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EFFECT OF DIETARY CHROMUM AND ASCORBIC ACID ON NUTRIENT DIGESTION, HEMATOLOGICAL TRAITS AND EGG QUALITY OF DOKKI4 CHICKENS UNDER WINTER CONDITIONS IN EGYPT

Hassan, R. A.[1]; E. M.A. Qota[1]; Y.Z. Eid[2] and Nasra B. Awadein[1]

1- Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

2- Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt.

ABSTRACT

The effect of chromium (Cr) and ascorbic acid (AA) as a dietary supplementation on digestion of nutrients, mineral concentration, egg quality and hematological parameters in laying hens were studied under winter condition (December– February). Total number of 275 (250 hens + 25 cocks) Dokki4 chickens, of 30 weeks old were divided into 5 groups of 5 replicates each (10 hens +1 cock/ replicate). Birds in the 1st group (Cont.+) were kept in controlled normal temperature (CNT,25°C) with 64±2% relative humidity (RH) and fed corn-soybean meal basal diet (16.4% crude protein, 2750 kcal ME/kg diet), without any supplementation. Birds in groups from 2 to 5 were exposed to natural low temperature (NLT, 8-18°C) with 65±2%. These groups were fed either basal diet (Cont.-), or the basal diet supplemented either with either 250 ppm AA, 400 ppb Cr or 250 ppm AA plus 400 ppb Cr.

Results obtained showed that except for crude fiber, cold stress impaired nutrient digestibility and mineral retention compared with those of birds exposed to normal ambient temperature. A combination of AA and Cr rather than each alone, provided the better digestibility of dry matter, crude protein, ether extract and mineral retention. The highest values of nutrient digestibility and Ca, P, Fe and Zn retention were observed with the combination of AA + Cr group while the lowest values were showed with the control group. It could be concluded that the combination between AA + Cr may improve the retention of nitrogen and mineral retention in laying hens under low ambient temperature. Low temperature decreased yolk weight, shell weight, yolk index, shell thickness and Haugh unit.

The results showed that there was no significant ($p \leq 0.05$) effect of treatments on blood hemoglobin. The results showed that low temperature decreased total lymphocytes cells while, erthrocytes, leukocytes, heterophil and H/L ratio were increased by cold weather compared to control birds at normal temperature. Lymphocytes cells are significantly higher ($p \leq 0.05$) in the group fed the combination of AA and Cr than groups fed AA or Cr alone. However, erthrocytes, leukocytes and heterophil were lower in the groups supplemented with AA and /or Cr than the low temperature control birds ($p \leq 0.05$).

The results of the present study conclude that a combination of 250 mg of AA and 400 mg of Cr provides the highest positive effect on nutrients digestion, mineral concentration, egg quality and hematological parameters of local laying hens under winter condition in Egypt .

Keywords: chromium, ascorbic acid, cold stress, laying chicken performance

INTRODUCTION

Poultry Production goes down at low ambient temperature. An increase in feed intake, decreased egg production, nutrient digestibility and feed conversion are believed to be associated with low ambient temperature in laying hens (Spinu and Degen, 1993). Environmental cold stress causes deficiencies of vit. C (Ascorbic acid) in poultry (Sahin and Sahin, 2001). Cold conditions cause decreases in plasma concentrations of some vitamins, minerals and insulin and increase in plasma corticosterone in poultry (Brown and Nestor, 1973; McDowell, 1989; Ensminger *et al.*, 1990; and Sigel, 1995). Ascorbic acid functions as a reducing agent and an antioxidant. Previous studies have shown that ascorbic acid is an indispensable micronutrient required to maintain the physiological processes of certain animals including poultry (McDowell, 1989). Poultry are known not be require a dietary source of vitamin C because of the ability of the birds to synthesize its own. Pardue and Thaxton (1986) have documented evidence that particular environmental stressors can alert ascorbic acid utilization or synthesis in avian species. It has been also reported that ascorbic acid synthesis inadequate under stress conditions such as low or high environmental temperature, humidity, high production rates and parasitic infestation (McDowell, 1989; Kutlu and Forbes, 1993; Sahin and Kucuk, 2001 and Sahin *et al.*, 2002). Several studies have indicated a beneficial effects of vitamin C supplementation on the performance of cold-stressed laying hens (Sahin and Sahin, 2002; and Kucuk *et al.*, 2003).

Trivalent chromium is a well known essential trace element for human and animals (Schwartz and Mertz, 1959). Trivalent chromium improve insulin effectiveness by enhancing its binding to receptors and the sensitiveness of the target cell (Anderson, 1997). This element is also involved in carbohydrate, lipid, protein and nucleic acid metabolic function (Ohba *et al.*, 1986; McCarty, 1991). Research on animals has confirmed that chromium from organic complex such as chromium picolinate, nicotinate and high chromium yeast is absorbed more efficiently, about 25–30% more than inorganic compounds like chromium chloride ($CrCl_3$), which are poorly absorbed (1-3%) regardless of does or dietary chromium status (Underwood and Suttle, 1999 and Mowat, 1994). National Research Council (NRC, 1989) has recommended an intake of 50 to 200 ppb of trivalent chromium for adult humans. However, an appropriate recommendation on the chromium requirement of poultry has not been made (NRC, 1997) and most poultry diets are basically composed of plant origin ingredients, which have usually low content of chromium (Giri *et al.*, 1990). Therefore, the objective of this study was to determine the effect of chromium (Postulated to function as an antioxidant) and vit. C supplementation in relation to digestion of nutrients, egg quality, some hematological parameters and yolk parameters in laying hens reared under cold weather (winter season) in Egypt.

MATERIALS AND METHODS

The present study was conducted at Sakha , Animal Production Research Station and Laboratories, APRI, ARC, Egypt during Dec. 2006 - Feb. 2007 to study the effects of dietary Ascorbic acid (AA) and/or Cr on digestion of nutrients, minerals concentration, egg quality and hematological parameters in laying hens during winter season. Total number of 275 (250 laying hens+25 cocks) chickens (Egyptian strain, Dokki4), 30 weeks old were divided into 5 groups of 5 replicates (10 laying hens+1 cocks / replicate). Hens in the groups 2 to 5 were housed in open-sided pens exposed to natural low environmental temperature prevails in Egypt during winter season, temperature varied between 8 and 18 °C during the experimental period with 65±2% relative humidity and 16 hours light per day. The first group (control +) was kept in controlled temperature room (25°C). The birds were fed either basal corn – soybean meal diet formulated according to Egyptian Feed Composition Tables (2001) containing 16.43% crude protein and 2750 kcal/kg diet, or the basal diet supplemented with either 250 ppm of AA (L-ascorbic acid, vit C), 400 ppb of chromium picolinate (Cr) or 250 ppm AA + 400 ppb Cr. The 1st (+) and 2nd (-) control groups were fed on the basal diet without any supplementation. Vitamin C was supplied as (Rovimix® STAY® 35) specifically produced for use as a stabilized source of vit C in feed by commercial company (Roche, Levent, Istanbul – Turkey) while Cr used in the form of CrPic, Chromax®, Prince Agri Products). Feed and water were offered *ad Lib*. during the experimental period (30-42 wks of age). At the end of the experiment, digestibility trail using total collection technique was conducted to determine nutrient digestibility, retention and excretion of dietary nitrogen (N), ash, calcium (Ca), phosphorus (P), iron (Fe) and zinc (Zn) by using five hens from each group. They were chosen randomly and housed individually in wire cages at normal and low temperatures. Birds of each group were fed on their respective experimental diet for preliminary period of 4 days to become adjusted to cages, and then the excreta were quantitatively collected for 3 days with recording feed consumption. After collection, the excreta were dried in an oven at 60°C for 36 hours, then ground. Three pooled samples of excreta voided from each group were taken for nutrients digestion coefficients and mineral retention and excretion determination. The chemical analyses of diets, excreta and egg samples were carried out according to AOAC (1995). At 34,38 and 42 weeks of age, ten eggs were randomly taken from each group for egg quality measurements. Egg shape index % (Carter, 1968) and yolk index % (Well, 1968) were calculated. Haugh units as indicator of albumen quality was calculated (Eisen *et al.*, 1962), shell thickness was also determined at three locations on the egg (air cell, equator , and sharp end) by using a dial pipe gauge. After measuring the egg quality , yolk samples for each treatment were separated from the broken eggs , calculated and extracted to determine cholesterol , LDL, HDL, triglycerides and total lipids according to Floch *et al* ., (1957). At the end of experiment , blood samples were collected from 6 birds taken randomly from each treatment. The blood received from each bird was collected in heparinzed tubes and used for the determination of Hematological Parameters . Hemoglobin concentration was determined in fresh blood samples using haemoglobinometer according to Pilaski (1972) . Also, Red blood cells (RBC,s) and white blood cells (WBC,s) were counted in fresh blood samples using hemocytometer and light microscope . Data were statistically analyzed using one–way ANOVA (SAS, 1996). Before analysis, all percentages were subjected to logarithmic or arcsine values transformation (log10x+1) to approximate normal distribution. Significant differences among means were detected by Duncan's new multiple range test (Duncan, 1955).

RESULTS

The effect of supplemental dietary AA and or/ or Cr during cold weather under low ambient temperature in winter in Egypt on nutrient digestibility, retention and excretion of some minerals of laying hens are shown in Table (1). Except for crude fiber, cold stress impaired nutrient digestibility and mineral retention compared with those of birds exposed to normal ambient temperature. A combination of AA and Cr rather than each alone, provided the greater digestibility of dry matter, crude protein, ether extract and mineral retention and low mineral execration rate in laying hens exposed to cold weather compared to the control (+) at normal temperature. The nutrient digestibility and retention of N , ash, Ca, P, Fe and Zn recorded the highest values with the combination of AA and Cr and the lowest values with the control (-) diet at low temperature. Accordingly, excretion of N, ash, Ca, P, Fe and Zn under cold stress were lower in the Cr and AA supplemented groups than the control (-) ($P \leq 0.05$), that of a combination of supplemental AA and Cr being the lowest. However , crude fiber digestibility was not influenced by dietary AA and / or Cr ($P \leq 0.05$) .

The effect of supplemental dietary AA and / or Cr during cold stress on egg quality are shown in Table 2 . Dietary supplement of AA and/or Cr under cold weather improved the egg quality in the terms of yolk hight, yolk index, but the combination of supplemental AA and Cr resulted in higher egg shell thickness and heaviest egg shell weight ($p \leq 0.05$). However, Haugh unit values were similar to the control birds kept under normal temperature.

Table (1): The effects supplemental ascorbic acid (AA) and chromium (cr) on nutrient digestibility and mineral retention and excretion of laying hens reared during winter season (means ±SE)

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+AA +Cr	
Nutrient digestibility:						
Dry matter %	74.00^{a}± 2.14	61.40^{d}± 0.07	64.10^{cd}± 1.08	67.50^{bc}± 1.56	71.33^{ab}± 0.61	0.0001
Organic matter %	76.40^{a}± 1.81	65.60^{c}± 0.42	68.73^{c}± 0.79	68.17^{c}± 1.13	72.80^{b}± 0.72	0.0001
Crude protein %	78.27^{a} 0.18	70.17^{b}± 1.30	75.23^{a}± 0.73	75.03^{a}± 1.33	75.77^{a}± 0.72	0.0005
Crude fiber %	21.17± 0.60	21.23± 0.73	21.83± 0.09	22.77± 0.43	21.70± 0.57	0.557
Ether extract %	78.27^{a}± 1.47	70.10^{c}± 0.83	73.90^{b}± 0.35	75.03^{b}± 0.84	76.43^{ab}± 0.58	0.001
Mineral retention:						
Nitrogen(g/hen/d, DM)	2.76^{a}± 0.15	2.02^{c}± 0.02	2.28^{bc}± 0.05	2.36^{bc}± 0.07	2.66^{ab}± 0.17	0.004
Ash(g/hen/d,DM)	9.40^{a}± 0.12	6.45^{c}± 0.09	7.5^{b}± 0.28	7.83^{b}± 0.07	9.40^{a}± 0.17	0.0001
Ca (g/hen/d, DM)	3.43^{a}± 0.19	2.00^{c}± 0.12	2.63^{b}± 0.09	2.80^{b}± 0.12	2.97^{b}± 0.09	0.0001
P (g/ hen/d, DM)	0.38^{a}± 0.02	0.24^{d}± 0.02	0.28^{bc}± 0.01	0.26^{cd}± 0.01	0.31^{b}± 0.01	0.0001
Fe (mg/hen/ d, DM)	13.90^{a}± 0.21	10.70^{c}± 0.15	12.20^{b}± 0.21	12.23^{b}± 0.43	12.83^{b}± 0.20	0.0001
Zn (mg/ hen/ d, DM)	3.63^{a}± 0.19	1.50^{c}± 0.29	2.43^{b}± 0.23	2.73^{b}± 0.15	3.03^{b}± 0.09	0.0001
Mineral excretion:						
Nitrogen(g/hen/d, DM)	1.54^{b}± 0.06	1.92^{a}± 0.04	1.75^{ab}± 0.14	1.73^{ab}± 0.13	1.49^{b}± 0.12	0.055
Ash (g/hen/d, DM)	9.75^{b}± 0.14	10.81^{a}± 0.12	10.80^{a}± 0.17	10.86^{a}± 0.12	9.91^{b}± 0.11	0.0001
Ca (g/hen/d, DM)	2.27^{c}± 0.15	3.00^{a}± 0.12	2.83^{ab}± 0.09	2.60^{bc}± 0.12	2.60^{bc}± 0.12	0.013
P (g / hen/d, DM)	0.48^{c}± 0.01	0.83^{a}± 0.09	0.65^{b}± 0.03	0.60^{bc}± 0.06	0.50^{bc}± 0.03	0.004
Fe (mg/hen/ d, DM)	37.23^{c}± 0.67	44.07^{a}± 2.02	41.83^{ab}± 0.88	42.43^{ab}± 0.46	39.60^{bc}± 0.67	0.010
Zn (mg/ hen/ d, DM)	6.57^{c}± 0.35	10.90^{a}± 0.38	9.57^{b}±0.23	9.70^{b}± 0.40	8.83^{b}± 0.23	0.0001

[abc] Means within each row with different superscripts are significantly different (p<0.05).

Table (2): The effects supplemental ascorbic acid (AA) and chromium on some egg characteristics of laying hens reared during winter season (means ±SE).

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+ AA+Cr	
Egg weight (g)	50.73 ±0.23	49.80 ±0.41	50.80 ±0.49	50.87 ±0.66	50.89 ±0.65	0.564
Yolk weight, g	18.80[a] ±0.18	17.33[c] ±0.13	18.13[b] ±0.19	18.07[b] ±0.23	18.44[ab] ±0.26	0.0001
Yolk weight, %	37.05[a] ±0.25	34.82[d] ±0.22	35.70[bc] ±0.19	35.53[c] ±0.25	36.25[b] ±0.24	0.0001
Albumen weight, g	26.07 ±0.21	27.40 ±0.34	27.07 ±0.30	26.87 ±0.51	26.39 ±0.45	0.120
Albumen weight, %	51.39[d] ±0.44	54.99[a] ±0.27	53.28[b] ±0.27	52.78[bc] ±0.45	51.82[cd] ±0.33	0.0001
Shell weight, g	5.87[ab] ±0.17	5.07[c] ±0.07	5.60[b] ±0.13	5.93[ab] ±0.15	6.06[a] ±0.06	0.0001
Shell weight, %	11.56[ab] ±0.31	10.18[c] ±0.15	11.02[b] ±0.23	11.70[ab] ±0.35	11.94[a] ±0.20	0.0001
Egg shape, %	76.82 ±0.18	76.09 ±0.19	76.40 ±0.38	76.51 ±0.26	76.71 ±0.27	0.357
Yolk index, %	49.18[a] ±0.45	45.64[c] ±0.40	47.47[b] ±0.56	48.57[ab] ±0.46	49.17[a] ±0.53	0.0001
Shell thickness(mm)	0.356[a] ±0.01	0.297[c] ±0.003	0.339[b] ±0.03	0.345[ab] ±0.03	0.354[a] ±0.03	0.0001
Haugh unit	77.13[a] ±0.45	73.45[c] ±0.52	75.13[bc] ±0.76	75.27[abc] ±0.70	76.17[ab] ±0.65	0.002

[abc] Means within each row with different superscripts are significantly different ($p<0.05$).

The effects of supplemental dietary Cr or AA on hematological parameters at the end of experimental period are given in Table (3). The results showed that there was no significant ($p\leq0.05$) effect of treatment on blood hemoglobin. Birds fed Cr and/or AA had slight increase in blood hemoglobin than control birds under low temperature. The results showed that low temperature decreased total lymphocytes cells while, erthrocytes, leukocytes, heterophil and H/L ratio were increased under cold weather compared to control birds at normal temperature. Lymphocytes cells are significantly higher ($p\leq0.05$) in the group fed the combination of AA and Cr than groups fed AA or Cr alone. However, erthrocytes, leukocytes and heterophil were lower in the groups supplemented with AA and / or Cr than the low temperature control birds ($p\leq0.05$).

Table (4) show no significant effects for treatments in yolk cholesterol, LDL, HDL and triglycerides at low ambient temperature while, total lipids significantly increased compared with the other treatments. No significant differences due to treatment were found for chemical composition of eggs (protein, ash and fat) as shown in Table (4) .

Table (3): The effects supplemental ascorbic acid (AA) and chromium on some hematological parameters of laying hens reared during winter season (means ±SE).

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+ AA+Cr	
Hemoglobin (g/100ml)	9.75 ± 0.14	9.48 ± 0.29	9.60 ± 0.23	9.75 ± 0.12	9.80 ± 0.12	0.758
Erthrocytes ($x10^6$)	1.55b ±0.86	2.30^{a} ±0.23	2.00^{ab} ±0.115	1.82^{ab} ±0.115	1.60^{b} ±0.173	0.340
Leukocytes ($x10^3$)	16.50^{d} ±1.15	28.50^{a} ±1.15	22.50^{b} ±1.154	21.60^{bc} ±0.577	18.50^{cd} ±0.866	0.0001
Heterophil (H)	29.00^{d} ±1.15	48.00^{a} ±1.732	39.00^{b} ±2.309	36.00^{bc} ±1.155	31.00^{cd} ±1.732	0.0001
Lymphocyte (L)	71.00^{a} ±2.31	55.00^{c} ±1.155	60.00^{b} ±2.88	62.00^{b} ±1.155	69.00^{a} ±1.732	0.0001
H / L ratio	0.408^{c} ±0.02	0.873^{a} ±0.103	0.656^{b} ±0.070	0.580^{bc} ±0.008	0.451^{c} ±0.036	0.0001
Eosinophil	----	2.00 ±0.288	----	-----	----	-----
Basophil	-----	1.00 ±0.00	-----	------	------	-----
Monocyte	-----	2.00 ±0.00	-----	----	-----	-------

abc Means within each row with different superscripts are significantly different ($p<0.05$).

Table (4): The effects supplemental ascorbic acid (AA) and chromium on some yolk parameters and egg chemical composition of laying hens reared during winter season (means ±SE).

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+ AA+Cr	
Fresh egg yolk analyses:						
Cholesterol (mg/dl)	17.39 ±1.732	18.47 ± 2.309	18.0 ± 1.154	18.03 ± 1.717	17.85 ± 1.709	0.995
LDL (mg /dl)	10.50 ± 1.155	11.05 ± 0.577	11.86 ± 1.155	11.00 ±1.155	10.77 ±1.155	0.916
HDL (mg / dl)	5.96 ± 0.577	4.70 ± 0.554	5.00 ± 1.155	5.25 ± 1.046	5.65 ±0.577	0.825
Triglycerides (mg/dl)	277.05 ±1.245	283.00 ± 1.732	280.00 ±2.887	281.00 ±3.756	277.00 ±4.041	0.593
T. lipids (g /l)	320.07^{b} ±5.781	339.00^{a} ±3.321	330.00^{ab} ±2.887	329.33^{ab} ±1.202	313.33^{b} ±8.819	0.042
Egg's chemical composition						
Protein %	50.46 ± 1.16	49.82 ± 0.58	50.0 ± 1.73	49.9 ± 1.16	50.2 ± 0.58	0.994
Fat %	46.64 ± 1.16	46.80 ± 0.58	46.90 ± 0.87	46.98 ± 0.58	46.65 ± 0.58	0.962
Ash %	3.30 ± 0.17	3.03 ± 0.29	3.18 ± 0.09	3.23 ± 0.15	3.15 ± 0.09	0.828

abc Means within each row with different superscripts are significantly different ($p<0.05$).

DISCUSSION

In the present study, the effects of AA and chromium supplementation on nutrient digestibility, retention and excretion of some minerals, some egg quality and yolk parameters in laying hens reared under cold weather were investigated. Significantly ($p \leq 0.05$) negative effects on nutrient digestibility, retention and excretion of some minerals, and egg quality were observed when birds were kept in cold weather compared with laying hens kept under thermal neutral temperature (25 °C). It was found that dietary AA and highest values were obtained if AA was supplemented with chromium. Stress increases the their excretion, and depresses ascorbic acid synthesis in poultry (Pardue and Thaxton, 1986; Anderson, 1987; Smith and Teeter, 1987; Mc Dwell, 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, 1986; 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; Njoku, 1986; Kutlu and Forbes, 1993). It is a well – known that growth rate and egg production decrease when ambient temperature goes below or above the thermally neutral zone (Ensminger *et al.,* 1990). 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 corticosteroids, 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 (Mc Dowell, 1989).

A low ambient temperature was reported to suppress nutrient digestibility in laying hens (Ensminger *et al.,* 1990). 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 and vitamin C alleviated these negative values. Environmental stress has been shown to increase mineral excretion (Smith and Teeter 1987) . El-Husseiny and Creger (1981) found that broilers reared under environmental stress had lower rates of Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn retention. Stress increase chromium mobilization from tissues and Zn retention. Stress increase chromium mobilization from tissues and its excretion and also depresses ascorbic acid synthesis (McDowell, 1989) ; thus stress may exacerbate a marginal chromium and ascorbic acid deficiency or an increased chromium and ascorbic acid deficiency or an increased chromium and ascorbic acid requirement, implying that both chromium and ascorbic acid should be supplemented as shown in the present study . Since ascorbic acid and chromium (Postulated to be antioxidants) have a protective effect on pancreatic tissue against oxidative damage (Mc Dowell, 1989; Preuss *et al.,* 1997), they may help pancreas to function properly including secretions of digestive enzymes, thus improving retention of nitrogen and minerals. Metwally (2004) found that increase in retention and a decrease in excretion of nitrogen, ash, calcium, phosphorus, and iron were proved for the birds fed vitamin C as compared to those fed the control diet under low temperature. The results of the current metabolic trial showed that retention of Ca, P, Zn and Fe is improved and excretion, decreased by supplemental chromium and ascorbic acid.

Similary to the results of the present study, El- Boushy *et al.*, (1988) reported that dietary vitamin C supplementation increased egg shell strength and interior egg quality in stressed laying hens. On the other hand, Sahin *et al.,* (2002) stated that higher doses of supplemental chromium improved 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.,* (1999) reported that the shell thickness was not effected by chromium picolinate supplementations (400 and 800 mg/kg) under thermally neutral conditions. It has been reported that ascorbic acid plays arole 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 in increases calcium mobilization from bones, suggesting vitamin C has an important role in egg shell formation (Dorr and Balloun, 1976).

Birds reared at low temperature showed significantly ($P \leq 0.05$) greater erythrocytes compared to the other treatments (Table 3). Social environment stress may be responsible for the increased erythrocytes seen in birds reared under low temperature. Heterophils (H) and leukocytes (L) cells, statistically, ($P \leq 0.05$) greater in low temperature group compared to normal temperature treatment (Table 3). As well as, a significant ($P \leq 0.05$) raise in heterophils cells number, together with a corresponding significant ($P \leq 0.05$) reduction in lymphocytes cells number. The H/L ratio were significantly ($P \leq 0.05$) increased at low ambient temperature (Table 3). These results suggest that low ambient temperature were more stressed compared to normal temperature . In response to supplementations, lymphocytes cells increased ($P \leq 0.05$) compared to control under low ambient temperature. This could be indicating that the tow supplements may increase the immunity of hens (Meydani and Blumberg, 1993). However, heterophil cells number responded significantly ($P \leq 0.05$) decreased due to low temperature supplemented treatments (Table 3) , indicating that the supplementations can ameliorate haematological stress of low ambient temperature , similarly, Lewis (1999). The alterations in heterophils, lymphocytes cells or their ratio suggest that hens under low temperature have a great effect on the stress response (Shini, 2003).

Firstly, the heterophil to lymphocyte ratio has become widely accepted as a reliable and accurate physiological indicator of the stress response in chickens , exposure to stressors causes it to increase progressively, the reference values for it of about 0.2 , 0.5 and 0.8 are characteristic of low , optimal and high degrees of stress, respectively (Gross and Siegel, 1993 ; Elston *et al.*, 2000 and Shini , 2003) .

The H / L ratios were significantly (P≤0.05) increased in control at low temperature compared to normal teperature . This result suggests that hens under low temperature were more stressed . In response to both supplementations , the H / L ratio was significantly (P≤0.05) lower compared with stressed – laying hens when Cr and / or AA was incorporated in diet , suggesting that they are effective to alleviate the adverse effect of low temperature . The significant decreases of the H / L ratio observed for treatments were due to increases in its denominator (lymphophilia). Similarly , Ascorbic acid reduced significantly the H / L ratio in birds under heat , cold , sound , fasting , or management stress (Gross, 1992; Mckee and Harrison, 1995; Zulkifli *et al* ., 2000). The significant reduction in stress levels, as indicated by the lower H/L ratio, observed in the hens supplemented with tocopherol is in agreement with the positive effects by decreased adverse effects of heat stress were reported by (Bollengier *et al.,* 1999; Lewis *et al.,* 1999; Smith 2000). The alterations in heterophils , lymphocytes cells or their ratio suggest that hens under low ambient temperature should have a great effect on the stress response (Shini, 2003).

Eosinophil, Basophil and Monocyte cells were associated only with low ambient temperature group without additives supplementation (Table 3), which has been reported previously by Maxwell *et al.*, (1992) and Tollba *et al.*, (2006). However, hens fed supplemented diets had no response on Eosinophil, Basophil and Monocyte cells, suggesting that diets supplemented with Cr and / or AA are effective to alleviate the adverse effect of low temperature . Maxwell *et al.*, (1992) and Shini , (2003) they found that an extreme stress Basophil and Monocyte cells becomes evident in avian species.

The blood hematology results show that the supplementations involved in adjustment of the H / L ratio and disappearance of Eosinophil, Basophil and Monocyte cells , indicating that they can attenuate the physiological stress of low ambient temperature .

Research on Cr and / or AA at low temperature and its effect on hematological, yolk parameters and eggs chemical composition in laying hens are very limited and it seems that present study is the first study about it

These results revealed additive effects of AA and chromium indicating that AA and chromium act synergistically. Sahin *et al.,* (2002b) reported that the combination of AA and chromium caused more significant changes than either vit. C or chromium alone and speculated a bout the synergistic a action of vitamin C and chromium. Similarly, Carol *et al.,* (1994) found an interaction between Cr and vit. 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 transpiration in red blood cells, and glucose competitively inhibits ascorbic acid transport (Mann and Newten, 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 concluding that a combination of 250 mg of AA and 400 ppb of Cr provides the highest positive effect on digestion of nutrients, egg quality, some hematological parameters of local laying hens under a low ambient temperature (winter season). Such a combination may offer a potential protective management practice in preventing cold stress – related losses in the performance of laying hens.

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تأثير الكروميوم وحامض الأسكوربيك فى العلف على هضم العناصر الغذائية وبعض صفات الدم وجودة البيض لدجاج دقى، تحت ظروف الشتاء فى مصر

رضا على حسن[1]، الشحات محمدعبد الحليم قوطة[1]، يحى زكريا عيد[2] و نصرة بديرعوضين[1]

١- قسم بحوث تغذيه الدواجن - معهد بحوث الأنتاج الحيوانى- مركز البحوث الزراعية -جيزه - مصر

٢- قسم أنتاج الدواجن - كلية الزراعة بكفرالشيخ – جامعة كفرالشيخ

اجرى هذا البحث بغرض دراسه تأثير أضافة الكروميوم وحامض الأسكوربيك فى العلف على هضم العناصر الغذائية وبعض صفات الدم وجودة البيض لدجاج دقى، تحت ظروف الشتاء (ديسمبر– فبراير) فى مصر بمحطه بحوث الانتاج الحيوانى بسخا– معهد بحوث الانتاج الحيوانى. استخدم فى هذه الدراسه عدد٢٧٥ طائر (٢٥٠ دجاجة بياضه +٢٥ ديك) من سلاله دقى، عمر٣٠ أسبوع وتم تقسيمها الى ٥ مجاميع بكل مجموعة ٥ مكررات وكل مكررة (١٠ دجاجات +١ ديك) . وتم وضع المجموعة الاولى فى حجرة التحكم الحرارى عند درجه الحراره العاديه ٢٥°م ورطوبه نسبيه ٦٤±٢% (كنترول ايجابى) وتم تغذيتها على علف الاساس بدون اضافات و الذى تم تكوينه على اساس الذره الصفراء وكسب فول الصويا ليحتوى على ١٦,٤% بروتين خام و ٢٧٥٠ كيلوكالورى طاقه ممثله/ كجم علف. وتم تعريض المجاميع من ٢الى٥ لدرجة الحرارة المنخفضة الطبيعيه خلال فصل الشتاء (متوسط درجة الحرارة العظمى نهارا ١٨°م ، والدنيا ليلا ٨°م) مع رطوبة نسبية ٦٥±٢% وتم تغذية تلك المجاميع الاربع اما على علف ألاساس بدون اضافات (كنترول سالب تحت ظروف الحراره المنخفضه) أو علف ألاساس مضافا اليه ٢٥٠ مليجرام حامض الاسكوربيك/ كجم علف أو ٤٠٠ ميكروجرام كروميوم (من بيكولينات الكروميوم)/ كجم علف أو ٢٥٠ مليجرام حامض الاسكوربيك +٤٠٠ ميكروجرام كروم/ كجم علف .أظهرت النتائج أن أنخفاض درجة الحرارة أدت الى تدهور فى هضم العناصر الغذائية ما عدا الألياف الخام والأملاح المحتجزة بالمقارنة بالطيور المعرضة لدرجة الحرارة المناسبة. الأتحاد بين الكروميوم وحمض الأسكوربيك أعطت أفضل معاملات هضم للمادة الجافة والبروتين الخام والدهن والأملاح المحتجزة عن وجودها منفردة . تم الحصول على أعلى قيم لهضم العناصر الغذائية والكالسيوم والفوسفور والحديد والزنك المحتجز عندما كان هناك أتحاد بين الكروميوم وحمض الأسكوربيك بينما كانت أقل قيم فى مجموعة الكنترول. أظهرت النتائج أن ليس هناك تأثير معنوى على هيموجلوبين الدم وأظهرت النتائج أن أنخفاض درجة الحرارة قللت خلايا lymphocytes بينما زادت خلايا Erthrocytes, Leukocytes , Heterophill فى الجو البارد عن المعاملة الموجودة فى الجو المناسب فى حين المجاميع التى تحتوى على الكروميوم وحمض الأسكوربيك زادت معنويا خلايا lymphocytes بينما أنخفضت خلايا Erthrocytes, Leukocytes , Heterophill بالمقارنة بمجموعة الكنترول الموجودة فى درجة الحرارة المنخفضة . نستخلص من هذا ألأتحاد بين الكروميوم وحمض الأسكوربيك تحسن النيتروجين المحتجز للدجاج البياض تحت ظروف البرد . أنخفض وزن الصفار والقشرة ودليل الصفار وسمك القشرة ووحدات البياض بأنخفاض درجة الحرارة . ومن نتائج هذة الدراسة دلت على أن الأتحاد بين ٢٥٠ مليجرام حمض الأسكوربيك + ٤٠٠ مليجرام كروميوم أعطت أعلى تأثير أيجابى لمعاملات الهضم وتركيز الأملاح وجودة البيض فى الدجاج المحلى تحت ظروف الجو البارد فى مصر.

قام بتحكيم البحث

أ. د/ السمرة حسن علي أبوعجلة	**كلية الزراعة – جامعة المنصورة**
أ. د/ تاج الدين حسن تاج الدين	**كلية الزراعة (دمياط) – جامعة المنصورة**