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Original article

WELFARE IMPROVEMENT IN LAYING HENS DURING THE HOT PERIOD UNDER A SEMI-OPEN REARING SYSTEM THROUGH DIETARY ARGININE AND VITAMIN C SUPPLEMENTATION

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Summary

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The welfare assessment model in poultry under different rearing systems has gained increasing importance. An economically profitable approach for improvement of laying hens welfare during the hot summer period is diet's supplementation with specific additives (microelements, vitamins, and minerals) to satisfy the body's needs under heat stress. The purpose of the present study was to evaluate the welfare of DeKalb Brown laying hens (n=295) whose feed was supplemented with either 10 mg/kg L-arginine or with the combination 10 mg/kg L-arginine and 250 mg/kg vitamin C during the hot summer days, using a mathematical assessment model. The laying hens' welfare was scored on the basis of birds' behaviour, plasma corticosterone levels, blood biochemical parameters (glucose, cholesterol, creatinine, total protein and triglycerides) and rearing microclimatic parameters. The behaviour was observed by a video camera accounting the number of birds engaged in specific forms of activity: ingestive (ingestion of water or food), gregarious (moving, resting, egg-laying, dust bathing and feather cleaning), sexual and agonistic behaviour. The plasma corticosterone levels were assayed by means of commercial ELISA kit. Blood biochemical indices – cholesterol, glucose, creatinine, total protein and triglycerides were determined on an automated biochemical analyzer. The welfare score of DeKalb Brown laying hens during the hot period from the control group was 33.33% that of the group supplemented with L-arginine – 66.67 %, and in birds supplemented with arginine and vitamin C – 73.33 % due to the synergic heat stress-reducing effect of both compounds.

Key words: arginine & vitamin C, behaviour, corticosterone, DeKalb Brown hens, heat stress, welfare assessment model

INTRODUCTION

The farmer producers from the transition continental and European Mediterranean areas are seeking cost-effective semi-open production systems for laying hens pro-

duction, using more sustainable and healthy hybrids (Gerzilov, 2011). In these regions the welfare of birds is more strongly associated with the environment (Jones *et al.,* 2005; Moura *et al.,* 2006). The high summer temperatures provoke thermal stress in hens and worsen their welfare. Under heat stress the released adrenal glucocorticosteroids in the organisms of birds incite substantial blood biochemical alterations – statistically significantly increased blood concentrations of cholesterol, glucose, creatinine and triglycerides (Puvadolpirod & Thaxton, 2000a,b,c; Sahin *et al.,* 2005; 2009). These changes, together with behavioural indices, are believed to be important parameters for welfare evaluation in poultry (Popova–Ralcheva *et al.*, 2002).

Poultry possess numerous thermoregulatory mechanisms to counteract thermal stress. During the hot summer days, the rate of heat loss is enhanced while the heat production is decreased. The thermoregulation in birds is difficult because of the high body temperature (41.5 ^{o}C) and limited heat exchange mechanisms at that time. The primary mechanism for heat loss is through evaporation from mucous coats during accelerated respiration (Ensminger *et al.,* 1990; Sahin *et al.,* 2009). Other behavioural traits include more frequent drinking, looking for shady places to hide etc. aimed at greater body heat loss (Star *et al.,* 2008). However, free-range and semi-open farming conditions may challenge the adaptation abilities of poultry to the changed environment.

According to Sossidou & Elson (2009) and Nääs *et al.* (2010) poultry welfare is associated with the behaviour, health, mortality, and physiology. Graves (1982) confirmed that behaviour is a window of the body for the outside, enabling better and precise estimation of the welfare of birds.

Appropriate dietary supplements (microelements, vitamins and minerals) to satisfy the body's needs under heat stress are among the approaches for reduction of environmental stress and therefore, poultry welfare improvement. One option according to Wiesinger (2001) and Heinzen (2003) is the dietary arginine intake. The main anti-stress effect of arginine is attributed to corticosterone (Adams *et al.,* 1991) and ACTH (Giordano *et al.,* 1996) secretion inhibition by nitric oxide (a metabolite of arginine). Apart reduction of adrenal glucocorticoid synthesis in the adrenal glands, arginine has an antioxidant activity (Gupta *et al.,* 2005) as well.

Vitamin C has also a strong anti-stress effect (Bains, 1996; Sahin *et al.,* 2002; Bartlett & Smith, 2003). Sahin *et al.* (2002) found that in Japanese quails subjected to heat stress, the supplementation of 250 mg/kg vitamin C lowered blood ACTH concentrations. McDowell (1989) affirmed that vitamin C alleviated the negative effects of stress by decreasing the synthesis and secretion of corticosteroids.

So far, no information is available for the combined effect of arginine and vitamin C dietary supplementation for alleviation of heat stress and welfare improvement in hens reared under semi-open farming systems during the hot period.

The purpose of this paper was to evaluate the welfare of DeKalb Brown hens under a semi-open rearing system during the hot summer period, after dietary supplementation of L-arginine or the combination L-arginine and vitamin C, on the base of hen's behaviour indices, plasma corticosterone and some blood biochemical parameters.

MATERIALS AND METHODS

The experiments were performed with 885 DeKalb Brown laying hens at the age of 42 weeks from March 26 to August 26, 2012 at a private poultry farm situated at 10 km east from Stara Zagora, Bulgaria.

The birds were reared under semi-open farming system and divided in 3 groups (n=295, ♀). They were housed in a semi-opening building with length 21 m and width 7 m. On the south side of the building there was a solid one-meter high wall, and protective metal mesh up to the roof of the building. Each group was located in a semi-open compartment 7×7 m (49 m^2), 6.02 birds/m^2 (norm 8 birds/m^2 as per Regulation 44/2006). Each compartment was covered with 20 cm soft bedding (litter) consisting of chopped straw and cobs and equipped with 7 round feeders and 5 drinkers ensuring feeding and drinking widths of 4 and 2.8 cm respectively (Regulation 44/2006). During the two observation subperiods – the thermoneutral subperiod (from April 26 to May 26, 2012) and the hot subperiod (from June 26 to July 26, 2012) all groups were fed freely with the same compound feed according to laying hens category (Table 1). The first group was used as control and received no supplements. During the hot experimental period the diet of experimental groups was supplemented with

Table 1. Composition of basal diet for DeKalb Brown hens

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*	2.0	
Nutrient analysis:		Reference values**
Metabolisable E, MJ/kg	11.89	10.88–11.71
Metabolisable E, kcal/kg	2842	2600–2800
Protein (N x 6.25), g	171	min.150
Crude fat, g	40	min. 30
Crude fibre, g	40.57	max. 60
Arginine, g	10.10	9.30–10.30
Methionine, g	6.00	5.80–6.00
Threonine, g	6.20	6.00
Tryptophan, g	1.9	1.9
Calcium, g	32.10	30–38
Phosphorus available, g	3.00	2.60–4.00
Zinc, mg	80	

*The vitamin and mineral premix Rovimix 15-C Layer provided per kilogram diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 30 mg; vitamin K_3, 3,0 mg; vitamin B_1, 2,0 mg; vitamin B_2, 5,0 mg; vitamin B_6, 5,0 mg; vitamin B_{12}, 0,016 mg; niacin, 30 mg; pantothenic acid, 12.0 mg; folic acid 1.0 mg; biotin, 0.05 mg; Co, 0.15 ng; I, 1 mg; Fe, 50 mg; Zn, 80 mg; Mn, 100 mg; Cu, 8 mg; Se, 0.2 mg; antioxidant, 25 mg; ** According to Chiba (2014).

either 1% (10 g/kg) L-arginine (Roanal, Budapest, Hungary) or with the combination of 10 g/kg arginine and 250 mg/kg vitamin C (L-acidum ascorbicum, Shijiazhuang Co. Ltd) per kg feed. The rearing conditions were fully compliant with minimum requirements for humane treatment of breeder hens (Regulation 25/ 2006; Regulation 44/2006).

The DeKalb Brown hens behaviour was recorded with 3 video cameras for 12 h over 4 consecutive days during each subperiod (May 22–25 and July 22–25, 2012), accounting the number of birds engaged in specific forms of activity: 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 recording thermohygrographs (ERZG, GDR, Type 405); the velocity of the air motion – with a catathermometer, on a weekly basis, during three consecutive days at 7:00 AM, 2:00 PM and 9:00 PM at 5 micro zones within each compartment. The light intensity was measured by a digital luxmeter (Taschen-luxmeter LM37, Germany); the concentration of ammonia – by indicator tubes (Ammonia 0.2-A, Hygitest Association, Bulgaria) and a Drager ammonia sensor.

Blood samples for corticosterone determination were collected twice – during the thermoneutral (on May 26) and hot subperiods (on July 26), from *v. subcutanea ulnaris* in sterile heparinised and plain vacutainers (Vacutainer® Plus plastic plasma tube 13×75 mm × 4.0 mL BD). They were collected always between 1:00 and 2:00 PM to avoid the influence of the circadian rhythm of corticosterone. Blood samples were collected from nine (three per replicate) birds randomly chosen from each treatment group.

The plasma corticosterone levels were assayed with immunoenzymatic ELISA kit (Corticosterone ELISA RE52211, IBL Gesellschaft fur Immunchemie und Immunbiologie MBH, Hamburg, Germany) and ELISA reader in the Laboratory of Innate Resistance at the Faculty of Veterinary Medicine – Stara Zagora. Blood biochemical indices – glucose, cholesterol, creatinine, total protein and triglycerides were determined on an automated Cobas Mira biochemical analyzer at an accredited biochemical lab, Sveti Georgi Diagnostic and Consultation Medical Centre, Plovdiv.

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

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 in the hens' living area with the veterinary requirements for animal breeding facilities (Regulation 44/2006), it was found out that the average ambient temperature in our experiment was 30.24±0.88 ^{o}C, i.e. substantially higher that the allowances of 18– 25 ^{o}C for this category birds. The data for the microclimatic indicators of DeKalb Brown laying hens are given in Table 2.

In the present experiment, the hot summer conditions provoked substantially

Table 2. Microclimatic parameters during the hot period under semi-open buildings for DeKalb Brown hens

Periods	Ambient temperature (°C)	Air humidity (%)	Air velocity (m/s)	NH_3 (ppm)	Light intensity (lx)
Thermoneutral period	20.50±2.50	69.07±4.59	1.10±0.45	traces	65.00±3.27
Hot summer period	30.24±0.88	65.75±1.25	0.71±0.03	traces	87.90±4.34
Minimum-maximum parameters	18.00–31.12	64.50–73.66	1.55	traces	92.24–61.73
Reference values*	18–25	50–70	0.2–1.0	< 15	30–60

*Reference values as per Regulation 44/2006 (Anonymous, 2006b)

Table 3. Blood corticosterone levels and biochemical indices in DeKalb Brown hens supplemented either with 10 mg/kg L-arginine (Arg group) or with 10 mg/kg L-arginine and 250 mg/kg vitamin C (Arg+it. C group) (mean ±SEM; n=36 ♀)

Parameters	Thermoneutral period	Hot summer period		
	Control group	Control group	Arg group	Arg+Vit. C group
Corticosterone, nmol/L	82.57±4.15	177.25±3.78^^^	125.53±6.47***	106.5±6.26***#
Total cholesterol, mmol/L	2.41±0.16	3.31±0.27^^	2.87±0.05	2.65±0.12*
Glucose, mmol/L	6.30±0.40	10.80±0.23^^^	7.97±0.54**	7.47±0.24***
Total protein, g/L	111.79±3.43	57.98±4.83^^^	81.49±5.03**	92.36±4.27***
Creatinine, µmol/L	69.59±3.84	99.09±3.97^^^	85.67±2.80*	77.26±2.43***#
Triglycerides, mmol/L	4.42±0.43	10.35±0.35^^^	8.67±0.26**	6.59±0.31***###

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

higher blood corticosterone (P<0.001), cholesterol (P<0.01), glucose (P<0.001), creatinine (P<0.001) and triglycerides (P<0.001) compared to the thermoneutral period were established (Table 3). The behavioural changes in laying hens were manifested with statistically significantly lower number of feeding (P<0.05), feather-cleaning (P<0.001), egg-laying (P<0.01), dust bathing (P<0.001) and mating birds (P<0.001) but increased number of drinking (P<0.001) and resting (P<0.001) control birds, compared to the thermoneutral period (Table 4).

On the basis of the behaviour, corticosterone levels and blood biochemical changes, the five freedoms were scored in our experiment (Table 5) and the total poultry welfare (PW) score of control hens under semi-open rearing system during the hot period was calculated – PW=33.33%.

Our results showed that in arginine-supplemented birds, blood corticosterone (P<0.001), glucose (P<0.01), creatinine

Table 4. Number of DeKalb Brown hens, supplemented either with 10 mg/kg L-arginine (Arg group) or with 10 mg/kg L-arginine and 250 mg/kg vitamin C (Arg+Vit. C group) exhibiting a specific type of behaviour (mean ±SEM, n=295♀)

Behaviour	Thermoneutral period		Hot summer period					
	Control group	%	Control group	%	Arg group	%	Arg+Vit. C group	%
Feeding	6.80± 0.63	23.45	4.91± 0.66$^{\wedge}$	16.93	7.86± 0.58***	27.11	7.50± 0.62**	25.86
Drinking	4.34± 0.30	14.97	7.16± 0.38$^{\wedge\wedge\wedge}$	24.69	7.16± 0.44	24.70	6.77± 0.45	23.34
Egg-laying	1.43± 0.23	4.93	0.61± 0.13$^{\wedge\wedge}$	2.12	1.09± 0.13*	3.76	0.96± 0.14*	3.31
Moving	8.09± 0.41	27.90	7.41± 0.23	25.55	3.00± 0.29***	10.34	2.84± 0.29***	9.79
Resting	2.30± 0.29	7.93	5.93± 0.56$^{\wedge\wedge\wedge}$	20.45	4.52± 0.58*	15.59	3.84± 0.57**	13.24
Feather cleaning	0.86± 0.13	2.97	0.39± 0.10$^{\wedge\wedge}$	1.34	1.14± 0.21***	3.93	1.73± 0.18$^{***\#}$	5.97
Dust bathing	0.64± 0.12	2.21	0.09± 0.04$^{\wedge\wedge\wedge}$	0.31	2.21± 0.36***	7.62	3.36± 0.44$^{***\#}$	11.59
Aggression	1.57± 0.13	5.41	1.23± 0.18	4.24	0.23± 0.07***	0.79	0.14± 0.05***	0.48
Sexual behaviour	1.91± 0.16	6.59	0.89± 0.11$^{\wedge\wedge\wedge}$	3.07	1.66± 0.17***	5.72	1.73± 0.17***	5.97

^P<0.05; ^^P<0.01; ^^^P<0.001: statistically significant difference in control group between thermoneutral and hot summer periods; *P<0.05; **P<0.01: statistically significant difference between control and experimental groups during the hot summer period; $^{\#}$P<0.05; $^{\#\#}$P<0.01$^{\#\#\#}$P<0.001: statistically significant difference between arginine-supplemented and arginine and vitamin C supplemented groups during the hot summer period.

(P<0.05) and triglycerides (P<0.01) were lower than respective concentrations in controls (Table 3). Also, there were more feeding (P<0.001), egg-laying (P<0.05), resting (P<0.05), feather cleaning (P<0.001), dust bathing (P<0.001) and mating (P<0.001) birds supplemented with L-arginine, as well as lower number of walking (P<0.001) and aggressive (P<0.001) hens compared to controls (Table 4). The reduced negative impact of heat stress has resulted in higher scores of F_1, F_2, F_4, and F_5 freedoms vs controls (Table 5). Thus the total poultry welfare score in arginine-supplemented birds was PW=66.67%.

The combination of 10 mg/kg arginine with 250 mg/kg vitamin C in our experiment provoked similar changes in blood corticosterone and biochemical parameters, as well as observed types of behaviour in laying hens (Tables 3 and 4). There were statistically significantly more cleaning (P<0.05) and dust bathing (P<0.05) hens compared to the arginine-supplemented group. The plasma corticosterone (P<0.05) and blood triglycerides (P<0.001) in arginine+vitamin C-supplemented birds have further decreased com-

Table 5. Welfare assessment scores of *DeKalb Brown* hens supplemented either with 10 mg/kg L-arginine (Arg group) or with 10 mg/kg L-arginine and 250 mg/kg vitamin C (Arg+vit. C group) during the hot summer period

Poultry welfare assessment		Hot summer period		
Freedom	Degree	Control group	Arg 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	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	0	2	2
Total score		5	10	11
Poultry welfare, %		33.33	66.67	73.33

pared to arginine-supplemented only (Table 3). The total poultry welfare score of the hens, supplemented with arginine+vitamin C during the hot period was PW=73.33% (Table 5).

DISCUSSION

During the hot summer period the ambient temperature in the living area of hens was substantially higher that the veterinary requirements. It induced a thermal stress in laying hens. It is a common cause of welfare worsening in hens triggering a chain of non-specific reactions and systemic mechanisms of defense. The stress 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). In heat stressed birds, the hypothalamus activates adrenal gland cortex and it reacts by enhanced secretion of glucocorticoids – corticosterone (Puvadolpirod & Thaxton, 2000a,b,c). These events generate numerous behavioural, hormonal and biochemical changes concerning welfare worsening of the hens. Our results confirmed that the behaviour, blood corticosterone and blood biochemical parameters in laying hens have changed during the

hot subperiod. At that time, blood corticosterone, cholesterol, glucose, creatinine and triglycerides were substantially higher compared to the thermoneutral period. Our data are in agreement with those reported by Popova-Ralcheva *et al.* (2002), Sahin *et al.* (2005; 2009), affirming that during heat stress, corticosterone, adrenaline and glucagon are primary regulators of blood glucose in birds and providing evidence for increased serum glucose, cholesterol and creatinine. The experiments of Star *et al.* (2008) and Sahin *et al.* (2009) demonstrated statistically significantly higher blood corticosterone, glucose and reactive oxygen species in four strains of laying hens. In birds with induced stress Puvadolpirod & Thaxton (2000a,b,c) reported higher blood plasma cholesterol and lower total protein concentrations.

The heat stress in our experiment was manifested with significantly lower number of feeding, egg-laying, feather-cleaning, dust bathing and mating birds but increased number of drinking and resting control birds, compared to the thermoneutral period, which are criteria for welfare worsening in birds (Ensminger *et al.,* 1990; Sahin *et al.,* 2009; Bozakova *et al.,* 2012). Similar results for decreased egg-laying during a hot period in breeder hens are reported by Star *et al.* (2008) and Yardibi & Turkay (2008). The elevated corticosterone, cholesterol and triglycerides compared to the thermoneutral period in our experiment resulted in lower total poultry welfare score in control hens during the hot period (PW=33.33%).

Our results confirmed the influence of the dietary supplementation with 1% arginine on behaviour, corticosterone and biochemical indices in the DeKalb Brown hens under semi-open rearing system. There were more feeding, egg-laying, resting, feather cleaning, dust bathing and mating experimental birds, as well as lower walking and aggressive chickens compared to controls, all of them criteria for better welfare. The blood corticosterone, glucose and triglycerides were lower than respective levels in controls. Olsson & Keeling (2005) and Dixon *et al.* (2008) found that the behaviour of taking dust baths is an important indicator of social welfare of birds. According to Sherwin & Kelland (1998), the higher welfare in turkeys increased the time spent in stretching, feather pecking, and dust bathing. The improved welfare of experimental birds was also evidenced by the lower frequency of aggression acts vs controls, as also stated by Popova-Ralcheva *et al*. (2002). The positive effects of arginine supplementation were attributed to the inhibiting role of its metabolite nitric oxide on ACTH and corticosterone secretion (Tsai *et al.,* 2002; Heinzen, 2003), the suppression of adrenal glucocorticoid synthesis and arginine antioxidant activity (Gupta *et al.*, 2005). Thus, the welfare score in arginine-supplemented hens in our experiment increased to PW=66.67%.

The dietary combination of 1% arginine with 250 mg/kg vitamin C, tested in our experiment, had a better positive effect on welfare in DeKalb hens during the hot period under the semi-open rearing system. There were more cleaning and dust bathing hens compared to the arginine-group. The plasma corticosterone and blood triglycerides have further decreased. The welfare score of birds receiving the two supplements in our experiment increased to PW=73.33%, which could be explained by their combined stress-reducing effect during the hot summer period. The lower plasma corticosterone concentration in hens was probably due to various anti-stress effect mecha-

nisms of the arginine and vitamin C combination (Bozakova & Gerzilov, 2014). Apart the discussed inhibiting role of arginine metabolite on ACTH and corticosterone secretion, vitamin C further reduces corticosterone levels by participating in gluconeogenesis during stress (Jones *et al.,* 1996; Sahin *et al.,* 2002). This way the results of the present experiment provide evidence for the synergistic activity of L-arginine and vitamin C for heat stress reduction and poultry welfare improvement.

In conclusion, the welfare score of *DeKalb Brown* hens under semi-open rearing system during the hot period e. g. under heat stress was PW=33.33%. In birds supplemented with 10 mg/kg L-arginine during the hot period, several behavioural changes were observed, as well as reduced blood corticosterone and some biochemical indices compared to controls, resulting in increased welfare score (PW=66.67%). After co-administration of 10 mg/kg L-arginine and 250 mg/kg vitamin C during the hot period, the welfare score of hens increased to PW=73.33% due to the various heat stress-reducing effects of both compounds.

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