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WELFARE ASSESSMENT OF BREEDER HENS SUPPLEMENTED WITH ZINC AND VITAMIN C DURING THE COLD WINTER PERIOD

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Summary

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The purpose of the study was to evaluate the poultry welfare of three groups New Hampshire hens – control, supplemented either with 35 mg zinc/kg feed or a combination of 35 mg zinc/kg feed with 250 mg/kg vitamin C during the cold winter period. Eighty seven New Hampshire breeders at the age of 48 weeks were divided in three groups (n=29) and were reared under a free range system (sleeping pens and outdoor walking yards). The welfare of flocks was scored on the basis of their behaviour, plasma corticosterone and several blood biochemical parameters, as well as microclimatic rearing parameters during the cold winter and thermoneutral period. The behaviour of breeders was recorded with a video camera for 12 h during 4 consecutive days accounting the number of birds engaged in specific forms of behaviour: ingestive, gregarious, sexual and agonistic. The plasma corticosterone levels were assayed by commercial ELISA kit. Blood biochemical indices were determined on an automated biochemical analyzer "Cobas mira". During the cold period, the poultry welfare (PW) score of control breeders was PW=40 %, of the Zn-supplemented flock – PW=60 % and of zinc and vitamin C supplemented flock – PW=73.33 %. The poultry welfare improvement was due to the stress-reducing effect of both Zn and vitamin C supplements. The combined supplement had a better alleviating stress effect due to synergic action of its ingredients.

Key words: behaviour, cold period, corticosterone, hens, welfare assessment, zinc and vitamin C

INTRODUCTION

Reliable welfare criteria and evaluation models in poultry production and welfare assessment models under different conditions in poultry have gained increasing importance (Tuyttens *et al.*, 2008; Linares & Martin, 2010).

For poultry reared in organic production systems, cold stress conditions are created during the winter period in temperate continental areas. They affect adversely the welfare and productivity of birds (Sahin *et al.,* 2002; Onderci *et al.,* 2003).

The range of thermal comfort in poultry is between 18 and 22 °C. Within it, there are no problems with their thermoregulation (Ensminger *et al.,* 1990, Sahin *et al.,* 2009). The thermoregulatory mechanisms are mobilised in conditions of low ambient temperature (below 6 °C)

Significant changes occur on the hypothalamic-pituitary-adrenal axis (Ensminger *et al.,* 1990; Sahin *et al.,* 2009), in orthosympathetic nervous system (Puvadolpirod & Thaxton, 2000a,b,c) and the bird behaviour. The feed consumption and locomotion level are increased, water drinking and the behaviour linked to heat loss – wing spreading, feather cleaning, dust bathing etc. are reduced (Ensminger *et al.,* 1990; Sahin *et al.,* 2003).

The application of appropriate additives (trace elements, vitamins, minerals) in rations to meet the specific needs of the organism in terms of cold stress (Rodenburg *et al.,* 2005; Lin *et al.,* 2006) is a cost-effective approach to protect the poultry welfare during the cold periods.

Sahin *et al.* (2002) and Onderci *et al.* (2003) have investigated the effect of 30 mg/kg zinc and 400 μg/kg chromium given either independently or in combination in laying hens subjected to cold stress (6.8 °C). The authors found out that the supplements helped to reduce cold stress in laying hens. At the same time trace element supplementation had also a positive effect on behaviour of laying hens, i.e. on welfare improvement in birds. Therefore, zinc is used as an antistress feed supplement of poultry to meet their specific needs in cold stress (Sahin & Kucuk, 2003; Kanakov, 2005; Sahin *et al.,* 2005; Kucuk, 2008). 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.

Additionally, the same authors found that the supplementation of 250 mg/kg vitamin C in Japanese quails, subjected to heat stress lowered concentrations of ACTH. Kutlu & Forbes (1993) established that vitamin C supplementation reduced the synthesis of corticosteroid hormones in birds. Similarly, McDowell (1989) affirmed that by decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress.

So far, no information is available for the combined effect of zinc and vitamin C supplementation for alleviation of cold stress and welfare improvement in breeder hens reared in free range systems during the cold period of the year. This was the incentive of the present reseach.

The purpose of the study was to evaluate the welfare of three groups New Hampshire breeder hens (control, supplemented either with 35 mg zinc/kg feed or a combination of 35 mg zinc/kg feed with 250 mg/kg vitamin C) during the cold winter period on the basis of their behaviour, plasma corticosterone levels and some blood biochemical parameters.

MATERIALS AND METHODS

The experiment was conducted in the Experimental Farm of the Agricultural University – Plovdiv from February 15 to May 15, 2011. Eighty seven New Hampshire breeders at the age of 48 weeks were divided in three groups (n=29) and reared under free range system (sleeping pens and outdoor walking yards).

Each group was housed in identical sleeping pens (3.50/2.50/2.75 m) equipped with perches, providing 0.20 m perch space per hen (norm 0.15 m/hen, Anonymous, 2006a) and two-floor wooden nests. Each yard was 9.20/24 m with perennial broadleaf trees in the middle, providing individual area of 7.613 m^2 (significantly more than the area according to Anonymous, 2006a; 2006b). Yards were provided with tubular feeders and with watering troughs ensuring feeding and drinking widths of 10 and 3 cm (Anonymous, 2006b). The rearing conditions were fully compliant with minimum requirements for humane treatment of breeder hens (Anonymous, 2006a).

The three groups were fed freely with the same compound feed according to birds' category. During the cold winter period (from February 15 to March 15, 2011) and thermoneutral period (from April 15 to May 15, 2011), the diet of experimental groups was supplemented with either 100 mg/kg Zinteral 35 (Lohmann Animal Health, Germany, containing 35 mg zinc/kg as zinc oxide) or with 100 mg/kg Zinteral 35 together with 250 mg/kg vitamin C (L-acidum ascorbicum, SHIJIAZHUANG Co. Ltd).

The microclimatic rearing conditions were determined by routine methods. They were measured in the living space of birds – in walking yards after 7.00 AM, and in sleeping houses after the birds returned for the night. The temperature and the relative air humidity were measured by a weekly thermohygrograph; the velocity of the air motion – with a catathermometer three times daily – 7.00 AM, 2.00 PM and 9.00 PM. The light intensity was measured by a digital luxmeter, the concentration of ammonia – by indicator tubes and a Drager ammonia sensor on indicated hours. The average microclimatic parameters in the living space of birds were determined on the basis of the pointed out indicators identified in the walk yards during the day and in the sleeping pens – during the night (Table 1).

The blood samples for analysis were taken from randomly selected six birds from each group during the cold (15 March) and thermoneutral (15 May) periods from *v. subcutanea ulnaris* in vacutainers.

The behaviour of breeders was recorded during the cold winter (11–14 March) and thermoneutral (11–14 May) periods with a video camera for 12 hours during 4 consecutive days accounting the number of birds engaged in specific forms of behaviour: ingestive (ingestion of food or water), gregarious (moving, resting, egg-laying, dust bathing and feather cleaning), sexual and agonistic behaviour during cold winter and thermoneutral periods.

The plasma corticosterone was assayed with an immunoenzymatic ELISA kit (Corticosterone ELISA RE52211. IBL Gesellschaft fur Immunchemie und Immunbiologie MBH, Hamburg, Germany). Blood biochemical indices – glucose, cholesterol, creatinine, total protein and triglycerides were determined on an automated biochemical analyzer "Cobas mira" at an accredited biochemical lab in the Diagnostic and Consultation Medical Centre Sveti Georgi – Plovdiv.

Table 1. Average microclimatic parameters in the birds' living area determined on the base of the pointed out indicators identified in the walk yards and in the sleep houses during the cold winter and thermoneutral period

Periods	Ambient temperature (^oC)	Air humidity (%)	Air velocity (m/s)	NH_3 (ppm)	Light intensity (lx)
Cold period	6.93±0.64	66.33±3.64	0.50±0.06	traces	55.00±4.12
Thermoneutral	19.44±2.5	68.07±2.59	0.49±0.08	traces	65.00 ±3.27
*Reference values**	*18–25*	*50–70*	*0.2–0.5*	*< 15*	*30–60*

As per Anonymous (2006b).

The poultry welfare assessment score was calculated by a modification of the system of Bozakova *et al.* (2012) based on the animal welfare concept of the UK Farm Animal Welfare Council (1995).

The statistical processing of the results was performed by means of ANOVA using the GraphPad InStat 3.06 software at level of significance P<0.05.

RESULTS

The average microclimatic parameters in the living space of New Hampshire breeders during the cold and thermoneutral period are given in Table 1. The microclimatic parameters were determined on the basis of the pointed out indicators identified in the walk yards during the day and in the sleeping pen – during the night, where the major part of birds were located.

During the cold period, the average temperature in the birds' living area was substantially lower that the veterinary requirements for this category birds.

During the cold winter period the behaviour changes in control birds were compared to the thermoneutral period (Table 2). The number of feeding birds was higher (P<0.001) and the number of egg-laying (P<0.001), resting (P<0.05), feather cleaning (P<0.01), aggressive (P<0.01) and mating birds (P<0.01) was

Table 2. Number (%) of breeders, supplemented either with zinc or zinc and vitamin C exhibiting a specific type of behaviour during the cold winter and thermoneutral periods (mean ±SEM, n=29)

Behaviour	Cold period						Thermoneutral period	
	Control group		Zn group		Zn + vitamin C group		Control group	
	number	percent	number	percent	number	percent	number	percent
Feeding	11.16±0.68$^{\wedge\wedge\wedge}$	38.48%	10.07±0.67	34.72%	10.30±0.64	35.50%	7.00±0.65	24.14%
Drinking	5.00±0.26	17.24%	4.50±0.17	15.52%	4.50±0.17	15.52%	4.61±0.27	15.91%
Egg-laying	0.57±0.10$^{\wedge\wedge\wedge}$	1.96%	0.89±0.15*	3.06%	1.11±0.16**	3.84%	1.57±0.23	5.41%
Moving	5.84±0.39$^{\wedge\wedge}$	20.14%	5.16±0.25	17.79%	4.64±0.29**	15.99%	7.43±0.45	25.63%
Resting	1.50±0.21$^{\wedge}$	5.17%	2.75±0.20***	9.48%	2.45±0.22**	8.46%	2.23±0.24	7.68%
Feather cleaning	0.59±0.12$^{\wedge\wedge}$	2.04%	0.84±0.12	2.90%	0.95±0.15*	3.29%	1.09±0.14	3.76%
Dust bathing	0.89±0.11	3.06%	1.36±0.11**	4.70%	1.84±0.15***$^{\#\#}$	6.35%	0.93±0.17	3.21%
Aggression	1.66±0.16$^{\wedge\wedge}$	5.72%	1.39±0.13	4.78%	0.86±0.11***$^{\#\#}$	2.98%	2.20±0.12	7.60%
Sexual behaviour	1.16±0.15$^{\wedge\wedge}$	4.00%	1.64±0.09**	5.64%	1.77±0.15**	6.11%	1.84±0.17	6.35%

$^{\wedge}$P<0.05; $^{\wedge\wedge}$P<0.01; $^{\wedge\wedge\wedge}$P<0.001: statistically significant difference between thermoneutral and cold period in control group; *P<0.05; **P<0.01; ***P<0.001 : statistically significant difference between control and experimental groups during the cold winter period; $^{\#\#}$P<0.01: statistically significant difference between Zn- and Zn+vitamin C supplemented groups during the cold winter period.

Table 3. Blood corticosterone levels and biochemical indices in breeders supplemented either with zinc or with zinc and vitamin C (mean ±SEM, n=6)

Parameters	Cold period			Thermoneutral period
	Control group	Zn group	Zn + vitamin C group	Control group
Corticosterone, nmol/L	110.58±10.28$^{\wedge\wedge}$	87.20±5.97*	80.36±4.30*	74.23±7.23
Glucose, mmol/L	10.29±0.37$^{\wedge\wedge\wedge}$	8.97±0.43*	7.30±0.20***$^{\#\#}$	6.47±0.55
Total cholesterol, mmol/L	3.67±0.19$^{\wedge\wedge}$	3.05±0.13*	2.47±0.08***$^{\#\#}$	2.76±0.16
Total protein, g/L	75.15±4.81$^{\wedge\wedge}$	78.16±5.34	89.02±3.63*	98.46±4.08
Creatinine, µmol/L	77.26±6.66	70.59±4.88	70.59±2.82	67.26±3.53
Triglycerides, mmol/L	7.83±0.71$^{\wedge\wedge}$	6 .00±0.62*	5.33±0.40**	4.08±0.58

$^{\wedge\wedge}$P<0.01: statistically significant difference between thermoneutral and cold period in control group; *P<0.05; **P<0.01; ***P<0.001 : statistically significant difference between control and experimental groups during the cold winter period; $^{\#\#}$P<0.01: statistically significant difference between Zn- and Zn+vitamin C supplemented groups during the cold winter period.

lower vs the thermoneutral period.

There were significantly more egg-laying, resting, dust bathing and mating supplemented chickens compared to controls. At the same time there were more dust bathing (P<0.01) and less aggressive birds (P<0.01) receiving both supplements compared to those supplemented only with zinc.

Similar differences were observed in the dynamics of blood corticosterone and the other biochemical parameters (Table 3). During the cold period, blood corticosterone (P<0.01), glucose (P<0.001), cholesterol (P<0.01) and triglycerides levels (P<0.01) in control birds were significantly higher compared to the thermoneutral period (Table 3). Simultaneously the total protein level (P<0.01) was decreased vs the thermoneutral period.

Under the influence of supplements (zinc and vitamin C) significant decrease in blood corticosterone, glucose, total cholesterol and triglycerides levels were established in experimental groups, compared to controls.

The blood glucose and total cholesterol levels in Zn and vitamin C-supplemented chickens were lower than in Zn-supplemented ones (Table 3).

On the basis of behavioural, corticosterone and blood biochemical changes (glucose, cholesterol, total protein and triglycerides) the five freedoms were scored and the total poultry welfare (PW) score in control breeders during the cold period was calculated to be 40.00% (Table 4).

The improved welfare of birds supplemented with either Zn or Zn/vitamin C combination during the cold period was confirmed by the statistically significant differences in poultry behaviour – there were more egg-laying, resting, dust bathing and mating experimental birds, compared to controls (Table 2). Blood corticosterone, glucose, cholesterol and triglycerides were lower than controls (Table 3).

The reduced negative impact of the cold stress in Zn-supplemented birds resulted in higher scores of $F_{1,}$ $F_{2,}$ $F_{4,}$ and

Table 4. Welfare assessment scores of the breeders, supplemented either with zinc or zinc and vitamin C during cold winter period (mean ±SEM, n=29)

Poultry welfare assessment		Cold period		
Freedom	Degree	Control group	Zn group	Zn + vitamin C group
Freedom from thirst and hunger – F_1	0 – excessive thirst and hunger 1 – limited thirst and hunger 2 – lack of thirst and hunger 3 – excessive feeding and drinking	1	2	2
Freedom from discomfort – F_2	0 – excessive discomfort 1 – limited discomfort 2 – limited comfort 3 – full comfort	1	2	2
Freedom from pain. injury disease – F_3	0 – exhausting disease 1 – limited disease 2 – occasional pain and injury 3 – lack of pain and injury	2	2	2
Freedom to express normal behaviour – F_4	0 – behaviour disturbance 1 – limited behaviour expression 2 – moderate expression 3 – full expression	1	2	3
Freedom from fear and distress – F_5	0 – fear and distress 1 – limited fear and distress 2 – partial freedom 3 – full freedom	1	1	2
	Total score	6	9	11
	Poultry welfare assessment, %	40.00	60.00	73.33

F_5 freedoms vs controls (Table 4). Thus the total poultry welfare score in Zn-supplemented birds was 60.00%.

Similar changes in the behaviour, corticosterone and biochemical parameters during the cold period were observed in birds receiving Zn+vitamin C. There were statistically significantly more dust bathing (P<0.01) and less aggressive birds (P<0.01), as well as reduced blood cholesterol and glucose levels (P<0.01), compared to the Zn-supplemented breeders. On that basis the total poultry welfare score of the Zn + vitamin C-upplemented group was PW 73.33%.

DISCUSSION

Under the free range system (sleeping pens and outdoor walking yards) during the cold periods, the average ambient temperature in the birds' living area was substantially lower that the veterinary requirements for this category birds. Cold stress is a common cause of poor welfare in fowls during the winter months and it triggers a chain of non-specific reactions and systemic mechanisms of defense. The stress response in birds is mediated mainly by activation of the hypothalamic-pituitary-adrenal axis, the orthosympathetic nervous system and poultry behaviour changes (Puvadolpirod & Thaxton, 2000a,b,c; Sahin *et al.,* 2009). Under the influence of low ambient temperatures the hypothalamus is triggered, the adrenal gland cortex is activated and reacts by enhanced secretion of glucocorticoids, the major among which in birds is corticosterone (Siegel, 1995). These events further generate numerous biochemical, behavioural, immunological and productive alterations resulting in worsened welfare of birds. Our experiments confirmed the data of cited reserachers that under the influence of the glucocorticoids blood corticosterone, glucose, cholesterol and triglycerides in chicken breeders changed to become substantially higher compared to the thermoneutral period. The cold stress was manifested with marked behavioural changes – significantly lower egg-laying, resting, feather-cleaning, and mating birds, which are criteria for the welfare worsening in birds (Bozakova, 2008; Bozakova *et al.,* 2012). On that basis the total poultry welfare (PW) score in control breeders during the cold period was 40.00 %.

Our studies confirmed the positive influence of the supplementation with 35 mg/kg zinc on blood corticosterone, biochemical indices and behaviour in the breeders. Zinc supplements are especially important for stress alleviation in birds (Sahin *et al.,* 2002; 2003; Star *et al.,* 2008). Stress triggers an excessive production of free radicals (Halliwel & Gutteridge, 1989) namely oxidative stress (Sahin *et al.,* 2002; Lin *et al.,* 2006). Zinc is known to play a key role in the endogenous antioxidant protection system. Being a co-factor of essential antioxidant enzymes – Cu/Zn superoxide dismutase and inhibiting NADPH-dependent lipid peroxidation, zinc limits the excessive secretion of corticosterone during stress (Prasad, 1997; Prasad & Kucuk, 2002). Thus, Zn-supplementation contributes to alleviation of cold stress and influences positively the poultry behaviour in our experiment – there were more egg-laying, resting, dust bathing and mating birds, resulting in a better level of welfare (60.00 %) compared to controls. Also, supplemental zinc is known to increase serum vitamin C (Onderci *et al.,* 2003).

The influence of the combination of 35 mg/kg zinc and 250 mg/kg tested in our experiment, had a better positive effect on poultry welfare in New Hampshire breeders. The blood corticosterone, glucose, total cholesterol levels were considerably lower than Zn-supplemented birds during the cold period. This effect was attributed to the corticosterone and anxiety-reducing effects of vitamin C in birds (Satterlee *et al.,* 1993; Jones *et al.,* 1996). Kutlu & Forbes (1993) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. Similarly, Sahin *et al.* (2002) reported low concentrations of ACTH in quails reared under temperature stress conditions and fed a diet supplemented with vitamin C. By decreasing the synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress (McDowell, 1989). Additionaly Bains (1996) reported a corticosterone-modulating effect of vitamin C via its involvement in the gluconeogenesis to enhance the energy supply during stress. This way, both supplements (Zn + vitamin C) act synergically in the reduction of cold stress and contribute to the imporved welfare of breeders (73.33%) during the cold period.

CONCLUSIONS

In New Hampshire breeders reared under an organic farming system, the cold winter period influenced negatively blood corticosterone levels, behaviour and some blood biochemical parameters reducing their poultry welfare score to 40.00%.

The zinc-supplemented birds at 35 mg/kg during the cold winter period were characterised with positive behavioural changes, reduced blood corticosterone and some blood biochemical indices compared to controls, as well as a higher welfare score of 60.00%.

Under the organic farming system, the supplementation with the combination 35 mg/kg zinc and 250 mg vitamin C improved the breeder's behaviour, blood corticosterone and some of studied blood biochemical indices during the cold period. Their welfare score increased to 73.33% due to the synergic cold stress reducing effect of both compounds.

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