Influence of Dietary Zinc and Vitamin C Supplementation on Some Blood Biochemical Parameters and Egg Production in Free-Range Laying Hens

Влияние на цинка и витамин С върху биохимични показатели на кръвта и носливостта при свободно отглеждани кокошки

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Abstract

The study aimed to follow out the effect of antistress dietary supplements Zinteral 35 and vitamin C on the levels of some blood biochemical parameters (corticosterone, total cholesterol, glucose, total protein and creatinine) and egg production in laying hens during cold (7^o C), thermoneutral (19^o C) and hot (31^o C) periods. The fowls were divided in three groups (26 females and 3 males in each group). They were reared in a free-range management system with elements of organic production. The experimental treatments were as followed: first (control) group without dietary supplement, second group with 100 mg Zinteral 35 per kg diet containing 35 mg/kg zinc oxide, third group with the same amount of Zinteral 35 together with 250 mg vitamin C per kg diet.
During the three periods with different ambient temperature, the hens supplemented either with zinc alone (second group) or co-administered zinc + vitamin C (third group) had significantly lower levels of plasma corticosterone ($P<0.001$), serum cholesterol ($P<0.05$) and glucose ($P<0.05$) than those from the first (control) group. The differences between the third and the first groups were bigger versus those between the second and the first groups.
For the entire period (March 1 and June 21), egg production was higher by 2.22 % and 4.60 % in the second and third groups respectively in comparison to the first group.
The combination of 100 mg Zinteral 35 and 250 mg vitamin C per 1 kg diet exhibited a synergistic effect in reducing cold and heat stress in laying hens and increased their egg production.

Keywords: blood biochemical parameters, corticosterone, egg production, laying hens, vitamin C, zinc supplement.

Резюме

Целта на настоящето проучване е да се изследва ефекта на антистресовите хранителни добавки Zinteral 35 и витамин С върху съдържанието на някои кръвни биохимични показатели (кортикостерон, общ холестерол, кръвна захар, общ белтък и креатинин) и носливостта на свободно отглеждани кокошки носачки с елементи на биологичното производство при студен (7 °С), термонеутрален (19 °С) и горещ (31 °С) период от годината. Експерименталните птици бяха разделени поравно в три групи (по 26 женски и 3 мъжки във всяка група) - първа (контрола) група без участие на хранителна добавка в комбинирания фураж, втората група с добавка на 100 mg Zinteral 35 съдържаща 35 mg/kg цинков оксид, и трета група с добавка на същото количество Zinteral 35 плюс 250 mg витамин С за всеки килограм комбиниран фураж.

По време на трите температурни периода, кокошките от втора група приемали като добавка само Zinteral 35 и тези от трета група приемали Zinteral 35 плюс витамин С са имали значително по-ниски нива на плазмен кортикостерон ($P<0.001$), серумен холестерол ($P<0.05$) и глюкоза ($P<0.05$) в сравнение с тези от първа (контролна) група, като разликите между трета и първа група са по-големи в сравнение с тези между втора и първа група.
За целия експериментален период (от 1 март до 21 юни) се отчете по-висока носливост съответно с 2,22 % и 4,60 % за втора и трета група в сравнение с първа група.
Комбинацията от 100 mg Zinteral 35 и 250 mg витамин С добавени към един килограм комбиниран фураж има синергичен ефект като намалява студовия и топлинен стрес и повишава носливостта на кокошките носачки.

Ключови думи: кокошки носачки, цинкова добавка, витамин С, кортикостерон, биохимични показатели на кръвта

Introduction

Free-range management systems and organic poultry farming create conditions for cold stress during the winter and heat stress during the summer. Temperature discomfort negatively affects laying capacity, weight and quality of eggs in various bird species (Wolfenson et al.**,** 1979, 2001; Emery et al., 1984; Özbey et al., 2004; Kucuk et al.**,** 2008).
Nutritional strategies are important with regard to maintaining egg productivity and limiting the temperature stress using appropriate supplements (micronutrients, vitamins, minerals) in rations (Wiesinger, 2001; Heinzen and Pollack, 2003; Rodenburg et al., 2005; Lin et al., 2006; Sahin et al., 2009).
In temperature stress conditions, many authors recommend the addition of zinc compounds alone or combined with other supplements in the diet because of its antioxidant and anti-stress effect (Kienholz et al., 1992; Sahin, et al., 2002, 2005; Nollet et al., 2008; Kucuk, 2008; Kucuk et al., 2003, 2008). Furthermore, the addition of this

element is justified by its reduced retention in the body during stress (Bartlett and Smith, 2003). Other authors included vitamin C in the diet to alleviate heat and cold stress (McDowell, 1989; Sahin et al., 2002).

The aim of this study was to investigate the influence of the anti-stress supplement Zinteral and vitamin C, either alone or together on blood corticosterone level, some blood biochemical parameters and egg production in free-range laying hens.

Material and Methods

This study was carried out with 87 New Hampshire strain NG hens from 36 to 51 weeks of age in the Poultry farm of the Department of Animal Science, Agriculture University – Plovdiv between March 1 and June 21, 2010.

The birds were divided in three equal groups - 26 females and 3 males in each group. They were reared in a free-range system with elements of organic production. The fowls were housed in small barns (size 3.50/2.50/2.75 m) with outdoor walking yards (size 9.20/24 m) and broad-leave trees in the middle (Figure 1). The barns were identically equipped with 3 perches, 2 m in length, and 8 two-floor wooden nests sized 30/30/40 cm each. The light intensity coefficient was 1:10. In the bottom of the southern wall of pens, there was a 30/40 cm rectangular opening for outdoor access.
The rearing conditions for the experimental hens were fully compliant with minimum requirements for humane treatment of breeder hens (Regulation No 25/2006, Regulation No 44/2006).
The average microclimatic parameters were measured automatically every day on the basis of the pointed out indicators identified in the walk yards during the day and in the sleep houses - during the night. The fowls from the first group were used as control. They were fed a diet presented in Table 1. The zinc in the diet of the fowls from second group was supplemented as Zinteral 35 (Lohmann Animal Health, Cuxhaven, Germany) containing 35 mg/kg zinc oxide at 100 mg per 1 kg diet.
The diet of the fowls from the third group was supplemented with the same amount of Zinteral 35 and 250 mg vitamin C (L-ascorbic acid, CSPC Weisheng Pharmaceutical, Shijiazhuang Co. Ltd) per 1 kg diet. According to Council Regulation (EC) No1804/1999, the used feed supplements are allowed in organic animal production systems.

Figure 1. Fowls in cold period (original)

During the study the laying capacity was determined on a weekly basis by universal formula:

$$I_{egg} = \frac{N_{egg}}{7.N_{hens.}}.100 \quad (\%)$$

where N_{egg} was the number of eggs produced per week, N_{hens} was the number of laying hens

Table 1. Composition of experimental basal diet

Ingredients	g/kg
Corn yellow	356.2
Wheat	200.0
Soybeans, toasted whole	170.0
Sunflower expeller	180.0
Limestone	80.0
Dicalcium phosphate	9.0
Sodium chloride	2.8
Vitamin and mineral premix [1]	2.0
Nutrient analysis:	
ME, kcal/kg	2842
Protein (N x 6.25), g	171
Fat, g	40
Lysine, g	7.4
Methionine + cystine, g	6.4
Threonine, g	6.2
Tryptophan, g	1.9
Arginine, g	
Calcium, g	32.1
Phosphorus available, g	3

[1] The vitamin and mineral premix Rovimix 15-C Layer provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 30 mg; vitamin K3, 3,0 mg; vitamin B1, 2,0 mg; vitamin B2, 5,0 mg; vitamin B6, 5,0 mg; vitamin B12, 0,016 mg; niacin, 30 mg; pantothenic acid, 12,0 mg; folic acid 1,0 mg; biotin, 0.05 mg; Co, 0,15; I, 1 mg; Fe, 50 mg; Zn, 80 mg; Mn, 100 mg; Cu, 8 mg; Se, 0.2 mg; antioxidant, 25 mg.

Blood for analysis (5 ml) was sampled from *v. Subcutanea ulnaris* of six hens from each group on March 15 (cold period), May 15 (termoneutral period) and June 21 (hot period) 2010. The manipulations related to blood collection did not exceed 2 min which is considered by Lagadic et al. (1990) as a duration having no effect on blood glucose levels in birds. Samples were transported to the lab in a cooling bag. By the blood taking from the birds we have complied with all the requirements of Regulation No 15/2006 on the minimum requirements for the protection and welfare of experimental animals and requirements to establishments, growing and / or supply.

Blood serum glucose and cholesterol were determined with the biochemical analyzer "BA-88" in the innate resistance research lab at the Faculty of Veterinary Medicine, Trakia University– Stara Zagora.

The statistical processing of the results was performed by means of one-way ANOVA using the GraphPad InStat 3.06 software at level of significance P<0.05 (t-test).

Results and Discussion

During the cold and the hot periods, registered ambient winter temperature was low (7.39 ^{o}C) and summer temperature was very high (over 31 ^{o}C), hence they were prerequisites for induction of stress (Table 2).

Table 2. Microclimatic parameters during the cold, thermoneutral and hot period (m±SEM)

Period	Average ambient temperature (^{o}C)	Average air humidity (%)	Average air velocity (m/s)	NH_3 (ppm)	Light intensity (lx)
Cold period	7.39±0.54	68.10±3.43	0.50±0.06	traces	43.21±4.34
Thermoneutral period	19.44±2.5	66.07±1.59	0.50±0.04	traces	65.00 ±3.27
Hot period	31.24±0.88	54.75±1.25	0.41±0.03	traces	87.90±4.34
Reference values*	18–25	50–70	0.2–0.5	< 15	30–60

Note: *Reference values as per Ordinance 44/2006

It was manifested with changes in blood plasma corticosterone and several serum biochemical indices (Table 3).

According to Sahin and Kucuk (2003), Sahin et al. (2004), Gonzales-Esquerra and Leeson (2006), Lin et al. (2006) increased blood corticosterone is a reliable indicator of the extent of stress provoked by extremely low or high temperatures in poultry farming. Sahin et al. (2002) outlined that under such conditions, higher serum glucose, cholesterol and triglycerides were established.

Birds supplemented with both zinc and vitamin C exhibited statistically significantly lower blood corticosterone concentrations (P<0.01 in thermoneutral period and P<0.001 in cold and hot periods) compared to untreated controls (Table 3).

The lower corticosterone level could be attributed to the antioxidant and antistress effect of zinc and vitamin C supplements (Sahin et al., 2002; Lin et al., 2006). The zinc limits the excessive secretion of corticosterone as a co-factor of essential antioxidant enzymes – Cu/Zn superoxide dismutase and inhibiting NADPH-dependent lipid peroxidation (Prasad, 1997; Prasad and Kucuk, 2002). In addition, Onderci et al. (2003) reported that supplemental zinc increased serum vitamin C.

During both the hot and the cold periods, statistically significant differences in serum glucose and total cholesterol were demonstrated – over P<0.05.

After supplementation of zinc only or Zn + vitamin C combination, statistically significant (over P<0.05) reduction in blood glucose and cholesterol occurred in birds from the second and the third group vs the first group. Similar data are reported by Sahin et al. (2002, 2009), Kucuk et al. (2003), Yanchev et al. (2007), which could be attributed to the antioxidant and antistress effect of zinc and vitamin C limiting excessive corticosterone production and corticosterone-induced systemic biochemical changes (Sahin et al., 2002; Kucuk et al., 2003).

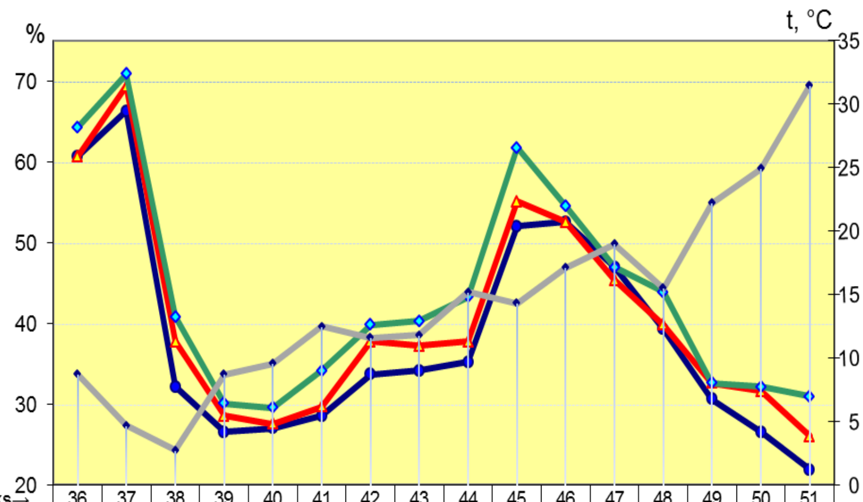
Table 3. Blood biochemical parameters in m±SEM (n=6)

Group	Cold period	Thermoneutral period	Hot period
		Corticosterone, mmol/L	
First group (control)	166.09±3.22 $A_1; a_1; a_2;$	107.31±11.98 $A_1; A_2; b_1; b_2;$	173.09±5.08 $A_2; a_3; a_4;$
Second group (Zn)	92.54±4.46 $A_3; a_1;$	53.44±10.14 $A_3; A_4; b_1;$	114.19±9.91 $A_4; a_3;$
Third group (Zn+ vit. C)	62.43±9.67 a_2	58.64±11.91 b_2	76.49±6.40 a_4
		Total cholesterol, mmol/L	
First group (control)	2.76±0.09 $A_5; a_5; a_6;$	2.29±0.04 $A_5; A_6; c_1; b_3;$	3.32±0.09 $A_6; c_2; c_3;$
Second group (Zn)	2.24±0.03 $a_5;$	2.16±0.04 $B_1; c_1;$	2.84±0.14 $B_1; c_2;$
Third group (Zn+ vit. C)	2.19±0.01 a_6	2.15±0.02 $b_3;$	2.79±0.16 c_3
		Glucose, mmol/L	
First group (control)	7.47±0.37 $a_7; b_4;$	6.86±0.17 $A_7; b_5; c_4;$	10.45±0.46 $A_7; a_8; b_6;$
Second group (Zn)	6.16±0.08 $b_4;$	6.31±0.13 $B_2; c_4;$	7.66±0.36 $B_2; b_6;$
Third group (Zn+ vit. C)	5.90±0.10 $a_7;$	6.10±0.10 $A_8; b_5;$	6.80±0.12 $A_8; a_8;$
		Total protein, g/L	
First group (control)	67.98±7.17 $C_1; c_5;$	87.32±2.72 $C_1; C_2;$	66.31±7.50 $C_2; c_6;$
Second group (Zn)	83.16±5.37	90.66±3.95 $C_4;$	76.49±4.35 $C_4;$
Third group (Zn+ vit. C)	88.12±4.30 $c_5;$	95.62±2.62	89.36±5.25 $c_6;$
		Creatinine, µmol/L	
First group (control)	77.34±6.67	72.34±5.52 $B_3;$	95.92±3.19 $B_3; c_7; b_7;$
Second group (Zn)	68.34±4.38	75.01±5.08	79.01±5.45 $c_7;$
Third group (Zn+ vit. C)	65.59±3.17	70.59±5.14	75.59±3.17 $c_7; b_7;$

Note: A_1-A_1 …. A_8-A_8 at $P<0.001$; B_1-B_1…B_3-B_3 at $P<0.01$; C_1-C_1…C_4-C_4 at $P<0.05$ statistically significant difference between cold and hot periods vs. termoneutral period (in the corresponding row)
a_1-a_1 …. a_8-a_8 at $P<0.001$; b_1-b_1…b_7-b_7 at $P<0.01$; c_1-c_1…c_7-c_7 at $P<0.05$ statistically significant difference between control and experimental groups for the period (in the corresponding column)

The substantially elevated blood glucose and cholesterol in layers subjected to cold and hot ambient stress could be explained by the enhanced flow of glucose toward cells under the influence of adrenal glucocorticoids (Popova–Ralcheva et al., 2002 a, b). The thesis is also supported by data provided by Morris (2002) stating that the stress response in birds is realised with the direct participation of corticosterone, adrenaline and glucagon, which are primary regulators of glucose concentrations.

The zinc and vitamin C, applied as a dietary supplement to compound feed, promote the reduction of serum corticosterone and cholesterol concentrations and this way, contribute to lower level of the adverse effect of ambient temperature-related stress in layers, helping to sustain their production potential and to preserve body condition. Over the entire experimental period, layers supplemented with both zinc and vitamin C (group III) exhibited the highest egg production (44.46 %), followed by those given zinc only (41.75 %) and last came control layers – 39.35 % (Figure 2). The greatest differences in egg production were detected during the cold period (by March 15) and hot period (by June 21) – between the first and the second ($p<0.05$), and first and third groups ($p<0.01$). The egg production rates of groups second and third did not differ significantly. Comparablee data to our results were reported by Yardibi and Turkay (2008) and Star et al. (2008), which demonstrated lower egg production in layer hens during the hot summer period. Therefore, the dietary supplementation of 35 mg/kg Zn as Zinteral 35 and 250 mg vitamin C during the hot and the cold periods of the year benefit egg production in hens. This could be explained by the antioxidant and antistress effect of zinc and vitamin C. Similarly, Sahin et al. (2002) reported that the addition of 30 mg Zn/kg feed in laying hens submitted to cold stress (6.8°C) decreased blood corticosterone, glucose and cholesterol. Under heat stress, the addition of 30 or 60 mg Zn to kg feed in quails contributed to maintaining lower corticosterone levels (Sahin and Kucuk, 2003; Sahin et al., 2005, 2009).



age in weeks→	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
I group - control	60,71	66,33	32,14	26,53	27,04	28,57	33,67	34,18	35,20	52,04	52,55	46,94	39,29	30,61	26,53	21,87
II group - 100 mg Zinteral in 1kg diet	60,71	69,39	37,76	28,57	27,55	29,69	37,76	37,24	37,76	55,10	52,55	45,39	39,80	32,65	31,63	26,02
III group - 100 mg Zinteral+250 mg vit C in 1kg diet	64,29	70,92	40,82	30,10	29,59	34,18	39,80	40,30	43,27	61,73	54,55	46,94	43,88	32,65	32,14	30,96
ambient temperature	8,7	4,7	2,7	8,7	9,6	12,5	11,6	11,8	15,2	14,3	17,1	19	15,5	22,2	24,9	31,5

Figure 2. Dynamics of egg production and ambient temperature

Low and high ambient temperatures influenced negatively the live body weight of birds (Figure 3). Furthermore, the average live weight of control layers was lower during the three studied periods compared to both supplemented groups. According to Star et al. (2008) and Yardibi and Turkay (2008), temperature-induced stress in birds is accompanied by adverse effects on their growth, statistically significant reduction in their productivity and poorer economic results due to the bigger and more inefficient energy expenditure.

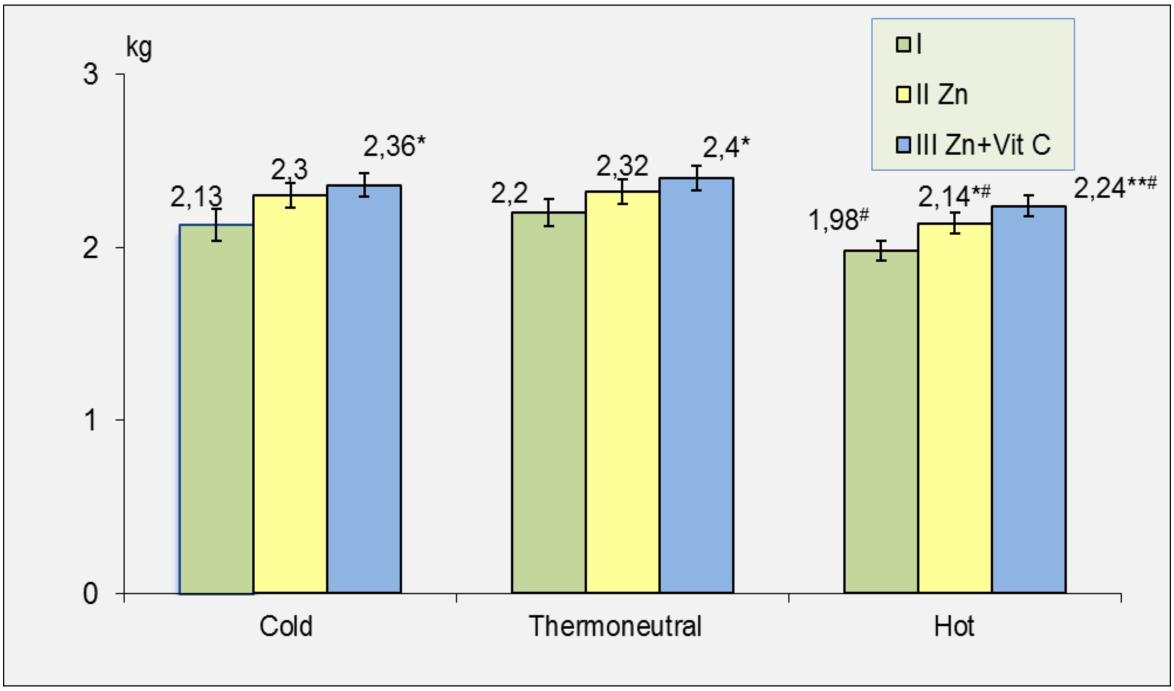


Figure 3. Body weight of layers

Note: * Significant difference between control and experimental groups at P<0.05;
Significant difference between thermoneutral and cold periods, and thermoneutral and hot period at P<0.05

Conclusion

The farming of layer hens in environment characterised with higher ambient temperature amplitudes was associated with temperature-induced stress, increased blood corticosterone and cholesterol concentrations and reduced egg production and live body weight.

The addition of zinc and vitamin C to the diet of layers reduced the negative impact of both cold and heat stress. Hens supplemented with 35 mg Zn /kg compound feed (second group) and 35 mg Zn + 250 mg vit. C /kg compound feed (third group) exhibited increased egg production by 2.22 % and 4.60 % vs control (first) group respectively.

The combination of 35 mg/kg zinc and 250 mg vitamin C had a synergistic effect in reducing both cold and heat stress in birds and for increasing egg production.

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