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EFFECT OF DIETARY CHROIUM AND ASCORBIC ACID ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF DOKKI$_4$ CHICKENS UNDER EGYPTIAN WINTER CONDITIONS

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SUMMARY

The effect of chromium (Cr) as chromium picolinate (Cr Pi) and ascorbic acid (AA) dietary supplementation on productive and reproductive performance of chickens were studied under winter conditions (December–February). Three hundred (250 hens+50 cocks) Dokki$_4$ chickens, of 30 weeks old were divided into 5 groups of 5 replicates each (10 hens +1 cock/ replicate). The remaining 25 cocks were also divided into 5 groups of 5 cocks each and housed separately for semen evaluation. Birds in the 1st group (Control(+)) was kept in controlled normal temperature (25°C) and fed on the 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 environmental temperature (8-18°C) with relative humidity 65±2%. These groups were fed either basal diet (Control(+)), or the basal diet supplemented with either of 250 ppm AA, 400 ppb Cr or 250 ppm AA plus 400 ppb Cr. Low temperature decreased (P<0.05) relative body weight change (8.6%), egg number (12.9%), egg mass (13.5%), laying rate (18.5%), hatchability of fertile eggs (4.6%), sperms motility (5.7%) and response to SRBCs post-injection at 6-days (13.6%) and at 9-days (25.8%), impaired feed conversion (28.9%) and economic efficiency (48.1%) and increased feed intake (9.3%), plasma cholesterol (21.3%) and glucose (12.2%) contents and malondialdehyde (MDA) concentrations in plasma (105.7%) and semen (54.1%) compared to the control group at normal temperature. All dietary supplementations alleviated (P<0.05) the adverse effects of the low temperature. Birds fed basal diet supplemented with AA+Cr under low temperature had significantly (P<0.05) improved body weight change (5.9%), feed conversion (17.1%), egg number (9.5%), egg mass (5.3%), economic efficiency (33.6%) plasma cholesterol (10.9%) and glucose (10.2%), semen MDA (25.8%), response to SRBCs post-injection at 6-days (19.8%) and at 9-days (38.3%), sperm motility (6.2%) and hatchability of fertile eggs (5.2%) compared with control group at low temperature. There was no significant effect on most semen quality, body temperature, eggs fertility, hatchability (based on total eggs set) and hatched chick weight It may be concluded that a dietary combination of 250 ppm of AA and 400 ppb of Cr provides positive effect on the productive and reproductive performance of local Dokki$_4$ chickens under low ambient temperature.

Keywords: chickens, chromium, ascorbic acid, cold stress, productive, reproductive, malondialdehyde

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INTODUCTION

Poultry performance goes down at low ambient temperature. An increase in feed intake and a decrease in 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 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 (Ensminger *et al*., 1990 and Sigel, 1995). Ascorbic acid is a reducing agent and antioxidant. Previous studies have shown that ascorbic acid is an indispensable micronutrient required to maintain the physiological processes of certain animals including poultry (Sahin *et al*., 2002). Poultry are known to have the ability to synthesize AA,. 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 and parasitic infestation (Kutlu and Forbes, 1993; Sahin and Kucuk, 2001 and Sahin *et al*., 2002). Several studies have indicated beneficial effects of ascorbic acid on the performance of cold-stressed laying hens (Sahin and Sahin, 2001; and Kucuk *et al*., 2003). Dietary Cr supplementation has positively affected the growth rate and feed efficiency of growing poultry (NRC, 1997 and Lien *et al*., 1999). These beneficial effects of Cr can be observed more efficiently under environmental, dietary and hormonal stresses (Sahin *et al*., 2001). Supplemental dietary Cr is also recommended by NRC (1997) for animals undergoing environmental stress. In addition, Cr is thought to be essential for activating certain enzymes and for stabilization of proteins and nucleic acids (Okada *et al*., 1984 and Anderson, 1987). It has been recognized that insulin metabolism influences lipid peroxidation (Gallaher *et al*., 1997). Cr (insulin Co-Factor) is therefore postulated to function as an antioxidant (Preuss *et al*., 1997). Cr deficiency causes disorders of carbohydrates and protein metabolism, reduction in insulin sensitivity in the peripheral tissues as well as a decrease in growth rate (Lindeman, 1996). Chicken and vertebrate sperms display high rates of metabolic activity and consequently reactive oxygen species (ROS) which is believed to be increased under stress conditions. They are rich in polyunsaturated fatty acids (PUFA), which renders them particularly susceptible to oxidation by ROS, especially under stress conditions in humans (Aitken *et al*., 1989), domestic chickens (Eid *et al*., 2006), and also in rabbits (Castellini *et al*., 2000). Over generations, ROS was associated with male infertility (Akiyama, 1999). McDaniel *et al*. (1995) found that when hens were inseminated with semen from stressed males, sperm-egg penetration and fertilized egg production decreased when compared to hens inseminated with semen from control males. As a result, a decrease in reproductive performance followed by significant financial losses occurs. It has been suggested that antioxidants reduce the physiological depress to stress in animals (Eid *et al*., 2003 and Eid *et al*., 2006). The antioxidant defense against ROS seems to be heavily influenced by nutrition. For this reason, the use of nutraceuticals in human and animal nutrition has considerably increased in the last decades (Young *et al*., 2000). The oxidative stability of rabbit semen increased in relation to dietary antioxidants like ascorbic acid (Yousef *et al*., 2003). Moreover, antioxidants can protect against

the damaging effect of leukocyte-derived ROS on sperm movement (Baker *et al*., 1996). Therefore, the objective of this study was to determine the effect of Cr and AA supplementations on the productive performance, lipid peroxidation, semen characteristics and reproductive parameters of $Dokki_4$ chickens reared under cold weather in Egypt during winter season..

MATERIALS AND METHODS

Total number of 300 (250 laying hens+50 cocks) chickens (Egyptian strain, $Dokki_4$), 30 weeks of age were divided into 5 groups of 5 replicates each (10 hens+1 cocks/replicate). The remaining 25 cocks were also divided into 5 groups of 5 cocks each and housed separately for semen evaluation. Birds were housed in open sided pens exposed to natural low environmental temperature prevails in Egypt during winter season (December–February). Natural temperature average was 18°*C* during day time and 8°C during night time with average relative humidity 65±2%. The birds were exposed to a light program of 16 hours of light: 8 hours of dark per day. Ingredients and chemical composition of the basal diet are shown in Table 1. The experimental diets were supplied to meet the nutrient requirements of the Ministry of Agriculture Decree (1996). The birds were fed either basal diet (1^{st} treatment: control, at 25°C normal room temperature, CN), the basal diet (2^{nd} treatment: control, at low temperature CL), the basal diet supplemented with 250 ppm AA (3^{rd} treatment), 400 ppb Cr as chromium picolinate (Cr Pi, (Chromax®, Prince Agric. Products) (4^{th} treatment) or 250 ppm AA plus 400 ppb Cr (5^{th} treatment). The dietary treatments lasted for 12 weeks from week 30 to week 42 of age. The AA was supplied as (Rovimix® STAY® 35) specifically produced for use as a stabilized source of AA in feed by commercial company (Roche, Levent, Istanbul, Turkey).

Productive parameters:

Initial and final body weight was recorded to calculate body weight change throughout the experimental period. Feed consumption was estimated weekly and feed conversion was calculated. The number of eggs and egg weights were recorded daily. At the end of the experimental period, nine birds per treatment were randomly chosen and slaughtered; blood samples were collected, and then centrifuged for 20 min at 3000 rpm. Plasma were collected and stored in -29°C until analysis. Plasma total cholesterol was determined according to (Watson, 1960), by using biochemical kits (Dammond Diagnostic, Cairo, Egypt). Lipid peroxidation as thiobarbituric acid reactive substances (TBARS) was determined in plasma or semen by method of Placer *et al.* (1966) as modified by Matkovics *et al.* (1989). Values of TBARS were expressed in terms of MDA nmol/ml plasma or semen).

Reproductive parameters:

Three cocks from each treatment were used for collecting semen samples at the 4^{th}, 8^{th} and 12^{th} week of the experiment. Semen was collected by means of abdominal massage. Semen volume was determined by using graded tubes. Sperm concentration ($x10^9$ sperm/ml) was estimated by a haemo-cytometer. Immediately after semen collection, sperm motility percentage was measured using a small droplet from each individual placed on a warm slide, covered with a slide and examined for sperm motility microscopically at 400X magnification using a stage warmer set at 39 °C. Motility was classified as described by Melrose and Laing (1970). Viability was

estimated as the percentage of sperm that were permeable to eosin; these were regarded as dead (Lake and Stewart, 1978). For fertility and hatchability estimation, eggs laid were collected for 7-day periods and were stored in an egg room at 15.5 °C dry bulb and 70% relative humidity (RH). They were incubated at 37.6°C and RH was 55–60% and hatched at 37.3°C and RH was 65-70% in automatic incubators. Fertility was calculated as number of fertile eggs as relative to total number of eggs set; meanwhile hatchability was calculated as number of healthy hatched chicks as relative to total fertile number of eggs set.

Table 1. Composition of the experimental chicken diet (30-42 wks old)

Ingredients	%
Yellow corn	66.0
Soybean meal (44%)	24.0
Sodium chloride	0.30
Limestone	7.59
Dicalcium phosphate	1.71
Vit. + Min. Mix.[1]	0.30
DL-Methionine	0.10
Total	100
Calculated analysis[2]:	
Crude protein, %	16.43
ME, Kcal/kg	2750
Crude fiber, %	3.20
Ether extract, %	2.70
Calcium, %	3.33
Avail. phosphorus, %	0.45
Total phosphorus, %	0.66
Methionine, %	0.39
Meth.+ Cyst., %	0.67
Lysine, %	0.86

[1]Premix contain per 3 kg: Vit. A 10, 000, 000 IU, Vit. D_3 2,000,000 IU, Vit. E 10,000 mg,Vit. K3 1,000 mg, Vit. B_1 1, 000 mg, Vit. B_2 5000 mg, Vit. B_6 1500 mg, Vit. B_{12} 10 mg, Niacin 3000 mg, Pantothenic acid 1000 mg, MnO 60, 00 mg, ZnO 50, 000 mg, Fe_2 SO_4 30,000 mg, $CuSO_4$ 4000 mg, Calcium iodide 300 mg, Co 100 mg, Choline chloride 250 mg, $CaCO_3$ carrier till 3000 g.
[2] According to Egyptian Feed Composition Tables (2001).

Economical Efficiency (EE):

It was calculated as follow: EE= [total revenue (number of newly healthy hatched chicks × its price (1.20 LE) + (useless eggs for incubation × its price (0.50 LE)) per hen – total feed cost (total feed intake ×its price, LE/hen) ÷ total feed cost] ×100.

Statistical analysis*:*

Data were statistically analyzed using one–way ANOVA of GLM procedure of Statistical Analysis Software (SAS,1996). Significant differences among treatment means were separated by Duncan's new multiple range Test (Duncan, 1955) with 5% level of probability.

RESULTS AND DISCUSSION

Productive performance:

The effects of supplemental dietary AA and Cr during cold weather under low ambient temperature in winter in Egypt on the performance of $Dokki_4$ laying hens are

shown in Table 2. Low temperature significantly (P<0.05) reduced final body weight (7.6%), relative body weight change (8.6%), egg number (12.9%), egg mass (13.5%) and laying rate (18.6%), increased feed intake (9.3%) and impaired feed conversion (28.9%) compared to control birds under normal temperature. There was a positive significant (P≤0.05) effect of AA and/or Cr supplementations on final body weight, relative body weight change, egg laying rate, egg number, egg weight under low ambient temperature. Dietary combination of AA. and Cr improved (P<0.05) final body weight (6.4%), relative body weight change (5.9%), egg number (9.5%), egg mass (5.3%) and laying rate (16.8%) compared to the control diet at low ambient temperature. No significant (P≤0.05) effect of either low temperature or any supplementations was noted on body temperature and egg weight. In the present study, significant adverse effects on body weight change, egg production, as well as on antioxidant status were observed when birds were kept in cold weather compared with laying hens kept under thermal neutral temperature (25°C). Stress depresses ascorbic acid synthesis in poultry (Anderson, 1987), thus may result in marginal chromium and AA deficiency or increased chromium and AA requirements implying that both Cr and AA should be supplemented in such conditions. Ascorbic acid and Cr 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 and 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 (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 AA reduces the synthesis of corticosteroids hormones in birds. By decreasing synthesis and secretion of corticosteroids, AA alleviates the adverse effects of cold stress such as related depression in poultry performance (Sahin and Sahin 2001).KuKcuk *et al*. (2003) reported that AA supplement increased egg production in laying hens under low temperature. Also, several researches have documented beneficial effects of AA supplementation on egg production in poultry kept under environmental stress (Orban *et al.,* 1993 and Bains, 1996). Sands and Smith (1999) also, reported that dietary chromium picolinate supplementation increased growth rate in stressed broilers. Lien *et al*. (1999) reported that 1600 ppb/kg or 3200 ppb/kg Cr Pi supplementation to a broiler diet increased feed intake and improved live weight gain. In addition, Steele and rose rough (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*. (2002) 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 Cr is involved in protein metabolism (Anderson, 1987). Chromium is thought to have a role in nucleic acid metabolism and in stimulation of amino acid incorporation into liver protein in vitro (Weser and Koolman, 1969). Okada *et al*.,

(1983) showed that an interaction of Cr with DNA templates resulted in a significant stimulation of RNA synthesis *in vitro*.

Table 2. Productive performance of Dokki$_4$ hens reared during winter season as affected by dietary supplemental AA or Cr

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+ AA+Cr	
Initial body weight, g	1503.33 ±8.82	1500.00 ±5.78	1501.67 ±10.14	1508.33 ±10.93	1513.33 ±6.67	0.809
Final body weight, g	1746.7^{a} ±3.33	1613.3^{d} ±6.66	1670.0^{c} ±10.00	1676.7^{c} ±14.53	1716.7^{b} ±8.82	0.001
Body weight change,%	16.19^{a} ±0.66	7.55^{d} ±0.19	11.21^{c} ±0.35	11.16^{c} ±0.38	13.43^{b} ±0.15	0.001
Body temperature,°C	41.47 ±0.067	4067 ±0.033	41.50 ±0.058	41.57 ±0.38	41.47 ±0.15	0.166
Egg number /h /12wk	55.00^{a} ±1.154	47.90^{d} ±0.001	49.67^{cd} ±0.882	51.00^{bc} ±0.577	52.33^{b} ±0.333	0.002
Egg weight, g	49.97 ±0.09	49.69 ±0.01	49.79 ±0.08	49.84 ±0.08	49.76 ±0.133	0.312
Egg mass, kg/h/12wk	2.75^{a} ±0.059	2.38^{d} ±0.001	2.47^{cd} ±0.041	2.54^{bc} ±0.033	2.60^{b} ±0.022	0.001
Egg production, %	65.48^{a} ±1.38	53.34^{d} ±0.12	59.13^{c} ±1.05	60.71^{bc} ±0.69	62.30^{b} ±0.39	0.001
Feed intake, g/h/d	100.7^{c} ±0.069	110.1^{a} ±0.061	106.8^{ab} ±0.68	106.9^{ab} ±0.78	102.21^{bc} ±3.34	0.013
Feed conversion, g	3.08^{d} ±0.06	3.97^{a} ±0.00	3.63^{b} ±0.04	3.53b^{c} ±0.06	3.29^{cd} ±0.13	0.001

^{abc} Means within each row with different superscripts are significantly different ($p<0.05$).
AA =ascorbic acid, Cr = chromium

Malondialdehyde, plasma constituents and immune response to SRBSs:

The effect of AA. and/or Cr on plasma and semen MDN, other plasma constituents and immune response to SRBSs under cold stress are presented in Table 3. The results show that low temperature increased plasma cholesterol (21.25%) and glucose (12.23%) contents and MDA concentrations in plasma (105.7%) and semen (54.14%) compared to control birds at normal temperature. On the other hand, low temperature decreased response to SRBCs post-injection at 6-days (13.61%) and at 9-days (25.77%) compared to control birds at normal temperature. Cholesterol, glucose and MDA concentrations were lower in the groups supplemented with AA and /or Cr than the low temperature control group ($p\leq0.05$). Post-injection responses to SRBCs at 6- and 9-days were higher in the groups supplemented with AA and AA+Cr than the low temperature control group ($P\leq0.05$). Results of the present study are in agreement with the findings of several researchers (Kucuk *et al*., 2003 and Metwally, 2004). Generally, supplementing AA decreased plasma concentrations of glucose and cholesterol. These results might be due to decreased corticosterone (Catabolic) and increased insulin (anabolic) concentrations upon AA supplementations. Chromium stimulates and regulates the action of insulin (Mowat 1994) which is involved in

anabolic processes (Colgan, 1993). Also, through increasing the effectiveness of insulin, Cr indirectly potentates AA transportation (Mann and Newton, 1975; Seaborn *et al*., 1994). Cr was shown to be a protective factor against heart disease by achieving a regression of cholesterol induced arteriosclerosis in rabbits (Abraham *et al.*, 1991).

Table 3. MDA, plasma constituents and immune response to SRBCs of Dokki$_4$ hens during winter season as affected by dietary AA or Cr.

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+ AA +Cr	
Plasma cholesterol, mg/dl	153.67^{d} ±2.33	186.33^{a} ±1.86	173.67^{bc} ±1.86	175.33^{b} ±2.91	166.00^{c} ±3.06	0.001
Plasma glucose, mg/dl	229.00^{c} ±1.00	257.00^{a} ±1.53	239.33^{b} ±2.040	235.00^{bc} ±2.089	230.07^{c} ±2.85	0.001
Plasma MDA, nmol/ml	1.23^{c} ±0.15	2.53^{a} ±0.15	1.83^{b} ±0.09	1.85^{b} ±0.08	1.53^{ab} ±0.15	0.001
Semen MDA, nmol/ml	4.23^{c} ±0.15	6.52^{a} ±0.13	5.43^{b} ±0.14	5.39^{b} ±0.11	4.84^{c} ±0.11	0.001
Post-injection responses to SRBCs at:						
3- days	5.12 0.13	4.54 0.15	4.28 0.11	4.18 0.13	4.57 0.14	0.105
6- days	7.35^{ab} 0.12	6.35^{c} 0.10	7.36^{ab} 0.10	6.60^{bc} 0.13	7.61^{a} 0.11	0.001
9- days	4.85^{a} 0.11	3.60^{b} 0.12	4.85^{a} 0.13	3.85^{b} 0.11	4.98^{a} 0.10	0.001

^{abc} Means within each row with different superscripts are significantly different ($p<0.05$).
MDA= Malondialdehyde, AA =ascorbic acid, Cr = chromium

It is well known that stress increases MDA concentration as a lipid peroxidation indicator (Halliwell and Gutteridge, 1989 and Sahin *et al.,* 2002). ntioxidant systems (glutathione peroxidase, superoxide dismutase and vitamins: E,C, A) are important in scavenging free radicals and their metabolic products, as well as in maintaining normal cellular physiology restoring depletion of various antioxidants in stressed poultry (Halliwell and Gutteridge 1989). In the present study, semen MDA decreased when dietary vitamin C and chromium were supplemented (Table 3). These results are in agreement with Gursu (2004) and Sahin *et al*. (2003). The presence of high concentrations of polyunsaturated fatty acid (PUFA) (Ravie and Lake, 1985) within the lipid fraction necessitates the presence of an efficient antioxidant system to protect against peroxidative damage and possible associated sperm dysfunction (Aitken, 1994). Because the actual mechanism of lipid peroxide formation by fowl spermatozoa remains unidentified, it is not yet known whether the presence or absence of some factors such as antioxidants could suppress the production of high concentrations of lipid peroxides and subsequently enhance the sperm quality under stress conditions. The lipid content of the seminal plasma and spermatozoa make it a

good target for free radical attacks under oxidative stress. This assumption is supported by the elevation of MDA under cold condition.

Semen characteristics:

The effects of AA. and/or Cr on semen volume, sperms concentration, motility, life sperms and abnormal sperms under the cold stress are illustrated in Table 3. There were no significant effects of low temperature on all pervious parameters of semen characteristics except for sperms motility were decreased (5.73%) compared with the control group under the normal temperature. Only dietary supplemented with AA+Cr significantly enhanced sperm motility comparing to the control under low temperature. On the other hand, this combination completely recovered the sperm motility to the same level of the control group under the normal condition. It is obvious that a decline in sperm motility was associated with accumulation of the MDA. The combination between AA and Cr decreased MDA in the seminal plasma because of its antioxidative properties and as a result of this; sperm motility was enhanced under stress condition. This is in accordance with the report of Surai *et al.* (1997) who showed that enhancement of the antioxidant capacity of semen could present a major opportunity for improving male fertility. This was clear in the present data too. Dietary AA + Cr protects sperms from free radical attacks under cold stress, which is translated into enhanced motility and insignificant reduction in dead sperm percentage, in addition to the reduced MDA value under the stress condition.

Fertility and hatchability:

Data concerning the effect AA. and/or Cr under low ambient temperature on reproductive traits of laying hens are presented in Table 4. No significant effects of either low temperature or dietary supplements were found on fertility, hatchability (based on total egg set) and chick weight at hatch. Significant decreases (4.6%, P=0.044) were found in hatchability percent based on fertile eggs by low temperature. Dietary supplemented with AA.+Cr showed higher hatchability percent based on fertile eggs compared to the control group under low temperature and equaled with that of the normal temperature. This significant effect may refer to the combined antioxidative effect of AA. + Cr. However, reverse reports (Whitehead and Keller, 2003 and McDaniel *et al*., 2004) did not find any positive effect for improving fertility or hatchability due to AA.

Economic efficiency:

Results in Table (6) indicated that laying hens exposed to low temperature (8-18°C) for 12 weeks impaired economic efficiency (48.09%). Dietary supplementations improved economic efficiency values by 33.55, 21.32 and 10.21% for groups fed basal diet with AA+Cr, AA and Cr, respectively, as compared with that of the control group which exposed to low temperature. This improvement could be due to improving the feed conversion or reducing the amount of feed required to produce one unit of egg mass.

Table 4. Semen characteristics of Dokki$_4$ cocks reared during winter season as affected by dietary supplemental AA or Cr

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+ (AA+Cr)	
Semen volume, ml	0.360 ±0.012	0.350 ±0.012	0.363 ±0.012	0.357 ±0.003	0.383 ±0.003	0.111
Concentration of sperm, 10^9/ml	2.19 ±0.106	2.18 ±0.107	2.20 ±0.173	2.27 ±0.145	2.40 ±0.058	0.651
Sperm motility, %	85.57^a ±1.18	80.67^b ±0.166	83.50^{ab} ±2.760	84.00^{ab} ±0.577	85.66^a ±1.202	0.048
Life sperms, %	86.00 ±1.16	86.00 ±1.16	88.00 ±1.16	88.70 ±1.33	90.00 ±2.57	0.166
Abnormal sperms,%	4.00 ±2.31	4.00 ±2.31	3.00 ±0.90	2.60 ±1.54	2.00 ±0.16	0.836

^{abc} Means within each row with different superscripts are significantly different (P<0.05).
AA =ascorbic acid, Cr = chromium

Table 5. Reproductive performance of Dokki$_4$ laying hens reared during winter season as affected by dietary supplemental AA or Cr

Item	(+) Cont. (25°C)	Low temperature treatments (8-18°C)				P value
		(-) Cont.	+AA 250ppm	+Cr 400ppb	+ (AA+Cr)	
Eggs fertility, %	88.00 ±1.16	88.00 ±1.16	88.00 ±1.51	88.50 ±1.25	90.00 ±1.73	0.734
Hatchability of total eggs set,%	75.40 ±1.26	74.40 ±1.24	76.93 ±1.05	76.53 ±0.87	77.27 ±1.59	0.766
Hatchability of fertile eggs, %	87.01^a ±0.84	83.00^c ±1.15	85.20^b ±0.87	85.00^b ±0.58	87.33^a ±0.49	0.044
Hatched chicks weight, g	35.20 ±0.82	35.10 ±0.83	35.86 ±0.82	35.53 ±0.48	35.90 ±0.23	0.804

^{abc} Means within each row with different superscripts are significantly different (P<0.05).
AA =ascorbic acid, Cr = chromium

Table 6. Economic efficiency of Dokki$_4$ laying hens reared during winter season as affected dietary supplemental AA or Cr

Items	(+) Cont. (25°C)	Low temperature treatments (8 -18°C)			
		(-) Cont	+AA 250ppm	+Cr 400ppb	+ (AA+Cr)
Price / kg feed, LE	1.500	1.500	1.515	1.600	1.615
Total feed intake /h, kg	8.46	9.25	8.97	8.98	8.59
Total feed cost /h, LE	12.690	13.875	13.590	14.368	13.873
Total revenue / h, LE*	22.00	19.16	19.87	20.4	20.93
Net revenue / h, LE	9.31	5.285	6.28	6.032	7.057
EE ,%	73.37	38.09	46.21	41.98	50.87
Relative EE, %**	100	51.91	62.78	57.22	69.33

*EE (Economic efficiency) = [total revenue (number of newly healthy hatched chicks × its price (1.20 LE) + (useless eggs for incubation × its price (0.50 LE)) per hen – total feed cost (total feed intake ×its price, LE/hen) ÷ total feed cost] ×100.
** Assuming the economic efficiency of (+) control equal 100.

In the present study, the magnitude of the increases of reproductive performance was greater when a combination of ascorbic acid and chromium was supplemented than when supplemented separately. These results revealed additive effects of ascorbic acid and chromium, indicating that ascorbic acid and chromium work together or act synergistically. Similarly, Carol *et al*. (1994) found an interaction between Cr and vitamin C on bone and brain Mn retention and distribution in guinea pigs, and stated 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 insuline, chromium indirectly promotes the ascorbic acid transportation (Seaborn *et al*., 1994). Based on the results of the present study, it may be concluded that dietary supplementation with a combination of 250 ppm AA and 400 ppb of Cr provides the highest positive effect on the performance and economic efficiency of local $Dokki_4$ chickens under 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 التغذية على الكروميوم وحمض الأسكوربيك على الأداء الأنتاجى والتناسلى لدجاج دقى، تحت ظروف الشتاء فى مصر

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اجرى هذا البحث بغرض دراسه تأثير أضافة الكروميوم وحمض الأسكوربيك على الأداء الأنتاجى والتناسلى لدجاج دقى، تحت ظروف الشتاء فى مصر(ديسمبر– فبراير). استجدم فى هذه الدراسه عدد٣٠٠ طائر (٢٥٠ دجاجة بياضه +٥٠ ديك) دقى٤ عمر ٣٠ أسبوع وتم تقسيمها الى ٥ مجاميع بكل مجموعة ٥ مكررات وكل مكررة (١٠ دجاجات +١ ديك) و ٢٥ ديك المتبقية قسمت أيضا الى ٥ مجاميع وكل مجموعة ٥ ديوك تم تسكينها فرديا لجمع السائل المنوى وتقيمة. وتم وضع المجموعة الاولى فى حجرة التحكم الحرارى عند درجه الحراره العاديه ٢٥°م (كنترول ايجابى) وتم تغذيتها على علف الاساس بدون اضافات و الذى تم تكوينه اساسا من الذره الصفراء وكسب فول الصويا ليحتوى على ١٦.٤% بروتين خام و٢٧٥٠ كيلوكالورى/ كجم علف. وتم تعريض المجاميع من ٢الى٥ لدرجة الحرارة المنخفضة الطبيعيه خلال فصل الشتاء (درجة الحرارة العظمى نهارا ١٨°م ، والدنيا ليلا ٨°م) مع رطوبة نسبية ٦٥±٢% وتم تغذية تلك المجاميع الاربع على علف ألاساس بدون اضافات أو علف ألاساس مضافا اليه ٢٥٠ مليجرام حامض الاسكوربيك/ كجم علف أو ٤٠٠ ميكروجرام كروميوم (من بيكولينات الكروميوم)/ كجم علف أو٢٥٠ مليجرام حامض الاسكوربيك +٤٠٠ ميكروجرام كروميوم/ كجم علف. ادى انخفاض درجه الحراره الى إنخفاضاً جوهريا فى كل من معدل التغير فى وزن الجسم، عدد البيض الناتج ، كتلة البيض، معدل الأنتاج، نسبه الفقس على اساس البيض المخصب، حركه الحيوانات المنويه، الاستجابه المناعيه ضد كرات الدم الحمراء للغنم بعد الحقن بسته ايام، تسعه ايام وذلك بمقدار: ٨.٦، ١٢.٩، ١٨.٦، ١٣.٥، ٤.٦، ٥.٧، ١٣.٦، ٢٥.٨% على التوالى وتدهورا فى كل من كفاءه تحويل العلف الى بيض، الكفاءه الاقتصاديه بمقدار ٢٨.٩، ٤٨.١% على التوالى وزياده فى كل من العلف الماكول ,محتوى بلازما الدم من الكلسترول ،الجلوكوز، وتركيز المالونالدهيد فى كل من بلازما الدم ،والسائل المنوى وذلك بمقدار: ٩.٣، ٢١.٣، ١٢.٢، ١٠٥.٧، ٥٤.١% على التوالى وذلك بالمقارنه مع مجموعه الكنترول الايجابى المرباه فى درجه الحراره العاديه. الاضافات الغذائيه تسببت فى تخفيف او تلاشى اثار الحراره المنخفضه على اداء الدجاج. سجلت الطيور المغذاه على علف الاساس مضافا اليه حامض الاسكوربيك مع الكروميوم افضل الاضافات وحسنت جوهريا اثار الحراره المنخفضه فى كل من معدل التغير فى وزن الجسم، الكفاءه الغذائيه، الكفاءه الاقتصاديه، عدد البيض الناتج، كتله البيض، محتوى بلازما الدم من الكلسترول، والجلكوز، وتركيز المالونالدهيد فى السائل المنوى، والاستجابه المناعيه بعد ٦ ايام، ٩ ايام، حركه الحيوانات المنويه، نسبه الفقس على اساس البيض المخصب وذلك بمقدار ٥.٩، ١٧.١، ٣٣.٦، ٩.٥، ٥.٣، ١٠.٩،

١٠.٢، ٢٥.٨، ١٩.٨، ٣٨.٣، ٦.٢، ٥.٢% على التوالى وذلك بالمقارنه مع مجموعه الكنترول السلبى المرباه فى درجه الحراره المنخفضه. لا توجد اختلافات معنويه بين المجموعات فى معظم صفات السائل المنوى، درجه حراره الجسم، خصوبه البيض، نسبه الفقس على اساس البيض الكلى، وزن الكتاكيت الفاقسه. وتخلص هذه الدراسه الى أن أضافه كل من حامض الاسكوربيك بمعدل ٢٥٠مليجرام والكروميوم بمعدل ٤٠٠ ميكروجرام معا لكل كجم علف أعطت تأثير أيجابيا على الأداء الإنتاجى والتناسلى والكفاءة الاقتصادية لدجاج سلاله دقى؛ المرباه تحت ظروف درجة الحرارة المنخفضة خلال فصل الشتاء فى مصر.