عند 40 أسبوع من العمر تم تقدير الكالسيوم والفسفور والكولسترول فى البلازما بالإضافة إلى كولسترول الصفار. أوضحت النتائج عدم تأثر وزن الجسم بإضافة عنصر الكروميوم أو فيتامين سي أو مخلوطهما. استهلكت الدجاجات المغذاة على عنصر الكروميوم كمية عليقة اقل معنويا من الكنترول، بينما شوهد عكس الاتجاه فى الدجاجات المغذاة على عليقة تحتوى على فيتامين سي. أدى زيادة مستوى عنصر الكروميوم وفيتامين سي ومخلوطهما إلى زيادة معنوية فى سمك القشرة ومستوى الكالسيوم والفوسفور فى بلازما الدم مقارنة بالكنترول. وعلى العكس من ذلك، فقد شوهد انخفاض معنوي فى مستوى الكولسترول فى البلازما وصفار البيض وذلك فى الدجاجات المغذاة على مستويات عالية من عنصر الكروميوم. أوضحت النتائج أن إضافة مستويات عالية من الكروميوم وفيتامين سي و مخلوطهما أدى إلى تحسن الاستجابة المناعية الخلوية بصورة معنوية مقارنة بالكنترول.