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Opportunities for the Welfare Improvement of Laying Hens under Semi-open Rearing during the Cold Period with Arginine and Vitamin C Supplementation

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Abstract

The purpose of the present study was to evaluate the welfare of DeKalb Brown laying hens whose feed was supplemented with 1% L-arginine or either with a combination arginine and vitamin C during the cold winter days, using a assessment model. The welfare was scored on the basis of hen's behaviour, plasma corticosterone and several blood biochemical parameters.

The extremely low environmental temperatures during the cold period provoked in DeKalb Brown hens a cold stress, manifested with excessive blood corticosterone concentrations, behavioural and blood biochemical changes and the welfare score of control hens was PW=46,67 %. The 1% L-arginine supplemented hens were characterized with positive behavioural changes and reduced blood corticosterone and some biochemical indices compared to controls, as well as an increased welfare score to PW=73,33 %. The supplementation with 1 % L-arginine and 250 mg vitamin C during the cold period was reflected positively on the behaviour, blood corticosterone and some biochemical indices in the DeKalb hens. Their welfare score increased to PW=80,00 % due to the synergic cold stress reducing effect of arginine and vitamin C.

Keywords: DeKalb Brown, welfare, behaviour, arginine and vitamin C, corticosterone

Introduction

The farmer producers from the transition continental and European Mediterranean areas are seeking cost-effective ways to adapt semi-open rearing system for laying hens production, preferably more sustainable and healthy hybrids. Concerning Jones et al. (2005) and Moura et al. (2006), the welfare of birds is more strongly associated with the environment in these zones. The cold winter temperatures provoke cold stress in hens and worsen their welfare. Poultry possess numerous thermoregulatory mechanisms to counteract thermal stress. But the thermoregulatory mechanisms mobilize in conditions of low ambient temperature (below 6 °C). The significant changes are going on in the hypothalamic-pituitary-adrenal axis (Ensminger et al., 1990; Sahin et al., 2009), in orthosympathetic nervous system (Puvadolpirod and Thaxton, 2000 a,b,c) and the bird behavior. The feed consumption and locomotion level are increased, water drinking reduces, the behaviour linked to heat loss reduces too – wing spreading, feather cleaning, dust bathing etc. (Ensminger et al., 1990).

For the hen's welfare improving it is attempting to reduce environmental stress by appropriate dietary supplements (microelements, vitamins, and minerals) to satisfy the body's needs under cold stress. One option according to Wiesinger (2001) and Heinzen (2003) is the dietary arginine intake. The most of the biological arginine effects are via nitric oxide (NO). The main antistress effect of the nitric oxide is due to the inhibiting the corticosterone secretion (Adams et al., 1991) and ACTH (Giordano et al., 1996). The nitric oxide inhibits the glucocorticoid`s synthesis in the adrenal glands. Also the arginine has an antioxidant activity (Gupta et al., 2005). Additionally the vitamin C owners strong antistress and antioxidant effect (Bartlett & Smith, 2003 and Sahin et al., 2002).

The objective of this research was to provide a welfare evaluation of DeKalb Brown hens under semi-open rearing system during the cold winter period, after dietary supplementation of either 1% L-arginine or the combination 1 % arginine and 250 mg/kg vitamin C (Arg.+ vit. C) using the changes in hen's behaviour, plasma corticosterone and some blood biochemical indices.

Materials and Methods

The experiments were performed with 996 DeKalb Brown laying hens at the age of 38 weeks from February 21 to May 21, 2012.

The birds were reared under semi-open farming system and divided in 3 groups (n=332; 295♀ and 37 ♂). The birds were placed in semi-opening building with a length 21 m and width 7 m. The south face of the building was thick to 1 m high and up the wool was carried out by protective metal mesh for the protection of the hens. The each group was located in a compartment with length and width 7 m, (49 m2). Each compartment was covered with 20 cm soft bedding (litter) consisting of chopped straw and cobs and provided with 7 round feeders and 5 drinkers ensuring feeding - 4 cm and drinking – 2,8 cm widths (Ordinance 44/2006). During the whole experimental period (from November 21 to May 21, 2012) including two observer subperiods – cold winter subperiod (from February 21 to March 21, 2012) and thermoneutral subperiod (from April 21 to May 21, 2012) all groups were fed freely with the same compound feed according to laying hen's category. The first group (I-st) was used as control and got a feed without supplements. During the cold winter subperiod the diet of experimental groups was supplemented with either 1% L-arginine ("Roanal", Budapest, Hungary) (II-nd group) and with a combination – 1 % arginine together with 250 mg/kg vitamin C, (L-acidum ascorbicum, SHIJIAZHUANG Co. Ltd) per kg feed (Arg.+vit. C = III-d group).

The DeKalb Brown hens behaviour was recorded with a 3 video cameras for 12 hours over 4 consecutive days during the each subperiod (from March 16 to March 19 and from May 16 to May 19, respectively), accounting the number of birds engaged in specific forms of behaviour: ingestive (ingestion of water or food), gregarious (moving, resting, egg-laying, dust bathing and feather cleaning), sexual and agonistic behaviour.

Microclimatic conditions were determined by routine methods. The temperature and the relative air humidity were measured by 3 weekly thermohygrographs; the velocity of the air motion – with a catathermometer. The light intensity - by a digital luxmeter; the concentration of ammonia – by indicator tubes and a Drager ammonia sensor, (Table 1).

Blood samples for corticosterone determination were collected during the cold subperiod (on March 20) and during the thermoneutral subperiod (on May 20), from v. subcutanea ulnaris in sterile vacutainers (Vacutainer® Plus plastic plasma tube 13 x 75 mm x 4.0 mL BD), containing 75 USP units sodium heparin. They were collected at the same time in all studied periods – between 1-2 h PM to avoid circadial influence on circulating corticosterone.

The plasma coricosterone levels were assayed with immunoenzymatic ELISA kit (Corticosterone ELISA RE52211, IBL Gesellschaft fur Immunchemie und Immunbiologie MBH, Hamburg, Germany) in the Laboratory of Innate Resistance Investigation in Faculty of Veterinary Medicine – Stara Zagora.

Blood biochemical indices – glucose, cholesterol, creatinine, urea, total protein and triglycerides were determined with an automated biochemical analyzer "Cobas mira".

The welfare assessment score was calculated by a system of Bozakova et al. (2012).

The statistical analysis was performed with the non-parametric Friedman's test for two-way repeated measures analysis. In case of significant P values (P<0.05), the non-parametric Tukey HSD test was then applied.

Results

Comparing microclimatic parameters with the parameters of veterinary requirements for animal breeding facilities, Regulation 44/ 2006, it was determined that the average ambient temperature in the hen's living area was 5.67±0.50, i.e. substantially lower that the allowances of 18-25 0C for this category birds. The data for the microclimatic indicators of DeKalb Brown hens are given in Table 1.

Table 1. Microclimatic parameters in the hen's living area under the semi-opening rearing during the cold winter period

Period	Ambient temperature (°C)	Air humidity (%)	Air velocity (m/s)	NH_3 (ppm)	Light intensity (lx)
Cold period	5.67±0.50	66,67±2,05	0.62±0.05	traces	59.00±3.00
Thermoneutral period	18.07±0.51	64.00±1.56	0.57±0.05	traces	65.30 ±3.10
Reference values*	18–25	50–70	0.2–1.0	< 15	30–60

*Reference values as per Regulation 44/ 2006

It provoked some marked behavioural changes - significantly lower number of drinking (P<0.001), egg-laying (P<0.01), moving (P<0.05), resting (P<0.001), feather-cleaning (P<0.001), dust bathing (P<0.001) and mating control birds (P<0.01), but increased number of feeding (P<0.001) compared to the thermoneutral period, Table 2. In arginine-supplemented group there were significantly more egg-laying (P<0.05), mating birds (P<0.05) and lower aggressive hens (P<0.01) compared to controls. In Arg.+Vit. C-supplemented group there were significantly more egg-laying (P<0.01), feather cleaning (P<0.001), dust bathing (P<0.05) and mating (P<0.01) compared to controls and more feather cleaning hens (P<0.05) compared to arginine- group. At the same time there were less moving (P<0.001) and aggressive birds (P<0.001) compared to control and arginine-group (Table 2).

Table 2. Number of *DeKalb Brown* hens, supplemented either with L-arginine or arginine and vitamin C exhibiting a specific type of behaviour during the cold period (mean ±SEM, n=295 ♀)

Behaviour	Thermoneutral period		Cold winter period					
	Control group	%	Control group	%	Arginine-group	%	Arg. + Vit. C group	%
Feeding	70,34±5,67	23,84	122,95±7,31^^^	41,68	129,77±5,11	43,99	125,68±4,90	42,60
Drinking	34,43±2,01	11,67	21,82±1,31^^^	7,40	21,82±1,31	7,40	22,62±1,32	7,67
Egg-laying	40,34±4,28	13,68	24,89±2,41^^	8,44	32,73±3,53*	11,09	34,89±3,33**	11,83
Moving	50,23±1,74	17,03	43,86±2,37^	14,87	37,73±2,39	12,79	29,32±2,04***##	9,94
Resting	33,18±3,12	11,25	16,36±2,17^^^	5,55	17,95±2,08	6,09	18,86±2,15	6,39
Feather ceaning	16,70±0,83	5,66	12,73±0,94^^^	4,31	15,45±0,93	5,24	18,64±1,19***#	6,32
Dust bathing	15,57±0,98	5,28	10,45±0,95^^^	3,54	11,71±0,93	3,97	13,07±1,02*	4,43
Aggression	16,36±0,82	5,55	16,36±0,82	5,55	13,41±0,79**	4,55	9,77±0,95***##	3,31
Sexual behaviour	19,32±1,00	6,55	16,25±0,78^^	5,51	18,64±0,79*	6,32	19,43±0,75**	6,59

^^P<0.01; ^^^P<0.001: statistically significant difference in control group between thermoneutral and cold period; *P<0.05; **P<0.01: statistically significant difference between control and experimental groups during the cold period; #P<0.05; ##P<0.01###P<0.001: statistically significant difference between Arginine- and Arginine + vitamin C- supplemented groups during the cold period;

During the cold period the blood corticosterone, cholesterol, glucose, total protein and triglyceride levels in control hens were higher than the thermoneutral period (table 3). In arginine-supplemented hens the corticosterone, cholesterol, glucose, and triglyceride's levels were lower than control one. The blood corticosterone and triglycerides levels in Arg.+Vit. C-supplemented hens were lower than in Arg.-supplemented ones.

Table 3. Blood corticosterone levels and biochemical indices in *DeKalb Brown* hens supplemented either with 1% L-arginine or 1% arginine and vitamin C during the cold period (mean ±SEM, n=9 ♀)

Parameter	Thermoneutral period	Hot period		
	Control group	Control group	Arginine-group	Arginine + vitamin C-group
Corticosterone, nmol/L	84,23±4,07	167,58±5,50^^^	152,58±3,40*	142,58±3,53**#
Total cholesterol, mmol/L	2,41±0,16	3,64±0,34^^	2,87±0,05*	2,82±0,19*
Glucose, mmol/L	6,47±0,43	10,14±0,40^^^	9,13±0,51	9,30±0,55
Total protein, g/L	112,29±2,70	103,29±3,54^	104,96±3,36	106,29±4,02
Creatinine, µmol/L	69,59±3,84	76,26±2,51	82,76±2,78*	91,42±3,79**#
Triglycerides, mmol/L	5,42±0,49	9,35±0,23^^^	8,67±0,26*	7,59±0,36**#

^^P<0.01; ^^^P<0.001 : statistically significant difference in control group between thermoneutral and cold period; *P<0.05; **P<0.01: statistically significant difference between control and experimental groups during the cold period; #P<0.05; ##P<0.01###P<0.001: statistically significant difference between Arginine- and Arginine + vitamin C- supplemented groups during the cold period

On the basis of behavioural, cortico¬sterone and blood biochemical changes the five freedoms were scored and the total poultry welfare (PW) score in control DeKalb hens during the cold period was calculated to be 46,67 % (Table 4).

The improved welfare of birds supp¬le¬mented with either arginin or arginin/vitamin C combination during the cold period was confirmed by the significant differences in poultry behaviour – there were more egg-laying, mating birds, feather cleaning, dust bathing, mating and lower aggressive hens (P<0.01) compared to controls, (Table 2). At the same time in supplemented birds the corticosterone, glucose, cholesterol and triglycerides were lower than controls (Table 3).

The reduced negative impact of the cold stress in arginine-supplemented birds resulted in higher scores of F1, F2, F4, and F5 freedoms vs controls (Table 4). Thus the total poultry welfare score in Zn-supplemented birds was 73,33 %.

Similar changes in the behaviour, corticosterone and biochemical parame¬ters during the cold period were observed in birds receiving Arg.+Vit. C. There were statistically significantly more feather cleaning hens and less moving and aggressive birds compared to arginine- group. At the same time the corticosterone and triglyceride’s levels in those hens were lower than in Arg.-supplemented hens. On that basis the total poultry welfare score of the Arg. + Vit. C-upplemented group was PW 80,00 %.

Table 4. Welfare assessment scores of *DeKalb Brown* hens supplemented either with L-arginine or with arginine and vitamin C during the cold winter period

Poultry welfare assessment Freedom	Degree	Free range system		
		Control group	Arginine-group	Arg. + Vit. 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	2	3	3
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	2
Freedom from fear and distress - F_5	0-fear and distress 1-limited fear and distress 2-partial freedom 3-full freedom	1	2	3
Total score		7	11	12
Poultry welfare assessment, %		**46,67**	**73,33**	**80,00**

Discussion

During the cold periods, the average ambient temperature in the hen’s living area was substantially lower that the requirements for this category birds. Cold stress is a common cause of poor welfare in fowls during the winter and it triggers a chain of non-specific reactions and systemic mechanisms of defence. The stress response in hens is mediated mainly by activation of the hypothalamic-pitui¬tary-adrenal axis, the orthosympa¬the¬tic nervous system and poultry behaviour changes (Puvadolpirod and Thaxton, 2000a,b,c).

Under the influence of low temperatures the hypothalamus is triggered, the adrenal gland cortex is activated and reacts by enhanced secretion of glucocorticoids, the major among which in hens is cortico¬sterone (Siegel, 1995). These events further generate numerous biochemicals, behavioural, immunological and produc-tive alterations resulting in worsened welfare of birds. Our experiments con¬firmed the data of cited researchers that under the influence of the glucocorticoids blood corticosterone, glucose, cholesterol and triglycerides in hens changed to become substantially higher compared to the thermoneutral period. The cold stress was manifested with marked behavioural changes – signifi¬cantly lower egg-laying, resting, dust bathing, feather-cleaning and mating birds, which are criteria for the welfare worsening in birds (Bozakova et al., 2009). On that basis the poultry welfare (PW) score in DeKalb hens under semi-open rearing system during the cold period was 46,67 %.

According to Wiesinger (2001) and Heinzen (2003), one of the ways to reduce stress in birds is the addition of L-arginine in their food. Our research confirmed the positive influence of the 1 % L-arginine diet supplementation on blood corticosterone, bio¬chemical indices and behaviour in the DeKalb hens. The arginine supplements are especially important for stress alleviation in birds (Wiesinger, 2001 and Heinzen, 2003. The most of the biological arginine effects are via nitric oxide (NO). Concerning Adams et al. (1991) the main antistress effect of the nitric oxide is due to the inhibiting the corticosterone secretion as well as to ACTH (Giordano et al., 1996). Thus, the arginine-supplementation contributes to alleviation of cold stress and influences positively the poultry behaviour in our experiment – there were more egg-laying, dust bathing, mating birds and lower aggressive hens. That is a evidence for the better welfare level of the arginine-supplemented birds (73,33 %.). Additionally Olsson et al. (2005) and Dixon et al. (2008) found that the behaviour of taking the dust bath is an important indicator of social welfare of the bird group. In turkeys, improved welfare is manifested with increased time spent in stretching, feather cleaning and dust bathing (Sherwin & Kelland, 1998).

The combination of 1 % arginine together with 250 mg/kg vitamin C, tested in our experiment, had a better positive effect on poultry welfare in DeKalb hens under semi-open rearing system during the cold period. The blood corticosterone and triglyceride's levels were considerably lower than arginine-supplemented hens. 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 about low concentrations of ACTH in quails reared under temperature stress conditions and fed a diet supple¬mented with vitamin C. By decreasing the synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress (McDowell, 1989). Additionally 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 (arginine + vitamin C) act synergically in the reduction of cold stress and contribute to the improved DeKalb hen's welfare of (80,00 %.) during the cold period.

Conclusion

The influence the cold winter temperature period provoked in DeKalb Brown hens under semi-open building a stress, manifested with excessive blood corticosterone concentrations, behavioural and blood biochemical changes. The welfare score of DeKalb hens suffering from cold stress was PW= 46,67.

During the cold period the 1% L-arginine supplemented DeKalb Brown hens under semi-open building were characterized with positive behavioural changes (increased number of the egg-laying, mating birds and decreased number of the aggressive hens) and reduced blood corticosterone and some biochemical indices compared to controls, as well as an increased welfare score to PW=73,33

The supplementation with 1% L-arginine and 250 mg vitamin C during cold winter period was reflected positively on behaviour (increased number of the egg-laying, feather cleaning, dust bathing and mating hens as well as decreased number of the aggressive hens), blood corticosterone and some biochemical indices in the DeKalb hens. Their welfare score increased to PW= 80,00% due to the synergic stress reducing effect of arginine and vitamin C.

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