Optimal dietary concentrations of vitamin C and chromium picolinate for alleviating the effect of low ambient temperature (6.2°C) on egg production, some egg characteristics, and nutrient digestibility in laying hens

N. SAHIN [1], K. SAHIN[2]

[1]*Veterinary Control and Research Institute of Ministry of Agriculture, Elazig, Turkey*
[2]*Department of Animal Nutrition, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey*

ABSTRACT: This experiment was conducted to evaluate the effect of vitamin C (L-ascorbic acid) and chromium (chromium picolinate, Cr Pic) on egg production, some egg characteristics, and digestibility of nutrients in laying hens (Hy-Line) reared under a low ambient temperature (6.2°C). Two hundred and ten laying hens (32 week-old) were divided into seven groups, 30 hens per group. The treatment groups were designed in a 2 × 3 factorial arrangement using two levels of vitamin C (125 and 250 mg/kg of diet) and three levels of chromium picolinate (200, 400, or 800 µg/kg of diet), and control group was fed basal diet. The highest values of performance were obtained if 250 mg/kg vitamin C was supplemented with either 400 or 800 µg Cr per kg of diet. An interaction between vitamin C and chromium for egg production ($P = 0.05$) and feed efficiency ($P = 0.02$) was detected. Similarly, egg weight, specific gravity, egg shell thickness, egg shell weight and Haugh unit improved with diet containing 250 mg vitamin C and either 400 or 800 µg Cr per kg of diet ($P \leq 0.05$). Digestibility of dry matter (DM), ash, organic matter (OM), crude protein (CP), and ether extract (EE) were higher with higher dietary vitamin C ($P \leq 0.05$) and also with higher Cr ($P \leq 0.05$). There were no interactions between vitamin C and chromium detected for any parameters measured for egg quality in terms of egg weight, specific gravidity, egg shell thickness, egg shell weight and Haugh unit and digestibility of nutrients ($P \geq 0.28$). Data obtained in the present study shows that a combination of 250 mg vitamin C and 400 µg chromium per kg of diet gave the best results in laying hens reared under a low ambient temperature and a conclusion is suggested that such a diet can be considered as a protective management practice in poultry to alleviate, at least in part, the depressive effect of cold stress on poultry performance.

Keywords: stress; chromium; vitamin C; performance; nutrient digestibility; laying hen

INTRODUCTION

An environmental temperature varies from –5 to +5°C in many regions of the world during winter months. Such cold conditions cause some adverse effects including increased feed intake, decreased egg production, egg quality, nutrient digestibility, and feed efficiency in laying hens (Sagher, 1975; Arad and Marder, 1982; Ensminger *et al*., 1990; Spinu and Degen, 1993; Sari, 1993). Such ambient temperatures also result in increased mineral excretion (Smith and Teeter, 1987). Attempts to improve productivity under cold climatic conditions were mostly focused on the ways of enhancing energy intake (Sari, 1993). It was reported that the negative effects of environmental stress could be prevented by the use of some minerals and vitamin supplements such as vitamin C and chromium (McDowell, 1989; Mowat, 1994; Lindeman, 1996; NRC, 1997; Sands and Smith, 1999; Sahin *et al*., 2001, 2002a; Sahin and Küçük, 2001).

The poultry does not require any dietary vitamin source as it is able to synthesize vitamin C. Pardue and Thaxton (1986) reported that particular environmental stressors could alter ascorbic acid utilization or synthesis in poultry. It has also been reported that under stress conditions such as low or high environmental temperatures, humidity, high productive rate, and parasite infestation, ascorbic acid synthesis is inadequate (Freeman, 1967; Sykes, 1978; Hornig *et al*., 1984; McDowell, 1989; Cheng *et al*., 1990). Several researchers documented a beneficial effect of ascorbic acid supplementation on growth rate, egg production, egg shell strength and thickness in stressed poultry (Thornton, 1962; McDowell, 1989; Bains, 1996). At temperatures above or below the thermally neutral zone (18–22°C), corticosteroid secretion increases as a response to stress (Brown and Nestor, 1973). By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the adverse effects of stress such as cold stress-related depression in

poultry performance (McDowell, 1989; Kutlu and Forbes, 1993).

Dietary chromium supplementation has been shown to positively affect the growth rate and feed efficiency of growing poultry (Cupo and Donaldson, 1987; NRC, 1997; Lien et *al*., 1999). These beneficial effects of Cr can be observed more efficiently under environmental, dietary, and hormonal stresses (Anderson, 1994; Wright *et al*., 1994; Sahin *et al*., 2001). Supplemental dietary chromium is also recommended by NRC (1997) for animals undergoing environmental stress. Being the active component of the glucose tolerance factor (GTF), Cr stimulates and regulates the action of insulin (Anderson, 1994; Mowat, 1994); thus it is involved in anabolic and catabolic processes (Colgan, 1993).Through increasing the effectiveness of insulin, Cr also indirectly empowers the ascorbic acid transportation (Kapeghian and Verlangieri, 1984; Seaborn *et al*., 1994). In addition, chromium is thought to be essential for activating certain enzymes and for stabilization of proteins and nucleic acids (Okada *et al*., 1984; Anderson, 1987; Linder, 1991). It has been recognized that insulin metabolism influences lipid peroxidation (Gallaher *et al*., 1993). Chromium (insulin cofactor) is, therefore, postulated to function as an antioxidant (Preus *et al*., 1997). Chromium deficiency causes disorders of carbohydrate and protein metabolism, reduction in insulin sensitivity in the peripheral tissues as well as a decrease in growth rate (Doisy, 1978; Pagan *et al*., 1995; Lindeman, 1996). In a previous study, we draw a conclusion that supplemental chromium and vitamin C significantly alleviated the cold stress-related decrease in performance suggesting that additional vitamin C and chromium supplementation into diets may be necessary under stress conditions (Sahin *et al*., 2002b). Therefore, the objective of this study was to determine the optimal dose of chromium (postulated to function as an antioxidant) and vitamin C supplementation in relation to egg production, egg quality and digestion of nutrients (DM, OM, CP, and EE), in laying hens reared under a low ambient temperature (6.2°C).

MATERIAL AND METHODS

Animals

Two hundred and ten 32-week-old Hy-Line laying hens were obtained from a commercial company recognized by Veterinary Control and Research Institute of the Ministry of Agriculture, Turkey.

Dietary treatments and experimental design

The experimental groups were designed in a 2 × 3 factorial arrangement using two levels of vitamin C (125 and 250 mg/kg of diet) and three levels of chromium picolinate (200, 400, or 800 µg/kg of diet) and the control group was fed basal diet. Vitamin C (Rovimix® Stay-C® 35; specifically produced for use as a stabilized source of vitamin C in feed) was provided by a commercial company (Roche, Levent-Istanbul). Chromium picolinate (CrPic, Chromax®, Prince Agri Products) was used as a Cr source. The ingredients and chemical composition of the basal diet are shown in Table 1. The basal diet was a typical layer diet containing 11.62 MJ/kg (2 780 kcal per kg) metabolizable energy (ME) and 17.65% crude protein (CP). The diet was calculated to meet or slightly exceed the nutrient requirements recommended by the National Research Council (1994). Water and the diets were offered *ad libitum*.

The hens were randomly assigned to cage units. Each cage unit consisted of six subcages (30 × 45 × 35 cm), 3 hens per subcage. The hen house was lit for 17 h per day. During the experiment, the temperature and humid-

Table 1. Ingredients and chemical composition of the basal diet fed to laying hens

Ingredients	%
Ground Corn	63.05
Soybean Meal	22.75
Wheat Bran	1.68
Animal Fat	1.50
Limestone	8.57
Dicalcium Phosphate	1.40
Vitamin Premix[a]	0.25
Mineral Premix[b]	0.20
DL-Methionine	0.20
Sodium Chloride	0.40
Metabolizable energy ME (MJ/kg)[c]	11.62
Chemical analyses (DM basis) (%)	
Dry Matter	91.10
Ash	9.33
Organic Matter	81.76
Crude Protein	17.65
Ether Extract	4.5
Crude Fiber	5.9
N free extracts	53.70
Calcium[c]	4.15
Phosphorus[c]	0.78

[a]mix supplied per 2 kg of diet: vitamin A, 15.500 IU; cholecalciferol, 2.500 IU; vitamin E, 15.500 IU; menadione, 2.000 mg; thiamin, 500 mg; riboflavin, 7.000 mg; d-pantothenic acid, 8.000 mg; pyrodixine, 2 mg; vitamin $B_{12,}$ 15 mg; folic acid, 1.5 mg; niacin, 30 mg

[b]mix supplied per 2 kg of diet: Mn, 80 mg; Fe, 60 mg; Zn, 50 mg; Cu, 7 mg; Iodine, 0.3 mg; Se, 0.150 mg; choline chloride, 400 mg

[c]calculated from the tabular values (NRC, 1994)

ity in the hen house were measured four times a day (06.00, 12.00, 18.00, and 24.00). Average ambient relative humidity inside the hen house was 67.4 ± 5.3%. The mean value of daily temperature in the hen house was 6.2 ± 3.2°C with the minimum temperature of 2°C and maximum temperature of 10°C.. The experiment was carried out between the October15 and February 20.

Performance and egg quality

Body weights were recorded at the beginning and end of the study to determine body weight changes. Feed consumption was measured weekly. The number of eggs and egg weight were recorded daily throughout the experiment. Random samples of 20 eggs from each treatment were collected biweekly to measure egg quality. Parameters measured for egg quality were specific gravity, egg shell thickness, egg shell weight, and Haugh unit. Specific gravity of eggs was determined by the saline flotation method of Hempe *et al*. (1988). Salt solutions were made in incremental concentrations of 0.005 in the range from 1.065 to 1.120. Haugh units were calculated from the HU formula (Eisen *et al*., 1962) based on the height of egg-white determined with a micrometer and egg weight (Saginomiya, TLM-N1010, Japan). Shell thickness was determined by measuring the thickness mean values taken at three spots on the egg (air cell, equator, and sharp end) using a dial pipe gauge (Mitutoyo, 0.01–20 mm, Japan).

From day 100 to 106, seventy birds were placed into individual battery cages and distributed into six treatments, 10 birds each, for nutrient digestibility. Digestibility of nutrients was measured by collecting excrement samples twice a day. The excrement samples were oven-dried at 60°C for 48 h, then they were ground for chemical analyses. Digestibility of nutrients was measured using Cr_2O_3 (0.2% of the grower diet) as indicator described by Petry and Rapp (1971). Levels of indicator and nutrients of feed and excrement samples were detected and coefficients of digestibility were calculated using formula below:

$$\text{Digestibility of a nutrient (\%)} = 1\,00 - \frac{\text{Indicator in feed (\%)}}{\text{Indicator in excrement (\%)}} \times \frac{\text{Nutrient in excrement (\%)}}{\text{Nutrient in feed (\%)}} \times 100$$

Table 2. The effects of supplemental vitamin C and chromium on the performance of laying hens reared under a low ambient temperature (6.2 ºC)

Treatments						
Supplemented						
Vitamin C (mg/kg)	Chromium (µg/kg)	Initial bwt* (kg)	Final bwt* (kg)	Feed intake (g/d)	Egg production (%)	Feed efficiency [a]
0	0	1.49	1.42	120	8 0.0	2.28
125	200	1.48	1.59	118	83.0	2.20
125	400	1.46	1.63	117	84.7	2.14
125	800	1.48	1.64	117	84.1	2.15
250	200	1.47	1.73	118	85.2	2.09
250	400	1.46	1.80	119	88.4	2.01
250	800	1.47	1.81	119	89.5	2.06
Pooled SEM		0.53	0.05	4.3	0.8	0.06
Main effects						
125		1.46	1.65	118	84.2	2.16
250		1.47	1.76	119	87.5	2.08
	200	1.47	1.60	117	83.3	2.16
	400	1.46	1.67	118	86.2	2.10
	800	1.46	1.68	118	86.0	2.12
ANOVA						
Source		Probabilities				
Vitamin C		0.677	0.05	0.15	0.01	0.05
Chromium		0.832	0.05	0.46	0.01	0.05
Vitamin C × chromium		0.672	0.48	0.76	0.05	0.02

*bwt = body weight

[a]kg of feed consumed for egg production/kg of egg production

Laboratory analyses

Chemical analyses of the diets and excrement samples were run using international procedures of AOAC (1990). In order to estimate protein digestibility, excrement N was chemically analyzed according to the method of Terpstra and De Hart (1974).

Statistical analyses

The data were processed by ANOVA of SAS (1989) with vitamin C and Cr as main effects.

RESULTS

As can be seen in Tables 2, 3, 4, better results were obtained in all experimental groups when compared to the control group. Average of initial body weight was similar between groups ($P > 0.05$). However, two hundred and fifty mg vitamin C/kg of diet compared with 125 mg/kg of diet resulted in higher body weight change ($P = 0.05$), egg production ($P = 0.01$) and feed efficiency ($P = 0.05$) . Similarly, higher dietary Cr supplements caused improved body weight change ($P = 0.05$), egg production ($P = 0.01$), and feed efficiency ($P = 0.05$). Although there was no difference in feed intake between groups ($P > 0.05$), an interaction between vitamin C and Cr for egg production ($P = 0.05$) and feed efficiency ($P = 0.02$) was detected. The highest values of performance were obtained if 250 mg vitamin C was supplemented with either 400 or 800 µg Cr per kg of diet. Similarly, egg weight, specific gravity, egg shell thickness, egg shell weight and Haugh unit improved with diet containing 250 mg vitamin C and either 400 or 800 µg Cr per kg of diet ($P \leq 0.05$) (Table 3). Digestibility of dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) were higher with higher dietary vitamin C ($P < 0.05$) and also with higher Cr ($P \leq 0.05$) (Table 4) but no effect on crude fiber (CF) was detected between groups ($P > 0.05$). There were detected no interactions for any parameters measured for egg quality and digestibility of nutrients ($P \geq 0.28$).

DISCUSSION

In the present study, the effects of vitamin C and chromium supplementation on egg production, egg quality

Table 3. The effects of supplemental vitamin C and chromium on some egg characteristics of laying hens reared under a low ambient temperature (6.2°C)

Treatments						
Supplemented						
Vitamin C (mg/kg)	Chromium (µg/kg)	Egg weight (g)	Specific gravity	Eggshell weight (g)	Eggshell thickness (µm)	Haugh unit
0	0	58.3	1.0884	5.30	356.0	91.50
125	200	59.2	1.0893	5.33	357.4	92.12
125	400	60.8	1.0896	5.42	359.1	93.36
125	800	60.5	1.0896	5.39	359.9	93.45
250	200	60.9	1.0901	5.47	360.2	93.83
250	400	61.7	1.0908	5.58	362.8	94.70
250	800	61.3	1.0905	5.59	362.2	94.77
Pooled SEM		0.20	0.050	0.06	0.86	0.08
Main effects						
125		60.4	1.0894	5.38	358.3	92.33
250		61.1	1.0902	5.48	361.0	94.25
	200	59.6	1.0893	5.35	357.9	92.18
	400	60.9	1.0899	5.44	359.7	93.46
	800	60.8	1.0897	5.45	360.0	93.52
ANOVA						
Source		Probabilities				
Vitamin C		0.01	0.01	0.01	0.01	0.01
Chromium		0.01	0.03	0.05	0.05	0.05
Vitamin C × chromium		0.58	0.25	0.70	0.36	0.50

Table 4. The effects of supplemental vitamin C and chromium on nutrient digestibility of laying hens reared under a low ambient temperature (6.2 °C)

Treatments							
Supplemented							
Vitamin C (mg/kg)	Chromium (μg/kg)	Dry matter (%)	Ash (%)	Organic matter (%)	Crude protein (%)	Crude fiber (%)	Ether extract (%)
0	0	61.32	27.15	64.00	67.27	1.56	67.06
125	200	62.46	27.32	65.30	68.38	1.55	68.53
125	400	63.29	27.56	66.15	68.98	1.51	70.33
125	800	63.30	27.88	66.14	69.13	1.48	70.15
250	200	63.42	27.94	67.13	70.60	1.47	70.81
250	400	64.58	28.55	68.39	71.51	1.59	71.43
250	800	64.45	28.73	68.55	71.34	1.55	71.26
Pooled SEM		0.6	0.2	0.9	1.1	0.25	1.3
Main effects							
125		63.10	27.66	65.73	68.35	1.53	69.80
250		64.25	28.61	68.03	71.31	1.54	71.09
	200	62.86	27.42	65.03	69.61	1.51	69.25
	400	63.34	27.83	66.10	70.48	1.54	70.72
	800	63.50	28.21	66.23	70.35	1.55	70.68
ANOVA							
Source		Probabilities					
Vitamin C		0.05	0.05	0.01	0.01	0.40	0.01
Chromium		0.05	0.05	0.01	0.05	0.36	0.05
Vitamin C × chromium		0.63	0.82	0.40	0.65	0.75	0.50

in terms of egg weight, specific gravity, egg shell thickness, egg shell weight and Haugh unit and digestibility of nutrients in laying hens reared under a low ambient temperature (6.2°C) were investigated. It was found that dietary vitamin C and chromium alleviated the detrimental effects of cold stress. The highest values of performance were obtained if 250 mg vitamin C was supplemented with either 400 or 800 μg Cr per kg of diet. Stress increases the mobilization of some minerals such as chromium from the tissues and their excretion, and depresses ascorbic acid synthesis in poultry (Sykes, 1978; Borel *et al*., 1984; Hornig *et al*., 1984; Pardue and Thaxton, 1986; Anderson, 1987; Smith and Teeter, 1987; McDowell, 1989), thus may result in marginal chromium and vitamin C deficiency or increased chromium and vitamin C requirements, implying that both chromium and vitamin C should be supplemented in such conditions. Ascorbic acid and chromium are known to increase the use of corticosteroids released during stress (Pardue and Thaxton, 1984; Sahin *et al*., 2001), thus playing an important role in responding to stress. With respect to dietary ascorbic acid supplementation under stress in terms of better poultry performance, the results of the present study are in agreement with the findings of several researchers (Orban *et al*., 1993; Kafri and Cherry, 1984; Njoku, 1986; Kutlu and Forbes, 1993). It is a well-known fact that growth rate and egg production decrease when ambient temperature goes below or above the thermally neutral zone (Arad and Marder, 1982; Ensminger *et al*., 1990; Sari, 1993). At temperatures above or below the thermally neutral zone, corticosteroid secretion increases as a response to stress (Brown and Nestor, 1973). Kutlu and Forbes (1993) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress such as cold stress-related depression in poultry performance (McDowell, 1989). Sands and Smith (1999) also reported that dietary chromium picolinate supplementation increased growth rate in stressed broilers. Lien *et al*. (1999) reported that 1 600 μg/kg or 3 200 μg/kg chromium picolinate supplementation in a broiler diet increased feed intake and improved live weight gain. In addition, Steele and Rosebrough (1981) found that an addition of 20 ppm chromium chloride increased weight gains of turkey poults. Moreover, Sahin *et al*. (2001) reported that a supplement of 400 ppb chromium to the diet of laying hens reared under a low ambient temperature increased egg production and improved feed efficiency. Sahin *et al*. (2002a) also reported that the decrease in live weight, feed intake, egg production and feed efficiency in laying hens reared under cold stress was alleviated by dietary chromium and zinc supplementation. It is obvious that chromium is involved in protein metabolism (Anderson,

1987). Chromium is thought to have a role in nucleic acid metabolism and an increase in stimulation of amino acid incorporation into liver protein *in vitro* was observed (Weser and Koolman, 1969). Okada *et al*. (1983) showed an interaction of chromium with DNA templates that resulted in a significant stimulation of RNA synthesis *in vitro*. The oligopeptide low-molecular-weight chromium-binding protein (chromodulin) tightly binds four chromic ions before the oligopeptide obtains a conformation required for binding to the tyrosine kinase active site of the insulin receptor (Vincent, 2000, 2001). The oligopeptide chromodulin binds chromic ions in response to an insulin-mediated chromic ion flux, and the metal-saturated oligopeptide can bind to an insulin-stimulated insulin receptor, activating the receptor's tyrosine kinase activity. Thus, chromodulin appears to play a role in an auto-amplification mechanism in insulin signalling. In addition, the release of chromium from chromium picolinate for use in cells requires a reduction of the chromic center, a process that can potentially lead to the production of harmful hydroxyl radicals (Vincent, 2000).

Similarly to the results of the present study, El-Boushy *et al*. (1968) reported that dietary vitamin C supplementation increased egg production, egg shell strength, and interior egg quality in stressed laying hens. On the other hand, Sahin *et al*. (2002a, b) stated that higher doses of supplemental chromium increased egg production, improved feed efficiency, egg weight, egg specific gravity, egg shell thickness, egg shell weight and Haugh unit in laying hens kept under low temperature. However, Lien *et al*. (1996) reported that the shell thickness was not affected by chromium picolinate supplementation (400 and 800 µg/kg) under thermally neutral conditions. It has been reported that ascorbic acid plays a role in bone maturation by improving hydroxyproline production which is required for collagen formation. Accordingly, in birds, it was postulated that ascorbic acid stimulates 1,25-dihydroxy-cholecalciferol and together with it increases calcium mobilization from bones, suggesting vitamin C has an important role in egg shell formation (Dorr and Balloun, 1976; Demir *et al*., 1995).

A low ambient temperature was reported to suppress nutrient digestibility in laying hens (Ensminger *et al*., 1990; Sari, 1993). Similarly, Sahin (2001) reported a decrease in the utilization of dry matter, crude protein, and ether extract in laying hens kept under a low temperature (6.9°C), and supplemental chromium alleviated these negative values. The benefits of supplemental vitamin C under heat stress conditions were also demonstrated. Sahin and Küçük (2001) found increased digestibility of nutrients after dietary vitamin C supplementation in Japanese quails reared under heat stress (34°C). Kornegay *et al*. (1997) found that the digestibility of dry matter and N-retention were increased by supplemental Cr in pigs, which was speculated to be due to increased secretion of digestive enzymes. The results of nutrient digestibility in the present study also support the beneficial effect of dietary vitamin C and chromium in laying hens reared under a low temperature.

These results revealed additive effects of vitamin C and chromium, indicating that vitamin C and chromium act synergistically. Sahin *et al*. (2002b) reported that the combination of vitamin C and chromium caused more significant changes than either vitamin C or chromium alone and speculated about the synergistic action of vitamin C and chromium. Similarly, Carol *et al*. (1994) found an interaction between Cr and vitamin C in bone and brain Mn retention and distribution in guinea-pigs, and postulated that dietary Cr may influence ascorbic acid metabolism via protecting ascorbate from oxidative destruction. In addition, insulin is known to play a role in ascorbic acid transportation in red blood cells, and glucose competitively inhibits ascorbic acid transport (Mann and Newton, 1975). Through increasing the effectiveness of insulin, Cr indirectly promotes the ascorbic acid transportation (Seaborn *et al*., 1994).

The results of the present study allow to conclude that a combination of 250 mg of vitamin C and 400 µg of Cr provides the highest positive effect on the performance of laying hens under a low ambient temperature. Such a combination may offer a potential protective management practice in preventing cold stress-related losses in the performance of laying hens.

Acknowledgement

The authors thank the Veterinary Control and Research Institute of Elazig, particularly Dr. Nurcan Arslan, Director, for providing the experimental facility.

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Received: 01–09–05
Accepted after corrections: 01–12–17

Corresponding Autor:

Dr. Nurhan Sahin, Veterinary Control and Research Institute of Ministry of Agriculture, 23100 Elazig, Turkey
Tel: +90 424 218 18 34, e-mail: ksahin@firat.edu.tr