Effects of dietary organic chromium and vitamin C supplementation on performance, immune responses, blood metabolites, and stress status of laying hens subjected to high stocking density

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ABSTRACT The present study was carried out to investigate the effects of dietary supplementation of chromium-methionine (CrMet) and vitamin C (VC) on performance, immune response, and stress status of laying hens subjected to high stocking density. A total of 360 Hy-Line W-36 leghorn hens (at 26 wk old) were used in a $2 \times 3 \times 2$ factorial arrangement that had 2 cage densities (5 and 7 hens per cage), 3 Cr levels (0, 500, and 1,000 ppb as CrMet), and 2 dietary VC levels (0 and 500 ppm as L-ascorbic acid). The trial lasted for 12 wk. The first 2 wk were for adaptation (26 to 28 wk of age), and the remaining 10 wk served as the main recording period. In addition to performance, immune response to Newcastle disease virus (NDV) was assessed at d 7 and 14 postvaccination. Also, the birds' stress status was evaluated by analyzing appropriate plasma metabolites. The results showed that hens in cages with higher stocking density had lower hen-day egg production, egg mass, and feed intake compared with those in normal density cages ($P < 0.05$). Dietary CrMet supplementation caused significant increases in egg production and egg mass ($P < 0.01$). There were significant Cr × VC interactions related to egg production and feed conversion efficiency ($P < 0.01$); dietary CrMet supplementation was more effective in improving egg production and feed conversion ratio in VC-unsupplemented diets. Although plasma concentrations of triglycerides and high-density lipoproteins were not influenced by dietary treatments, supplemental CrMet decreased plasma cholesterol levels ($P < 0.05$). Plasma insulin and glucose levels of hens kept at a density of 7 hens/cage were significantly higher than those of hens in normal cage density ($P < 0.01$), and dietary CrMet supplementation decreased plasma concentrations of insulin ($P < 0.001$) and glucose ($P < 0.01$), with higher impacts in high stocking density-challenged hens. While high stocking density caused a marked increase in plasma corticosterone ($P < 0.01$), both supplemental CrMet and VC decreased it to near normal levels. There were significant stocking density × Cr interactions related to plasma insulin and corticosterone concentrations ($P < 0.01$); supplemental CrMet was more effective in lowering these hormones in high stocking density–challenged hens. The high stocking density challenge suppressed NDV antibody response ($P < 0.001$), while dietary supplementation of CrMet improved antibody titers against NDV at d 14 post vaccination particularly in hens kept at a density of 7 hens/cage ($P < 0.01$). From the present observations, it can be concluded that CrMet can improve laying performance largely because it alleviates harmful responses to stressful conditions.

Key words: laying hen, chromium-methionine, vitamin C, stressful condition, corticosterone

2015 Poultry Science 94:281–288
http://dx.doi.org/10.3382/ps/peu074

INTRODUCTION

That chromium (**Cr**) is an essential mineral was first demonstrated by Schwartz and Mertz (1959) in rats. Chromium is an integral component of an oligopeptide low molecular weight Cr-binding substance, chromodulin, which acts as part of the insulin signaling process across cell membranes. Stimulation of insulin's action, which is directly proportional to the Cr content of the chromodulin, occurs without a change in the insulin concentration required for half-maximal activity (Vincent, 2000). Chromium is also involved in carbohydrate, lipid, protein, and nucleic acid metabolism in different animal species (Ohba et al., 1986; McCarty, 1991; Anderson, 1997). Trivalent Cr is a component of glucose tolerance factor, which participates in glucose metabolism by enhancing the effect of insulin on various tissues (Mertz, 1993). Dietary Cr supplementation has been shown to positively affect growth performance and feed conversion ratio in growing poultry (Jackson et al., 2008; Samanta et al., 2008). Also, improved immunological responses have been reported as

Received July 26, 2014.
Accepted October 31, 2014.
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the result of Cr supplementation in broiler diets (Luo et al., 1999). In broilers, trivalent organic Cr supplementation could result in improved growth rate, feed efficiency, meat yield, and carcass quality with reduced carcass fat (Gursoy, 2000). Despite these beneficial effects, the exact nutritional recommendations for Cr in different poultry species have not been established (NRC, 1994), and the issue needs further investigation.

It has been demonstrated that stress conditions and disease occurrence increase urinary excretion of Cr (Anderson et al., 1988) and may exacerbate a marginal Cr deficiency. It has been reported that organic sources of Cr can be absorbed 20 to 30 times more efficiently than inorganic ones (Starich and Blincoe, 1983). It has also been demonstrated that stressful conditions may retard growth, change hormone release, increase disease susceptibility, lead to behavior changes, or a combination of all these items. Poultry studies have reported that dietary supplementation of Cr can alleviate the detrimental effects of heat stress (Sahin et al., 2002b, 2005) and cold stress (Sahin et al., 2001a).

Vitamin C (**VC**) is another functional nutrient involved in managing stress in farm animals (McDowell, 2000; Naseem et al., 2005). Amakye-Anim et al. (2000) reported that VC supplementation for infectious bursal disease–challenged chicks improved antibody response. Similarly, Naseem et al. (2005) observed that dietary VC supplementation for heat stress–exposed broiler chicks beneficially affected feed conversion efficiency, immune status, and relative weights of the bursa of Fabricius, thymus, and spleen. Few, if any, studies on dietary Cr × VC interaction in high stocking density–stressed laying hens have been conducted. Therefore, the purpose of this study was to investigate the effects of supplemental organic Cr (Cr-methionine, **CrMet**) and VC on performance, stress status, and immune responses of laying hens under high stocking density.

MATERIALS AND METHODS

Birds, Diets, and General Experimental Procedure

The present study was performed in the Poultry Research Station of Isfahan University of Technology (Isfahan, Iran), and all procedures used were approved by the Isfahan University of Technology Animal Care and Use Committee. A total of 360 Hy-Line W-36 leghorn hens (at 26 wk old) were used in a $2 \times 3 \times 2$ factorial arrangement of treatments that included 2 cage densities (5 and 7 hens per cage; 40 by 45 cm), 3 levels of supplemental Cr (0, 500, and 1,000 ppb as CrMet), and 2 VC levels (0 and 500 ppm as L-ascorbic acid). Chromium levels in the basal diets were 2,083 and 2,016 ppb during 26 to 32 wk and 32 to 38 wk of age, respectively, as analyzed by atomic absorption spectrophotometer (Analyst 100, Perkin-Elmer, Norwalk, CT). The first 2 wk (26 to 28 wk of age) was the adaptation period. The main trial period lasted for a total of 10 wk, from 28 to 38 wk of age. The basal experimental diets (Table 1) were formulated to provide all nutrients specified by Hy-Line W-36 recommendations (Hy-Line International, 2007). All of the diets were mixed using a micromixer, and the samples were analyzed according to AOAC (2002) standard procedures for basic chemical composition.

The birds were housed in a windowless space that had artificial light (16L:8D) throughout the duration of study. Feed and water were provided ad libitum. House temperature was maintained at 21 to 24°C throughout the trial period.

Production Performance

All eggs were collected and weighed daily on a cage basis. Feed consumption and hens' BW were measured at 33 and 38 wk of age. Mortality was recorded once observed to adjust for hen-day egg production, average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**).

Blood Biochemical Indices

At d 60 of the trial, plasma samples were obtained from 3 birds of each replicate to measure plasma concentrations of high-density lipoproteins (**HDL**), triglycerides (**TG**), cholesterol, glucose, and insulin. Plasma metabolites were analyzed with a biochemical analyzer (ERBA CHEM-5, Beijing Biochemical Instrument Company, Beijing, China) using the standard kits (Pars Azmoun, Tehran, Iran) described by Jahanian and Rasouli (2014).

Also, the plasma samples were analyzed for corticosterone levels according to the method developed by Puvadolpirod and Thaxton (2000). Plasma corticosterone concentration was determined by enzyme-linked immunoassay using a Coat-a-Count rat corticosterone RIA kit (Diagnostic Products Corporation, Los Angeles, CA 90045-5597). Because the binding property of chicks' corticosterone is different from that of rats, the standards were prepared by diluting 2,000 ng/mL corticosterone rat serum included in the kit with stripped chick plasma.

Briefly, the stripped chick plasma was prepared by collecting 200 mL of blood. The blood sample was centrifuged, and plasma was decanted into a 500 mL bottle. Then 25 g activated charcoal was added, and the mixture was incubated overnight at 4°C with automatic mild shaking. The plasma was centrifuged at $5,000 \times g$ and 4°C for 10 min and decanted. Another 25 g activated charcoal was added, and the mixture was incubated at 4°C overnight, again with mild shaking. The plasma was centrifuged for 10 min at $4,500 \times g$ and 4°C, filtered through Whatman #3 paper, and sequentially filtered through the 5 and 0.45 μm filters. The stripped

Table 1. Ingredient composition and chemical analysis of basal diets during different age (as fed basis).

Items (% unless stated otherwise)	26 to 32 wk of age	32 to 38 wk of age
Ingredients		
Corn, yellow	48.75	47.68
Soybean meal	24.55	23.21
Alfalfa meal	3.00	3.00
Barley	5.00	7.00
Wheat bran	3.00	3.00
Soybean oil	2.22	2.66
Dicalcium phosphate	1.82	1.82
Limestone	5.67	5.81
Oyster shell	4.00	4.00
Common salt	0.27	0.21
Na bicarbonate	0.20	0.20
Mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.25	0.25
DL-Methionine	0.20	0.17
L-Lysine.HCl	0.06	—
L-Threonine	0.06	0.04
Zeolite[3]	0.70	0.70
Nutrient composition		
ME (kcal/kg)	2700	2700
Crude protein	16.80	16.20
Methionine	0.45	0.42
Methionine + cysteine	0.73	0.69
Lysine	0.89	0.82
Threonine	0.70	0.63
Calcium	4.00	4.05
Non-phytate P	0.44	0.43
Sodium	0.18	0.17
Chromium (ppb)	2083	2016

[1]Mineral premix provided per kilogram of diet: Mn (from $MnSO_4.H_2O$), 66 mg; Zn (from $ZnSO_4.H_2O$), 60 mg; Fe (from $FeSO_4.7H_2O$), 30 mg; Cu (from $CuSO_4.H_2O$), 8.8 mg; I (from Ca $(IO_3)_2.H_2O$), 0.9 mg.

[2]Provided per kilogram of diet: vitamin A (from retinyl acetate), 7,700 IU; cholecalciferol, 3,300 IU; vitamin E (from DL-α-tocopheryl acetate), 12 IU; vitamin B_{12}, 0.009 mg; riboflavin, 4.4 mg; niacin, 22 mg; calcium pantothenate, 5.5 mg; menadione (from menadione dimethyl-pyrimidinol), 0.75 mg; folic acid, 0.2 mg; thiamine, 3 mg; pyridoxine, 5.5 mg; biotin, 0.04 mg; choline (from choline chloride 60%), 275 mg.

[3]Variable amounts of zeolite, chromium-methionine, and vitamin C so that the nutrient composition was similar among the experimental diets.

plasma was aliquoted and stored at −20°C. Assays were performed by adding 200 μL plasma samples or standards of stripped chick plasma to each tube, followed by 1 mL ^{125}I-labeled corticosterone. The samples were incubated overnight at 4°C. The unbound label was aspirated, and the remaining radioactivity was counted on an auto gamma counter (Puvadolpirod and Thaxton, 2000).

Antibody Response

LaSota strain vaccine was used to vaccinate the chicks against Newcastle disease virus (**NDV**) via spraying method at d 56 of the main trial period, and serum samples were collected for antibody assay at d 7 and 14 postvaccination from 3 birds per replicate. The hemagglutination inhibition test (Marquardt et al., 1984) was used to determine the antibody production response against NDV as $\log_2$ of the reciprocal of the last dilution.

Yolk Composition

Six eggs produced per cage in the last 2 d of the trial period were stored until analysis of yolk cholesterol and TG could be performed. All yolk lipids were extracted with chloroform and methanol (2:1 vol/vol) following the procedure of Folch et al. (1957). Yolk cholesterol and TG were analyzed according to the methodology of Hamil and Soliman (1994).

Statistical Analysis

An ANOVA of the data using the general linear model procedures of SAS software (SAS Institute, 1999) was done according to the $2 \times 3 \times 2$ factorial arrangement of the treatments that included stocking density and dietary Cr and VC levels as the main effect and respective 2- and 3-way interactions. Cage was the experimental unit for all measurements. The treatment means were separated by Duncan multiple range tests (Duncan, 1955) at $P < 0.05$.

Table 2. Effects of dietary vitamin C (VC) and organic chromium[1] (Cr) supplementation on the performance of high stocking density–challenged laying hens during 28 to 38 wk of age.

Cage stocking density	VC (ppm)	Cr (ppb)	Egg weight (g)	Egg production (%)	Egg mass (g/d per bird)	Feed intake (g/d per bird)	Feed conversion (g feed/g egg)
5	0	0	59.8	80.7	47.9	106.5	2.22
		500	59.4	86.4	51.3	108.4	2.15
		1,000	60.8	84.7	51.6	107.8	2.09
	500	0	59.5	83.3	50.2	105.7	2.11
		500	60.4	86.3	52.2	107.7	2.07
		1,000	59.6	86.3	51.4	109.8	2.08
7	0	0	59.4	77.3	46.1	106.6	2.33
		500	59.8	84.6	50.6	106.9	2.12
		1,000	59.9	84.8	49.8	106.9	2.12
	500	0	60.1	82.3	49.8	103.5	2.08
		500	59.9	84.2	50.4	108.4	2.15
		1,000	59.6	83.0	49.4	107.0	2.16
Stocking density (hens/cage)		5	59.9	84.4[a]	50.7[a]	107.4[a]	2.13
		7	59.8	82.9[b]	49.5[b]	106.7[b]	2.16
VC (ppm)		0	59.9	83.2	49.7	107.1	2.17
		500	59.8	84.2	50.6	106.9	2.11
Cr (ppb)		0	59.7	80.7[b]	48.5[b]	105.7[b]	2.19
		500	59.9	85.2[a]	51.0[a]	107.8[a]	2.12
		1,000	60.0	84.7[a]	50.7[a]	107.7[a]	2.12
SEM			0.53	1.10	0.95	0.80	0.04
Probability							
Stocking density			0.692	0.024	0.047	0.029	0.232
VC			0.970	0.112	0.138	0.757	0.053
Cr			0.741	0.001	0.009	0.001	0.142
Stocking × VC			0.600	0.687	0.998	0.511	0.918
Stocking × Cr			0.770	0.981	0.888	0.451	0.927
VC × Cr			0.234	0.045	0.128	0.037	0.033
Stocking × VC × Cr			0.350	0.427	0.721	0.119	0.235

[1]Chromium was added to the diets as chromium-methionine complex (Zinpro Corporation, Edina, MN).

[ab]Means with no common superscripts within the column of each classification (stocking density or Cr) are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Performance

Data on the effect of dietary supplementation of CrMet and VC on the production performance of high stocking density–challenged hens are shown in Table 2. As presented, the high stocking density–challenged hens had lower hen-day egg production, egg mass, and ADFI compared with those in normal density cages ($P < 0.05$). Consistent with the present findings, Sarica et al. (2008) observed that egg production, egg mass, viability, and live weight were significantly decreased by higher stocking densities. The same authors reported that hens housed at lower stocking densities reached sexual maturity significantly earlier than those in higher densities. Similarly, Onbasilar and Aksoy (2005) measured that hen-day egg production was 94.1, 89.3, and 78.5% at the stocking densities of 1,968, 656, and 394 cm^2/hen, respectively. Decreasing egg production has been shown to be attributable to the reduced feeding area per hen, cannibalism occurrence (Lee and Moss, 1995; Sohail et al., 2001; Onbasilar and Aksoy, 2005; Jalal et al., 2006), and stressful conditions caused by higher stocking densities.

Dietary CrMet supplementation caused significantly increased egg production, egg mass, and ADFI ($P < 0.01$); however, egg weight and FCR were not influenced by supplemental CrMet. Dietary inclusion of 500 ppm VC tended to improve FCR ($P = 0.053$). There were significant Cr × VC interactions related to egg production and FCR ($P < 0.05$); dietary CrMet supplementation was more effective in improving egg production and FCR values in VC-unsupplemented diets. Consistent with our results, Jackson et al. (2008) reported that dietary supplementation of Cr as Cr picolinate improved feed efficiency in the later phases of growth and decreased mortality in broiler chicks. Improved egg production in layers and quails as the result of Cr-supplemented diets has been observed in several studies (Sahin et al., 2001a,b, 2002b). In broilers, trivalent organic Cr supplementation could improve growth rate, feed efficiency, and carcass quality with more lean tissue (Gursoy, 2000).

In contrast with our findings, Naseem et al. (2005) observed that VC supplementation for heat-stressed broiler chicks improved feed conversion efficiency. Similarly, Sahin et al. (2003) reported that VC and folic acid supplementation for heat-stressed quails improved

Table 3. Effects of dietary vitamin C (VC) and organic chromium[1] (Cr) supplementation on blood biochemical indices of high stocking density–challenged laying hens.

Cage stocking density	VC (ppm)	Cr (ppb)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	High-density lipoproteins (mg/dl)	Glucose (mg/dl)	Insulin (μIU/dl)	Corticosterone (pg/ml)
5	0	0	1,449	153	14.3	307	7.1	6,178
		500	1,334	144	20.3	261	5.8	5,965
		1,000	1,305	130	22.0	252	5.9	5,716
	500	0	1,366	142	18.3	289	6.9	6,218
		500	1,183	96	17.3	257	6.0	5,226
		1,000	1,355	118	26.0	268	6.2	5,410
7	0	0	1,281	123	20.0	399	11.3	9,878
		500	1,401	159	25.5	303	7.3	7,017
		1,000	1,292	107	22.8	315	7.0	6,569
	500	0	1,411	155	20.5	415	11.1	8,518
		500	1,183	98	20.0	347	6.8	6,223
		1000	1,134	95	20.8	321	7.5	6,654
Stocking density (hens/cage)		5	1,326	131	19.7	272[b]	6.3[b]	5,578[b]
		7	1,283	123	21.6	350[a]	8.5[a]	7,476[a]
VC (ppm)		0	1,333	136	20.8	306	7.4	6,887[a]
		500	1,276	118	20.5	316	7.4	6,375[b]
Cr (ppb)		0	1,367	143[a]	18.3	353[a]	9.1[a]	7,698[a]
		500	1,280	124[ab]	20.8	292[b]	6.5[b]	6,108[b]
		1000	1,270	113[b]	22.9	289[b]	6.7[b]	6,087[b]
SEM			63.72	15.15	2.77	27.40	0.45	241.7
Probability								
Stocking density			0.298	0.500	0.290	0.001	0.001	0.001
VC			0.126	0.101	0.853	0.528	0.990	0.004
Cr			0.127	0.048	0.121	0.002	0.001	0.001
Stocking × VC			0.820	0.646	0.270	0.449	0.751	0.298
Stocking × Cr			0.390	0.513	0.269	0.372	0.001	0.001
VC × Cr			0.201	0.065	0.300	0.865	0.543	0.244
Stocking × VC × Cr			0.178	0.568	0.919	0.738	0.786	0.089

[1]Chromium was added to the diets as chromium-methionine complex (Zinpro Corporation, Edina, MN).

[ab]Means with no common superscripts within the column of each classification (stocking density, VC, or Cr) are significantly different($P < 0.05$).

growth performance compared to birds that did not receive the supplements.

Plasma Metabolites

As shown in Table 3, plasma concentrations of TG and HDL were not influenced by the experimental treatments; dietary supplementation of CrMet, however, caused a linear decrease in plasma cholesterol levels ($P < 0.05$). Plasma insulin and glucose levels of high stocking density hens were significantly greater than those of low stocking density hens ($P < 0.001$). Because plasma glucose is an early indicator of the stress condition, these high glucose levels show that high stocking density can increase hens' stress. The plasma levels of glucose and insulin found in this study indicate metabolic changes associated with stress (Siegel, 1995). It is well known that glucocorticoids can inhibit glucose movement into peripheral tissues by antagonizing insulin signaling (Buren et al., 2002). Of course, caution must be taken when considering results based on plasma glucose levels because several factors, including hormone secretion and nutrition level, can affect blood glucose levels (Smith and Bright-Taylor, 1974; Chamblee et al., 1989).

Dietary inclusion of Cr up to 500 ppb resulted in markedly decreased plasma concentrations of glucose ($P < 0.01$) and insulin ($P < 0.001$). There was a significant stocking density × Cr interaction related to insulin concentration ($P < 0.001$); supplemental CrMet was more effective in lowering plasma insulin levels in high stocking density–challenged hens. While high stocking density increased plasma corticosterone levels ($P < 0.001$), both CrMet and VC decreased corticosterone concentration ($P < 0.01$). A significant stocking density × Cr interaction was seen for plasma corticosterone ($P < 0.001$); supplemental CrMet decreased this stress hormone to a greater extent in hens exposed to high stocking density. The marked effect of CrMet on plasma corticosterone levels of high stocking density–challenged hens in the present study is not unexpected because poultry studies have shown that supplemental dietary Cr alleviates the detrimental effects of stressful conditions (Sahin et al., 2001a, 2002b, 2005). It seems that at least part of the effect of Cr is because of the Cr-binding molecule, chromodulin, which promote insulin signaling across cell membranes.

Puvadolpirod and Thaxton (2000) simulated stress responses in broilers by continuously administering adrenocorticotropin and described that stress caused less weight gain, hypertrophy of the liver due to

Table 4. Effects of dietary vitamin C (VC) and organic chromium[1] (Cr) supplementation on Newcastle antibody titers (log_2) of high stocking density–challenged laying hens.

Cage stocking density	VC (ppm)	Cr (ppb)	7 d postvaccination	14 d postvaccination
5	0	0	4.83	5.36
		500	5.01	5.65
		1,000	4.69	5.41
	500	0	4.93	5.36
		500	5.17	5.68
		1,000	4.87	4.72
7	0	0	3.24	2.87
		500	3.79	4.83
		1000	3.86	4.68
	500	0	3.62	4.02
		500	4.28	5.06
		1000	4.05	4.76
Stocking density (hens/cage)		5	4.92[a]	5.36[a]
		7	3.81[b]	4.37[b]
VC (ppm)		0	4.24	4.80
		500	4.49	4.93
Cr (ppb)		0	4.15	4.40[b]
		500	4.56	5.30[a]
		1,000	4.37	4.89[a]
SEM			0.29	0.31
Probability				
Stocking density			0.001	0.001
VC			0.136	0.461
Cr			0.142	0.001
Stocking × VC			0.534	0.055
Stocking × Cr			0.303	0.002
VC × Cr			0.941	0.145
Stocking × VC × Cr			0.913	0.557

[1]Chromium was added to the diets as chromium-methionine complex (Zinpro Corporation, Edina, MN).

[ab]Means with no common superscripts within the column of each classification (stocking density or Cr) are significantly different($P < 0.05$).

accumulated lipids, and increased circulating levels of corticosterone, glucose, total protein, TG, HDL, and cholesterol.

Immunological Responses

The effects of supplemental dietary CrMet and VC on antibody response to NDV are shown in Table 4. As indicated, the density of 7 hens/cage significantly diminished NDV antibody titers at both 7 and 14 d postvaccination ($P < 0.001$). Consistent with this finding, reports show that physiological stress is frequently associated with degeneration of lymphoid organs (Thaxton et al., 1982; Gray et al., 1989) and suppression of humoral and cell-mediated immune responses (Thaxton et al., 1968; Murray et al., 1987). Dietary inclusion of CrMet improved NDV antibody response at d 14 postvaccination ($P < 0.001$) and caused a numerical ($P = 0.142$) increase in NDV titers at d 7 postvaccination. Improved antibody production against NDV has been reported in heat-stressed broiler chicks with dietary supplementation of 2 or 10 ppm Cr, either in the form of $CrCl_3$ or yeast (Guo et al., 1999). Similarly, Lee et al. (2003) reported that antibody response to infectious bronchitis virus was improved in broiler chicks fed 400 ppb Cr picolinate. The exact mechanism by which Cr enhances the immune system is not yet known. However, it is likely that Cr decreases serum cortisol levels. Cortisol and corticosterone have been found to be immunosuppressive, inhibit the production and action of antibodies, impair lymphocyte function, and decrease leukocyte proliferation (Roth and Kaeberle, 1982; Munck et al., 1984; Gross, 1992). Decreased antibody titers could be indirectly due increased inflammatory cytokines in times of stress that stimulate the hypothalamic production of corticotrophin releasing factor (Ogle et al., 1997), which stimulates corticosterone production from the adrenal glands. Sahin et al. (2002a) reported reduced serum corticosterone levels in heat-stressed broilers fed Cr picolinate.

Although dietary supplementation of VC had no marked effect on the birds' immune response against NDV, dietary supplementation of CrMet for high stocking density–challenged hens was more effective in elevating antibody response compared with hens in normal stocking density; there was a significant stocking density × Cr interaction ($P < 0.01$). It seems that the effect of Cr supplementation on the antibody response of stressed hens is due to suppressing the adrenal glands' production of corticosterone (Sahin et al., 2002a).

In contrast to the present findings, Amakye-Anim et al. (2000) observed that dietary VC supplementation increased the immune response to infectious bursal

Table 5. Effects of dietary vitamin C (VC) and organic chromium[1] (Cr) supplementation on yolk components (mg/g) of high stocking density–challenged laying hens.

Cage stocking density	VC (ppm)	Cr (ppb)	Triglycerides	Cholesterol
5	0	0	57.3	14.8
		500	42.2	20.2
		1,000	37.9	19.7
	500	0	52.2	14.6
		500	25.7	16.9
		1,000	38.4	16.2
7	0	0	44.4	18.0
		500	51.9	10.7
		1,000	28.6	18.5
	500	0	34.9	15.3
		500	48.2	11.8
		1,000	43.2	16.0
Stocking density (hens/cage)		5	41.9	17.1
		7	41.6	15.1
VC (ppm)		0	42.7	17.0
		500	46.4	15.1
Cr (ppb)		0	47.2[a]	14.0
		500	42.5[ab]	14.9
		1,000	37.0[b]	17.6
SEM			4.52	2.63
Probability				
Stocking density			0.896	0.243
VC			0.309	0.282
Cr			0.043	0.423
Stocking × VC			0.246	0.781
Stocking × Cr			0.002	0.088
VC × Cr			0.053	0.888
Stocking × VC × Cr			0.120	0.717

[1]Chromium was added to the diets as chromium-methionine complex (Zinpro Corporation, Edina, MN).
[ab]Means with no common superscripts within the column of Cr level are significantly different ($P < 0.05$).

disease virus. In addition, Naseem et al. (2005) reported that dietary VC supplementation for broiler chicks subjected to heat stress improved immune status and increased the relative weights of the bursa of Fabricius, thymus, and spleen compared with birds that did not receive such supplementation.

Yolk Composition

As shown in Table 5, subjecting hens to high stocking density had no marked impact on yolk TG and cholesterol concentrations. Similarly, VC supplementation had no effect on the yolk's lipid profile. Dietary CrMet supplementation decreased yolk TG concentrations ($P < 0.05$) but had no clear effect on yolk cholesterol. Lien et al. (1999) reported that inclusion of Cr picolinate in laying hens' diets decreased yolk cholesterol. The exact mechanism by which Cr could decrease yolk TG is not known; however, the higher egg production of CrMet-supplemented hens likely accounts for part of this effect.

The present results indicate that Cr supplementation of laying hens under stressful conditions could beneficially affect production performance and improve humoral immunity, probably through modulating stress status. The synergistic effect of VC, however, needs further investigation.

ACKNOWLEDGMENTS

The authors would like to especially thank Pejman Ajilchi (chairman of Sana Dam Pars Co., Tehran, Iran) and Zinpro Corporation (Edina, MN) for providing us with chromium-methionine supplement and financial support.

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