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ETHOLOGICAL ASPECTS OF HENS WELFARE SUPPLEMENTED WITH IMMUNOMODULATOR *IMMUNOBETA*

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Summary

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The purpose was to study the behaviour and plasma corticosterone changes in Lohmann Brown laying hens after *Immunobeta* – supplementation. The behaviour was observed on 4 consecutive days for 12 hours by video cameras counting 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) and agonistic behaviour. The plasma corticosterone levels were assayed by means of commercial ELISA kit. Two types of behaviour were indicative for hens' welfare: comfort behaviour (dust bathing, feather cleaning, improved egg-laying) and aggression/fear behaviour. After 2-months of Immunobeta-supplementation, there were statistically fewer hens in the control group vs the experimental for the following activities: egg-laying $P<0.05$; feather cleaning $P<0.05$; dust bathing, $P<0.01$. Concurrently, the amount of aggressive hens in control group was higher than in the Immunobeta-group, $P<0.05$.

Key words: behaviour, corticosterone, hen welfare, yeast additive

INTRODUCTION

Heat stress is the major cause for the worse welfare of birds reared in subtropical areas (Hai *et al.*, 2006). The stress response in birds is mediated by activation of hypothalamo-pituitary-adrenal system and the sympathetic vegetative system and accompanied by several changes in the behaviour and metabolism of birds (Linares & Martin, 2010). In such instances, a good alternative for preserving the welfare of poultry in hot areas is the use of dietary supplements (trace elements, vitamins, minerals) for satisfying the needs of birds under heat stress. This action has provoked the interest of experts towards the use of immunomodulators as yeast additives. As a result of their administration, hens' non-specific humoral immunity has been stimulated.

Linares & Martin (2010) outlined that the natural behaviour of birds was the most relable welfare indicator. Tree types of behaviour are indicative for welfare improvement in hens: improved comfort behaviour (dust bathing, feather cleaning), improved egg-laying and reduced aggressiveness. Yalçin *et al.* (2010), Gürbüz *et al.* (2011), Cai *et al.* (2016) have found

that adding yeast lysate (*Saccharomyces cerevisiae*) can increase egg-laying ($P<0.001$) and the egg weight ($P<0.001$), and also improve fodder efficiency in laying hens. The immunomodulator *Immunobeta* was obtained from selected strains of yeast *(Saccharomyces cerevisiae)* through enzyme autolysis and a process of natural extraction of yeast cell components. It contains three active ingredients: 30% beta-glucans, 25% mannan oligosaccharides and 5% nucleotides.

However, there are only scarce data on the effect of *Saccharomyces cerevisiae* on poultry behaviour and corticosterone levels, which is the most accurate criteria for their welfare. This motivated our choice of topic for the present study. The purpose of the present study was to investigate the behaviour and plasma corticosterone changes in Lohmann Brown laying hens after 2 months *Immunobeta* supplementation at a doses of 4 kg/ton and 2 months after the end of *Immunobeta* treatment.

MATERIAL AND METHODS

The experiment was performed with 1056 hybrid Lohmann Brown divided in two groups (n=528, ♀) – one control and one experimental. The poultry farm owned by Divex-2-Georgi Georgiev Co, was located in the village of Malka Polyana, Aytos municipality. The laying hens were housed in enriched cages within a large battery. The size of the cages was 200 cm/100 cm. Each hen was given 750 cm^2 of usable area. The cages were provided with an egg-laying compartment, a moving area, perches, dust bath and nipple drinkers. Control hens received the usual diet for their age. Present surveys have been conducted according to animal welfare documents (Anonymous, 2006).

The behaviour of the hens was recorded with video cameras for 12 hours 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 behaviour: ingestive (ingestion of water or food), gregarious (moving, resting, egg-laying, dust bathing and feather cleaning), sexual and agonistic behaviour.

Blood samples for corticosterone determination were collected 2 months after Immunobeta- treatment (on May 26) and two months after the treatment ended (on July 26), from v. subcutanea ulnaris in sterile vacutainers. The plasma corticosterone levels were assayed with ELISA kit and ELISA-reader in the Laboratory of Innate Resistance Investigation in Faculty of Veterinary Medicine – Stara Zagora.

Microclimatic conditions' data about the temperature, relative humidity, gas composition and ventilation were recorded in the reporting card. The light intensity was measured by a digital luxmeter (Taschen-luxmeter LM37, Germany).

One-way ANOVA - GraphPad InStat 3.06 was performed to examine the differences among groups. The significance of mean differences between groups were tested as per Tukey. The values were given as mean and standard error of mean. Level of significance was $P<0.05$

RESULTS

During *Immunobeta* treatment microclimate conditions were within the norm: t^o =20.05±0.29 ^oC; R=64.63±0.54%; air velocity –0.27±0.01 m/s; light intensity-8.96±0.30 lx; NH_3-concentrations were 7.30±0.25 ppm (Anonymous, 2006a).

Table 1 presents the averaged results for the different types of behaviour on the basis of ethogrammes of control and Immunobeta – groups after 2 months of

Immunobeta-treatment and 2 months after the end of the treatment.

After 2 months of treatment, the number of egg-laying ($P<0.05$), feather cleaning ($P<0.05$) and dust bathing hens ($P<0.01$) in experimental group was statistically higher than the control one. Simultaneously, the aggressive hens in that group were less than the control one ($P<0.05$). Two months after the ending of *Immunobeta* treatment, the number of egg-laying ($P<0.05$), feather cleaning ($P<0.001$) and dust bathing hens ($P<0.01$) in experimental group was statistically higher than the control one.

During the two studied periods corticosterone levels in control and *Immunobeta*-treated hens were 25.54± 4.01 nmol/L (control group) and 27.15 ± 4.40 nmol/L (*Immunobeta* group) 2 months after treatment and 22.98 ± 2.60 nmol/L (control group) and 24.46 ± 5.80 nmol/L (*Immunobeta*-group) 2 months after treatment end. The differences in blood corticosterone concentrations between groups were not significant.

Table 1. Number of hens from control and Immunobeta groups exhibiting a specific type of behaviour after 2 months Immunobeta-treatment and two months after the end of treatment . Data are presented as mean±SEM, (n=528)

	After 2 months Immunobeta-treatment				Two months after the end of treatment			
	Control group	%	Immunoβ group	%	Control group	%	Immunoβ group	%
Feeding	224.18 ±12.85	42.46	194.18± 14.49	36.78	187.09± 12.89$^{\#}$	35.43	178.91± 13.86	33.88
Drinking	58.36± 4.01	11.05	55.64± 3.29	10.54	68.73± 2.15$^{\#\#}$	13.02	70.91± 2.46$^{\#\#\#}$	13.43
Egg-laying	57.27± 6.45	10.85	80.73± 7.03*	15.29	69.27± 6.55	13.12	88.36± 6.47*	16.74
Moving	141.27 ±11.74	26.76	111.27± 12.92	21.07	109.60± 5.76$^{\#\#}$	20.76	108.50± 5.63$^{\#\#}$	20.56
Resting	52.36± 9.76	9.92	50.18± 10.52	9.50	86.73± 6.81$^{\#\#}$	16.43	92.18± 7.36$^{\#\#\#}$	17.46
Feather cleaning	4.91± 1.67	0.93	11.46± 2.53*	2.17	1.64± 0.92$^{\#}$	0.31	10.60± 2.47***$^{\#}$	2.01
Dust bathing	2.18± 1.05	0.41	7.64± 2.03**	1.45	0.23± 0.10$^{\#}$	0.04	3.00± 1.11**	0.57
Aggression	14.73± 3.04	2.79	7.64± 1.71*	1.45	22.3± 4.77	4.24	9.00± 2.02	1.70

*P<0.05; **P<0.01; ***P<0.001 statistically significant difference for a given type of behaviour between control and treated groups. # P<0.05; ##P<0.01; ###P<0.001 indicates statistically significant difference for a given type of behaviour between thermoneutral and hot periods.

DISCUSSION

The immunomodulator *Immunobeta* had a positive influence on the hens' behavior. After 2 months of the treatment and two months after the treatment end, the number of egg-laying, feather cleaning and dust bathing hens in experimental group was statistically higher than the control one. Simultaneously, the aggressive hens in *Immunobeta* group were less than the control one. The number of egg-laying hens in our experiment was improved in *Immunobeta* group during the two subperiods. In line with our results, Yalçin *et al.* (2010), Gürbüz *et al.* (2011) have found that adding yeast lysate can increase the egg-laying and egg weight in laying hens.

In turkeys, improved welfare is manifested with increased time spent in stretching, feather cleaning and dust bathing (Sherwin & Kelland, 1998). Additionally Olsson & Keeling (2005) and Dixon *et al.* (2008) found that the behaviour of taking a dust bath is an important indicator of social welfare of the poultry. The improved behaviour of experimental hens was also evidenced by the lower number aggressive hens vs controls, as also stated by Popova–Ralcheva *et al.* (2002). *Immunobeta* did not affect corticosterone concentration of hens. Immunobeta active ingredients stimulate the non-specific systemic immune response as confirmed by Shao *et al.* (2013), and Huff *et al.* (2013). Additionally Czech *et al.* (2014) reported that yeast-based immunomodulators increased lysozyme concentration in turkeys. This could be attributed to the effect of *Immunobeta* which led to an increase in non-specific and specific resistance of poultry, is related to improving egg production and hens behaviour. All these facts provided evidence that the *Immunobeta* supplementation (4 kg/ton) improved hen's behaviour, stimulating their egg-laying, feather cleaning and dust bathing.

REFERENCES

Anonymous, 2006. Regulation 25 on the minimum requirements for the protection and welfare under laying hens rearing. *State Gazette,* **42** (BG).

Anonymous, 2006a. Regulation 44 for veterinary medical requirements of animal rearing facilities. *State Gazette*, **7**, 57–58 (BG).

Cai, L., C. M. Nyachoti , J. D. Hancock, J. Y. Lee , Y. H. Kim , D. H. Lee & I. H. Kim , 2016. Rare earth element-enriched yeast improved egg production and egg quality in laying hens in the late period of peak egg production. *Journal of Animal Physiology and Animal Nutrition* **100**, 492–498.

Czech, A., M. Merska & K. Ognik, 2014. Blood immunological and biochemical indicators in turkey hens fed diets with a different content of the yeast *Yarrowia lipolytica*. *Annuals of Animimal Science*, **14**, 935–946.

Dixon, L.M., I. J. H. Duncan & G. Mason, 2008. What's in a peck? Using fixed action pattern morphology to identify the motivational basis of abnormal feather-pecking behavior. *Animal Behaviour*, **76**, 1035–1042.

Gürbüz, E., T. Balevi, V. Kurtoğlu & Y**.** Öznurlu, 2011. Use of yeast cell walls and *Yucca schidigera* extract in layer hens' diet. *Italian Journal of Animal Science*, **10**, 134–138.

Hai, L., E. Decuypere& J. Buyse. 2006. Acute heat stress induces oxidative stress in broiler chickens. *Comparative Biochemistry and Physiology, part A*, **144**, 11–17

Huff, G. R, W. E. Huff & S. Jalukar, 2013. The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/*Escherichia coli* challenge. *Poultry Science Journal*, **92**, 655–662.

Kucuk, O., 2008. Zinc in a combination with magnesium helps reducing negative effects of heat stress in quails. *Biological Trace Element Research*,**123**, 144–153.

Linares, J. A. & M. Martin, 2010. Encyclopedia of Animal Behavior. Poultry, Behavior and Welfare Assessment, *Academic Press*, USA, pp. 750–756.

Martrenchar, A., Huonnig, D., Cotte, J. P. 2000. Influence of environmental enrichment on injurious pecking and perching behaviour in young turkeys. *British Poultry Science*, **42**, 161–170.

Olsson, I. A.& L. J. Keeling, 2005. Why in earth? Dustbathing behaviour in jungle and domestic fowl reviewed from Tibergian and animal welfare perspective. *Applied Animal Behaviour Science*, **93**, 259–282.

Popova–Ralcheva, S. V., V. Hadjiiliev, D. Gudev, A. Alexandrov & V. Sredkova, 2002. Ethological and physiological indices for well-being in broilers under different systems of management. *Bulgarian Journal of Agricultural Science*, **8**, 635–639.

Shao, Y., Y. Guo & Zh Wang, 2013. β-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica* serovar Typhimurium. *Poultry Science Journal*, **92**, 1764–1773.

Sherwin, C. M. & A. Kelland, 1998. Time-budgets, comfort behaviours and injurious pecking of turkeys housed in pairs, *British Poultry Science*, **39**, 325–332.

Yalçin, S., S. Yalçin, K. Cakin, O. Eltan & L. Dağaşan, 2010. Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) on performance, egg traits, egg cholesterol content, egg yolk fatty acid composition and humoral immune response of laying hens. *Journal of Science of Food and Agriculture,* **90***,* 1695–1701.