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EFFECT OF BETAINE ON EGG PERFORMANCE AND SOME BLOOD CONSTITUENTS IN LAYING HENS REARED INDOOR UNDER NATURAL SUMMER TEMPERATURES AND VARYING LEVELS OF AIR AMMONIA

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Abstract

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A research was initiated to determine the effect of supplemental dietary betaine on egg performance and some blood indices in 2-year-old laying hens reared under natural summer temperatures and varying ammonia levels. 99 laying hens were allocated into three groups: I - control; II - supplemented with 0.7 g/kg betaine and III - supplemented with 1.5 g/kg betaine. The hens were kept on deep litter in a windowless poultry house for 23 days. The results of this study indicated no change in terms of egg quality (albumen weight, albumen index, yolk weight, yolk index, yolk color score, Haugh unit, egg specific gravity), hematocrit and leukocyte counts.

The hens given 0.7 g/kg supplemental dietary betaine had higher erythrocyte number ($P<0.05$) than those given 1.5 g/kg supplemental betaine. Feeding 1.5 g/kg supplemental betaine resulted in significant decrease of heterophil percentage ($P<0.05$) and increase of lymphocyte percentage ($P<0.05$) at d 22, when air ammonia level was 3 times higher than permitted. These changes in leukocyte subpopulations led to a decrease of heterophil/ lymphocyte ratio. Plasma corticosterone levels at d 22 were lower in the I ($P>0.05$), II ($P<0.05$) and III group ($P>0.05$) relative to the correspondent values at d 8. Both levels of supplemented betaine increased ($P<0.001$) the average egg production.

Our results suggest that supplemental betaine has a positive effect on egg performance in hens reared under high air ammonia conditions.

Key words: betaine, egg performance, laying hens, hematocrit, erythrocytes, leukocytes, air ammonia, heterophil/ lymphocyte ratio, corticosterone, stress, egg specific gravity

Introduction

Dietary betaine supplementation on animal performance and carcass characteristics has been widely studied during the last decades. Betaine is trimethyl derivative of the aminoacid glycine and donates its labile methyl group, which can be used in transmethylation reactions (Simon, 1999). Observations on the biological effects of betaine revealed that betaine might have the potential to

improve the digestibility of specific nutrients (Eklund et al., 2006a, b). Betaine is shown to be the most effective osmoprotectant among the organic osmolytes (Hammer and Baltz, 2002). According to Clow et al. (2008), dietary betaine increases the concentration of betaine in the intestinal epithelium. It is involved in the osmoregulation of duodenal epithelium of broiler chicks and affects the movement of water across the small intestinal epithelium in vitro (Kettunnen et al., 2001). Betaine is a chemical chaperone and helps to stabilize proteins in their natural conformation. Therefore, it may be helpful for survival of intestinal microorganisms under stress conditions (Chattopadhyay, 2001). Furthermore, betaine acts as a modulator of nitric oxide synthesis, thus stimulating host defense and superoxide anion scavenging (Messadek, 2010). Supplemental dietary betaine improved weight gain and feed conversion in some poultry studies (Mathews and Southern, 2000; Hassan et al., 2005) whereas other studies showed minimal or no effect of betaine on animal performance (Zulkifi et al., 2004; Feng et al., 2006).

These controversial results are attributed to the occurrence of osmotic stress and the concentration of methyl group donors in the diet. The objective of this study was to investigate the effect of supplemental dietary betaine on egg performance, egg quality, hematocrit, erythrocyte number, leukocyte number, plasma corticosterone, white blood cell differential count and rectal temperature in laying hens reared indoor, under natural summer temperatures and fluctuating levels of air ammonia.

Material and Methods

Ninety-nine two years old laying hens of Label breed were allocated randomly into three groups consisting of 33 hens each. All birds were fed commercial layers feed throughout the trial which lasted 23 days. The second and III group, unlike I group (control), were fed supplemental dietary betaine (0.7 and 1.5 g/kg respectively). Fresh water was supplied ad libitum. The birds were housed on deep litter, under natural temperature conditions. Nest boxes were located along the sidewalls. The trial was conducted in windowless poultry house during the period between July and August 2010. The indoor temperature readings were taken in the morning (8.30 am) and afternoon (14.00 h) throughout the experimental period. Aeroqual ammonia monitor measured air ammonia, model S-200. Relative humidity was measured by psychrometer. Both indices were registered on d 1, 3, 8, 15 and 22 of the experimental period at about 0.3 m above the litter.

During the experimental period the following data were collected: daily egg production; egg weight measured on d 4, 13 and 23 of the experimental period; egg shape = (maximum width/maximum length) x 100; albumen index = (albumen height/ average albumen width) x 100; Haugh unit = $100 \text{ Log } [H - (\sqrt{G(30^{0.37} - 100)}/100 + 1.9]$; H – albumen height in millimeters, G – 32,2 gravitational constant , W- weight of egg, g; yolk index = (yolk height/ yolk diameter) x 100; yolk weight, yolk color score (assessed by Roche color fan) and shell thickness (measured by micrometer). The enumerated egg quality indices were determined in all eggs collected on d 23. Egg specific gravity was measured on d 4, 13 and 23 of the experimental period by floatation of the egg in various salt solutions. Rectal temperature was measured at 9 am on d 3, 9 and 23 by a digital thermometer. Venous blood samples were collected by brachial vein puncture on d 8 and d 22 between 10 and 12 am. Hematocrit, leukocyte count and erythrocyte count were determined on d 8 and d 22. Plasma corticosterone and white blood cell differential count were determined immediately after catching of each hen at d 8 and d 22. Peripheral blood leukocytes were counted on smears. Two drops of blood were taken via brachial vein puncture, 1 drop being smeared on each of two glass slides. The smears were stained using May-Grunvald and Giemsa stains (Lucas and Jamros, 1961). Two hundred leukocytes including heterophils, eosinophils, basophils, lymphocytes

and monocytes were counted microscopically on one slide of each bird.

Plasma corticosterone was determined using enzyme immunoassay kit (IBL gesellchaft fur immunchemie und immunbiologie, MBH, D 22335 Hamburg, Germany) Total erythrocyte and leukocyte count was determined by manual haemocytometer chamber count. Hematocrit was determined by centrifuging heparinized blood in a capillary tube. The results are expressed as means ± S.E.M. and were analyzed by ANOVA.

Results and Discussion

Hematocrit level in betaine supplemented groups tended to be lower ($P<0.05$) relative to those in control birds at d 8 following the start of the experimental period (Figure 1). The observed decline of hematocrit could be associated with the reported regulatory effect of betaine on erythrocyte membrane ATP-ases, via conformational changes which results in cell volume control (Craig, 2004). In addition, this finding is consistent with the hypothesis that the initial reduction of hematocrit is caused by increased blood volumes associated with osmoregulatory adjustments to elevated levels of yolk precursors (Williams et al., 2004). This hypothesis is in agreement with the higher ($P<0.05$) erythrocyte number in the II group as compared to III group against the background of insignificant hematocrit decline at d 8 of the experimental period (Figure 2) .

Hematocrit levels among the groups did not differ significantly at d 22 (Figure 1). These findings could be associated with the higher values of air temperature (Figure 3) and air ammonia (Table 1) on d 22 as compared to the corresponding values at d 8. Air temperature at d 8 was within the thermoneutral range, whereas at d 22 it reached 26 ^{0}C. Air ammonia level at d 8 was 10 times lower than that at d 22. It is worth to note that blood erythrocyte number in the III group at d 22 was lower ($P> 0.05$) as compared to that in I and II group in spite of the similar hematocrit levels among the experimental groups (Figure 2). These data suggest that the higher level of supplemental betaine has role in hematocrit maintenance and exerts its effect by increasing erythrocyte volume. The underlying mechanism of the observed erythrocyte number decline is probably related with the elevated level of air ammonia. Ammonia was reported to increase intracellular osmolarity, evoked by the elevation of glutamine because of the enhanced metabolic activity associated with ammonia detoxification in glioma cells (Zwingmann et al., 2000). Betaine is considered the most effective organic osmoprotectant (Hammer and Baltz, 2002). Consequently, it could be assumed that the observed decline of erythrocyte number in III group of birds was related with the osmoprotective properties of betaine. Leukocyte number tended to decline ($P>0.05$) at d 22 in I and II group, whereas in III

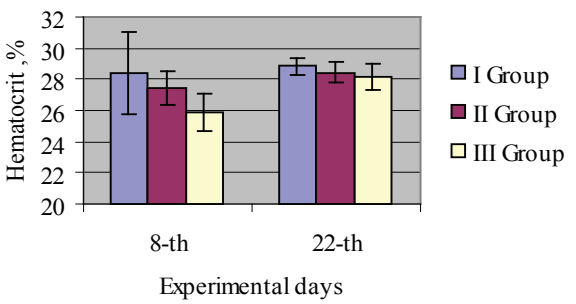

Fig. 1. Hematocrit in betaine supplemented laying hens, %

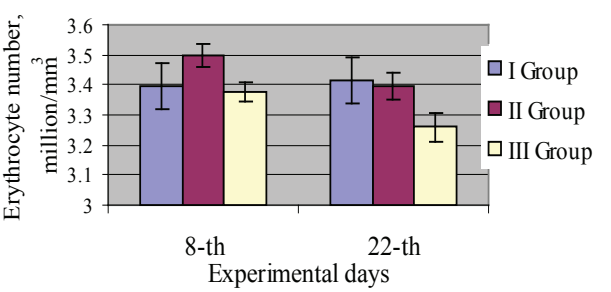

Fig. 2. Erythrocyte number in betaine supplemented laying hens

Table 1

Indoor air ammonia and relative humidity measured four times per day between 11.00 and 15.00 h

	Days through the experiment				
	1 day	3 day	8 day	15 day	22 day
Air ammonia range, ppm	9.2 – 23.8	23.6 – 58.0	5.2 – 7.1	27.8 - 84.2	32.8 – 78.0
Relative humidity, %	61 – 62	56 -59	70 – 76	69 – 71	66 - 68

group it increased slightly (P>0.05) as compared to the corresponding values at d 8 (Figure 4). The observed trend in I and III group is consistent with the reported decrease of leukocyte number in horses exposed to atmospheric ammonia (Katayama et al., 1995) and suggests that the increased air ammonia concentration at d 22 was probably the underlying cause of the observed leukocytes decline. The decrease of leukocyte number in I and II group at d 22 coincided with a sizable decline of lymphocyte percentage (Figure 5). It is known that ammonia gas interacts immediately upon contact with available moisture in the respiratory tract and mucosa surfaces to form caustic ammonium hydroxide (Rollins, 2010). Therefore, bearing in mind that air ammonia level at d 22 was relatively high, we suggest that the registered trend of leukocyte decline in I and II group was probably due to lymphocytes migration towards the affected tissues. The observed insignificant increase of leukocyte number in III group unlike the other two groups, coincided with the increase of lymphocyte percentage at d 22 (P<0.05), shown in Figure 5. This finding could be explained with the reported osmoprotective properties of betaine (Hammer and Baltz, 2002). Betaine supplementation reduced plasma corticosterone levels in both experimental groups at d 8 (Figure 6). As far as air temperature at that day was within the thermoneutral range and air ammonia level was low, we assume that betaine exerted its effect by augmentic nitric oxide production (Messadek, 2010) which on its turn inhibited glucocorticoid synthesis (Monau, 2010; Cymeryng et al., 1999). The relatively high level of basal corticosterone in the control group of birds at d 8 was probably due to the handling induced stress during the process of blood sampling. This assumption is further supported by the fact that heterophil/lymphocyte (H/L) ratio in the control birds was within the normal range (Figures 7 and 8), which is reported to be 0.381 in laying hens reared under intensive free range system (Shini, 2003). It is well known that the change in H/L ratio begins after a certain lag period following the start

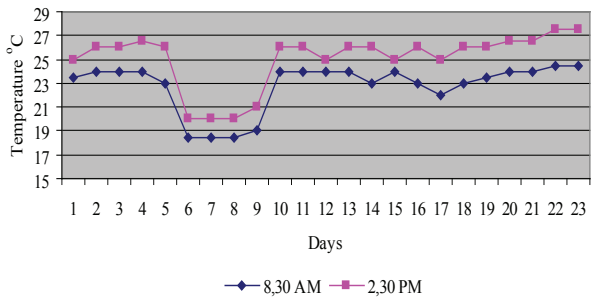

Fig. 3. Indoor temperature fluctuation measured throuhgout the experimental period

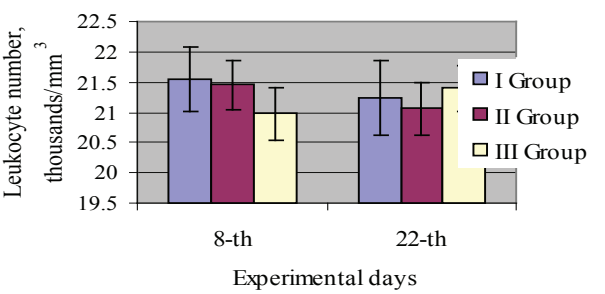

Fig. 4. Leukocyte number in betaine supplemented laying hens

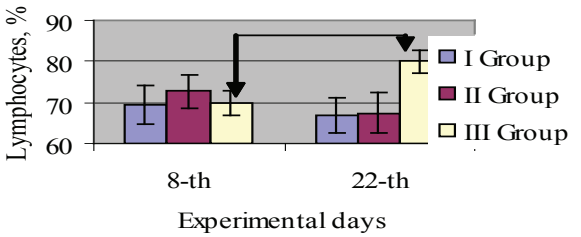

Fig. 5. Effect of betaine on blood lymphocyte percentage in laying hens kept indoor under natural summer temperatures

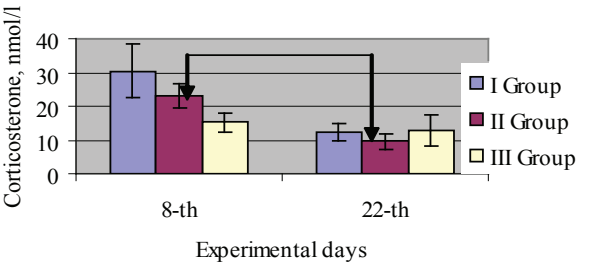

Fig. 6. Effect of betaine on plasma corticosterone level in laying hens kept indoor under natural summer temperatures

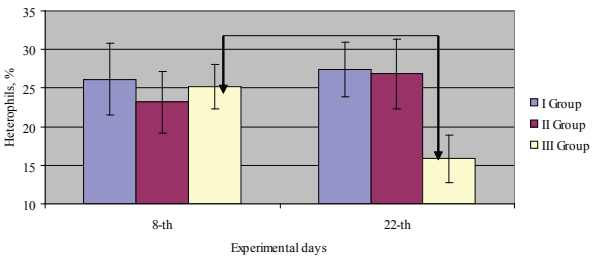

Fig. 7. Effect of betaine on blood heterophil percentage in laying hens kept indoor under neutral summer temperatures

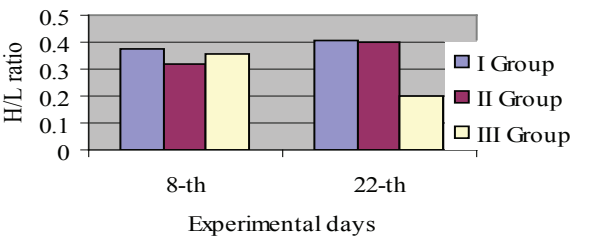

Fig. 8. Effect of betaine on heterophil to lymphocyte ratio in laying hens kept indoor under natural summer temperatures

of the stress episode and the rise of corticosterone level (Dhabhar et al., 1995). Plasma corticosterone levels at d 22 declined in all groups of birds. Air ammonia concentration at that day was 3 times higher than the admitted level (25 ppm). Ammonia level in poorly ventilated poultry houses has been shown to reduce plasma total trijodthyronin (Fidanci et al., 2010). Furthermore, hypothyroid Japanese quills exposed to confinement stress had blunted adrenal response (Weigel, 2007).

These data suggest that the lower plasma corticosterone in I group was probably mediated by an ammonia-induced hypothyroidism. Yet, this assumption remains to be elucidated. It should be noted that heterophil to lymphocyte ratio (H/L) in the III group unlike that in the II group declined sharply at d 22 as compared to that at d 8 (Figure 8). These data come to show that along with its osmoprotective effect betaine influences white blood cell distribution. The strength of the exerted effect seems to depend on the level of betaine supplementation. Besides, the effect of glucocorticoids on H/L ratio is well-documented (Dhabhar et al., 1995) but it doesn't seem to be the factor that affected H/L ratio in our case. Both levels of dietary betaine supplementation increased ($P<0.01$) egg production (Table 2) and daily egg mass, but reduced the mean egg weight ($P>0.05$). These results are consistent with those reported by Ryu et al. (2002) in laying hens reared under heat stress. Our finding indicates that betaine exerts positive effect on the performance of laying hens,

Table 2

Effect of betaine on egg performance of laying hens

Items	Groups		
	I group	II group	III group
Daily mean number of eggs [1]	20.782 ± 0.222	24.174 ± 0.228*	24.869 ± 0.259*
Mean egg weight[2], g	59.794 ± 0.761	57.429 ± 0.578	56.561 ± 0.647
Total egg mass (1 x 2)	1242.639	1388.288	1406.615

Values are means ± SEM

*Means differ significantly from the 1st group within an item (P<0.05)

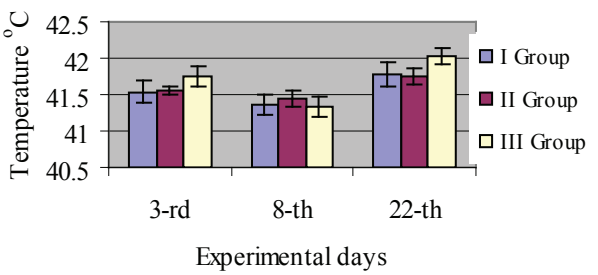

Fig. 9. Main rectal temperature of laying hens fed supplemented dietary betaine

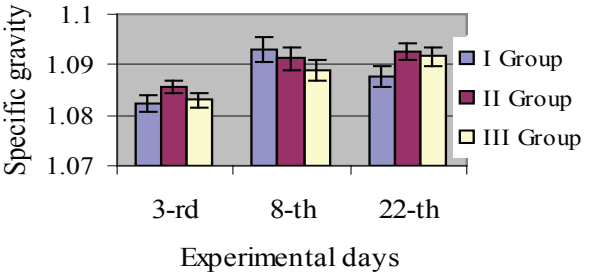

Fig. 10. Specific gravity of eggs in laying hens fed supplemental betaine

exposed to osmotic stress. It has been demonstrated that exposure of laying hens to high ammonia concentration (200 ppm) for 17 days causes a sharp decrease of egg production (Deaton et al., 1982). Indoor ammonia level in our experiment was not as high as that reported by Deaton et al. (1982) yet it was 3 times higher than the permitted level. Taken together our data support the commonly accepted view that betaine exerts positive effects on the performance mainly in animals exposed to osmotic stress.

Rectal temperature was not influenced by either dietary supplementation of betaine or ambient temperature fluctuation (Figures 3 and 9), though if there was any increase of rectal temperature it would certainly be masked by oviposition and ovulation-induced rise in body temperature (Kadono et al.,1981; Kadono and Yamade, 1985). Egg specific gravity was not influenced by betaine, though it tended to be slightly higher in the group given 0.7 g/kg supplemental betaine at d 3 and 22 when air temperature was high (Figures 3 and 10). Similar trend (P>0.05) was observed in respect to eggshell thickness (Table 3). The registered trend in both indices is consistent with the widely recognized fact that specific gravity is an excellent test of estimating shell thickness (Bennet, 1993). Our data concerning shell quality as assessed by egg specific gravity are not consistent with the reported enhanced eggshell quality in laying hens, given betaine-supplemented diet (Ryu et al., 2002). According to Kidd et al. (1997), the beneficial

Table 3
Effect of betaine on egg performance of laying hens

Items		Groups					
		I group		II group		III group	
		Mean	SEM	Mean	SEM	Mean	SEM
Egg weight, g		56.533	0.838	56.266	0.997	55.266	0.934
Shape index, %		75.705	1.013	76.252	0.655	77.288	0.687
Albumin	Index, %	101.112	3.565	97.037	6.591	96.99	5.452
	Weight, g	32.926	0.788	33.833	1.024	32.02	1.094
Yolk	Index, %	43.135	0.533	41.47	0.801	42.472	0.504
	Weight, g	17.273	0.348	16.566	0.303	16.633	0.386
Shell	Weight, g	6.333	0.26	5.866	0.245	6.6	0.221
	Thickness, mm	0.348	0.008	0.37	0.005	0.368	0.011
Haugh unit, %		88.266	1.193	85.733	2.412	86.666	1.65
Yolk color score		8.066	0.294	8.4	0.316	8.066	0.188

Values are means ± SEM

effect of dietary betaine on animal health and performance depends on the occurrence of osmotic stress and the specific betaine requirement relating to each particular case.

There were no significant differences between the groups with respect to egg quality traits (Table 3), though Haugh unit, which provides satisfactory measurement of albumen condition, tended to be lower in the II group.

Conclusion

Our results suggest that betaine supplementation could be used to increase egg production in laying hens reared under high air ammonia concentration.

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