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Effect of GABA on Hematological, Biochemical, Antioxidant and Immunological Parameters in Laying Hens

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Research Article

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Abstract

The present work investigated the effect of GABA on hematological, biochemical, antioxidant and immunological parameters in laying hens. Sixty Lohmann Brown laying hens (32 weeks old) were randomly allotted to 3 groups (each 20) and given the medications daily for 4 weeks in drinking water. The groups were; control (without drug), GABA (40 mg/kg b.wt) and GABA (80 mg/kg b.wt). Layers were estimated to complete blood picture, Hb, differential leucocytic count, antioxidant parameters, cholesterol, HDL, LDL, cortisol, haptoglobin, IGG, and epinephrine. There was a significant increase in RBCs, Hb, HDL, SOD, GSH, and IGG while a decrease in MDA, cholesterol, and LDL following GABA supplementation. In conclusion, GABA had a beneficial effect on blood picture, differential leucocytic count, antioxidant parameters, IGG, and haptoglobin.

Keywords: Antioxidant; Blood picture; Chickens; GABA; Layers; Immunity

Abbreviations: GABA: Gamma-Aminobutyric Acid; LDL-cholesterol: Low-Density Lipoproteins; GSH: Glutathione Peroxidase; SOD: Superoxide Dismutase; CAT: Catalase; FFA: Free Fatty Acid: TG: Triglycerides.

Introduction

Gamma-aminobutyric acid (GABA) is a kind of inhibitory transmitter compound in vertebrates [1]. The

inhibitory effects of GABA in the endocrine system are consistent with its well-known suppressive actions as a neurotransmitter in the nervous system [2]. Recently, it has been suggested that GABA acts in increasing voluntary feed intake in mammals and avian [3,4] and regulation of endocrine secretion and alleviation of stress in humans [5]. GABA treatments had no significant effect on WBC, RBC, or lymphocytes [6]. An increase in plasma free fatty acid and triglycerides and decreased cholesterol and HDL provided evidence that GABA altered the carbohydrate and lipid metabolism in layers [7]. This might partly explain the improved feed efficiency in layers fed GABA. Heat-stress stimulated corticosterone and catecholamines release and start cell membranes lipid peroxidation, including membranes of T and B lymphocytes, leading to over-production of oxygen free radicals OH and O_2 [8,9]. Furthermore, GABA has widely varying effects on animals and plays a great role in regulating appetite and improving nutrition utilization efficiency. Therefore, this study evaluated the effect of GABA supplementation on hematological, biochemical, antioxidant and immunological parameters in laying hens.

Materials and Methods

Experimental Birds and Design

All birds- based procedures in this study were approved by the Animal Health Research Institute (Tanta lab.). In total, 60 Lohman brown commercial layers (32 to 36 weeks of age), a photoperiod of 16 h light and 8 h dark/day. The diet used in the experiment was a corn-soybean meal, as a basal diet and Feed and water were provided *ad libitum*. Birds were assigned randomly to three treatment groups as follows: (1) control (no GABA), (2) 40 mg GABA/kg b.wt and (3) 80 mg GABA/kg b.wt. The GABA was provided as an oral solution under the tradename of GABA-L®, which was provided by AMECO-BIOS. (USA). Ethical Committee Faculty of Veterinary Medicine, Benha University, approved the study protocol (approval number; 20417).

Blood Collection

Two blood samples from the wing vein of 10 hens in each group were collected at the end of the experiment; 1st one was taken on EDTA tubes for estimation of hematological parameters and the 2nd sample was taken on plain tube without anticoagulant for estimation of serum biochemical parameters.

Determination of Hematological Parameters

Total erythrocytic and the leukocytic count was determined by the method of Natt MP, et al. [10], Hemoglobin concentration was determined using Drabkin's solution according to the method of D Armour, et al. [11], Packed cell volume (PCV) [12]. Blood indices: MCV, MCH, and MCHC were determined according to Jain [13]. Determination of differential leucocytic count according to Schalm, et al. [14].

Serum Biochemical Parameters

Total cholesterol and low-density lipoproteins (LDL-cholesterol) were estimated [15,16], respectively. High-density lipoproteins (HDL-cholesterol) were determined according to Lopes-Virella, et al. [17]. Serum triglycerides were determined according to Fossati & Prencipe [18].

Adrenaline (epinephrine) and noradrenaline (norepinephrine) were determined by ELISA. Cortisone was assayed according to the method described by Mason, et al. [19]. Haptoglobin was determined using kits based on sandwich enzyme-linked immunosorbent assay technology.

Oxidant and Antioxidant Parameters

These include serum Glutathione peroxidase (GSH; Paglia & Valentine [20] superoxide dismutase SOD; Marklund & Marklund [21], malondialdehyde MDA; Okhawa, et al. [22] and catalase CAT; Zamocky & Koller [23]).

Statistical Analysis

Data were subjected to One-way ANOVA using SPSS Statistics 22 statistical package (SPSS Inc., Chicago, IL, USA) and expressed as mean ± SE. Significant differences between groups were determined using Duncan's test at $P \leq 0.05$.

Results

Blood Picture

Significant increase in RBCs, WBCs, Hb, and PCV in laying hens in group 2 when compared to control. While in group 3 there were non-significant increases in RBCs and PC when compared to control (Table 1).

Experimental treatment			
Parameters	Group1 (Control)	Group 2	Group 3
RBCs (×10^6)	1.58±0.10^b	2.00±0.09^a	1.88±0.07^a
WBCs (×10^3)	24.20±2.07^a	28.10±4.11^a	31.20±2.26^a
Hb (mg/dl)	8.35±0.56^b	11.58±1.09^a	10.37±0.86^{ab}
PCV (%)	25.90±0.50^b	29.48±0.79^a	28.90±0.67^a
Haptoglobin (mg/dl)	153±11.58^a	151±9.27^a	128±12.81^a

Table 1: Effect of GABA on some blood picture of laying hens (n=10).
Values are means ± standard error. Means within the same row of different superscript letters are significantly different at (P ≤ 0.05).

Differential Leukocytic Counts and IGG

Table 2 showed the effect of GABA on differential leukocytic counts and serum IGG concentration of laying hens. It has been observed that there was a significant increase in lymphocyte % in laying hens in group 2 when compared to control. While there was a non-significant increase in heterophil, basophil, eosinophil and monocyte between treated groups and control.

Experimental treatment			
Parameters	Group1 (Control)	Group 2	Group 3
Heterophil (%)	52.52±3.06^a	46.47±1.94^a	45.64±1.51^a
Basophil (%)	3.06±0.47^a	2.39±0.23^a	2.40±0.29^a
Eosinophil (%)	3.75±0.71^a	2.60±0.36^a	2.59±0.19^a
Lymphocyte (%)	36.35±2.14^b	44.64±2.07^a	44.81±1.40^a
Monocyte (%)	4.32±0.85^a	3.91±1.33^a	4.56±0.80^a

Table 2: Effect of GABA on differential leukocytic counts and serum IGG concentration of laying hens (n=10).

Blood Serum Lipids Profile

Table 3 showed that the effect of GABA on serum lipids profile in laying hens. It was noted that in treated groups there was a substantial reduction in total cholesterol, LDL and a considerable rise in HDL when compared to control. Also, there was when compared to control. While there was a non-significant decrease in triglycerides, VLDL and CHO/HDL ratio between treated groups and control.

Experimental treatment			
Parameters	Group1 (Control)	Group 2	Group 3
Triglycerides (mg/dL)	202.14±2.77^a	197.10±1.25^a	187.44±8.05^a
Total cholesterol (mg/dl)	210.43±1.74^a	200.43±0.89^b	199.12±0.87^b
HDL (mg/dl)	52.58±0.92^b	55.28±0.22^a	55.18±0.17^a
LDL (mg/dl)	117.37±2.10^a	105.73±0.92^b	106.45±2.27^b
VLDL (mg/dl)	40.43±0.55^a	30.42±0.25^a	37.49±1.61^a
CHO/HDL (%)	4.01±0.10^a	3.63±0.02^a	3.61±0.01^a

Table 3: Effect of GABA on serum lipids profile of laying hens (n=10).
Values are means ± standard error. Means within the same row of different superscript letters are significantly different at (P ≤ 0.05).

Effect on Some Antioxidant Enzyme Activities

Table 4 showed the effect of GABA on some serum antioxidant enzyme activities in laying hens. It has been observed that there was a non-significant increase in CAT, SOD, and GSH of laying hens in treated groups when compared to control and non-significant increase between treated groups.

Experimental treatment			
Parameters	Group1 (Control)	Group 2	Group 3
MDA (µmol/l)	12.01±1.05[a]	11.83±0.88[b]	11.20±0.35[b]
CAT (U/ml)	0.10±0.02[a]	0.18±0.03[a]	0.18±0.02[a]
SOD (U/mgHb)	229.47±28.97[a]	253.72±36.68[a]	287.25±30.18[a]
GSH (mg/dl)	47.95±0.31[a]	51.60±2.14[a]	49.93±0.70[a]

Table 4: Effect of GABA on some serum antioxidant enzyme activities of laying hens (n=10).
Values are means ± standard error. Means within the same row of different superscript letters are significantly different at ($P \leq 0.05$).

Effect on Some Hormones Concentrations

(Table 5) showed that the effect of GABA on some serum hormones concentrations in laying hens. It has been observed that there was a significant decrease in cortisol and norepinephrine of laying hens in treated groups when compared to control. While there was a non-significant decrease in epinephrine and haptoglobin between treated groups. But compared to control, there was a considerable elevation in IGG in treated groups.

Experimental treatment			
Parameters	Group1 (Control)	Group 2	Group 3
Cortisol (ng/ml)	3.66±0.09[a]	3.08±0.12[b]	3.14±0.16[b]
Epinephrine (µg/liter)	89.20±8.92[a]	76.20±0.97[a]	78.40±1.69[a]
Norepinephrine (µg/liter)	163.40±3.93[a]	147.80±3.48[b]	146.20±2.22[b]
Haptoglobin (mg/dl)	153±11.58[a]	151±9.27[a]	128±12.81[a]
IGG (mg/dl)	536±28.39[b]	670±39.62[a]	720±24.49[a]

Table 5: Effect of GABA on some serum hormones concentrations of laying hens (n=10).
Values are means ± standard error. Means within the same row of different superscript letters are significantly different at ($P \leq 0.05$).

Discussion

Gamma-aminobutyric acid (GABA) is a kind of inhibitory transmitter compound in vertebrates [1] and is widely distributed throughout nature. Stress leads to a reduction of defense mechanisms, induced a state of immunosuppression in birds and inhibition of immune function in hens [24,25]. These effects caused suppression of circulating WBCs [26], rises in the heterophil/lymphocyte ratio [27]. Our results showed that there was a significant increase of RBCS, WBCS, lymphocytes, which means that increasing RBCs increases vitality and performance of laying hens. These results were inconsistent with the results recorded by Park & Kim [5] who found that GABA treatments had no significant effect on WBC, RBC, or lymphocytes.

However, increased plasma free fatty acid (FFA) and triglycerides (TG) and decreased cholesterol and HDL provided evidence that GABA altered the carbohydrate and lipid metabolism in layers. This might partly explain the improved feed efficiency in layers fed GABA. The increased plasma FFA and TG revealed that GABA increased fat mobilization [7]. Corticosterone and catecholamines release and lipid peroxidation initiation in cell membranes, including T and B lymphocytes membranes, were stimulated by heat-stress, leading to over-production of oxygen free radicals OH and O_2 [8,9]. The important aspects of radical scavenging system (GSH and SOD) are embedded in the process of anti-oxidation [28,29]. The rise in glutamate level (valuable for the synthesis of GSH) was a result of an increased GABA content increasing the activity of the anti-oxidation enzyme; this might explain the above results. In this study, it was observed that no significant increase in the level of GSH and SOD. The GSH and SOD activities in heat-stressed chickens have been improved by GABA [30]. GABA supplementation to pigeons could enhance GSH and SOD activities [31].

Cell membrane damage is caused by Malondialdehyde (MDA). In this research, reduced MDA with GABA supplementation was recorded, and reduced levels of MDA were always accompanied by elevated antioxidant enzyme activity [28,32]. Our findings were supported by Zhu, et al. [33] who reported an increase in activity of

antioxidant enzymes and decreased MDA level with increasing level of dietary GABA in Hy-Line Brown hens. Thus, the increase of nutrition utilization and antioxidant activities in response to GABA are likely the main cause of the improvements in laying performance and egg quality in layers.

Epinephrine and norepinephrine are the main neurotransmitters of the sympathetic nervous system and cortisol secretion is partly controlled by GABA [34,35]. To evaluate the effect of GABA on the functional the ability of the sympathetic nervous system, epinephrine, norepinephrine, and cortisol concentrations were evaluated in the serum of layers, our results showed that no significant decrease in epinephrine and norepinephrine. Thus, our results indicate that the GABA had no direct effect on the sympathetic nervous system in comparison with the controls.

Haptoglobin is an acute-phase protein that is rapidly increased in the blood by disease-causing agents and many physiological changes and plays an important role in immunity. GABA has positive effects on cellular immune function [36]. The results of our study were similar to the previous report of Zhang, et al. [37], which documented an increase in immunity (serum IgG and IgA) between GABA treatments and control and decreased serum haptoglobin concentrations were reduced in GABA-supplemented birds, compared with the control, although the layers in this study were not subjected to stress. Additionally, in our study, an increase in serum IgG concentrations were observed with GABA supplementation, showing the opposite pattern to serum haptoglobin concentrations which agree with Park & Kim [5] and the probable reason is the inhibition of GABA on somatostatin and adrenal corticosteroid hormone secretion [38], which impairs immunoglobulin production. Thus, it might be expected that GABA may have modulatory effects on humoral immune responses by activating IgG and decreasing haptoglobin.

High temperature can reduce the immune function and Ig concentration in laying hen; the reason was probably that high temperature can produce a sustained high level of corticosterone, which induces long lysis reaction in cells, resulting in reduced Ig synthesis [39]. Consistently, our findings showed considerable improvement in the plasma concentrations of IgG and IgM in layers supplemented with 40 mg/kg GABA. Improving in body's immune function by GABA probably because of that as a neurotransmitter or local hormone, GABA can control the body's endocrine activity, and alter the GABA concentration in the body, irrespective of where it is located, will influence the hormone secretion of the endocrine system [40]. In conclusion, GABA had a beneficial effect on blood picture, differential leucocytic count, antioxidant parameters, IGG, and haptoglobin.

References

1. Roberts E, Frankel S (1950) Aminobutyric acid in brain and its formation from glutamic acid. Journal of Biological Chemistry 187: 55-63.

2. Kuffler SW, Edwards C (1958) Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. J Neurophysiol 21(6): 589-610.

3. Jonaidi H, Babapour V, Denbow DM (2002) GABAergic control of food intake in the meat-type chickens. Physiology and Behaviour 76: 465-468.

4. Tajalli S, Jonaidi H, Abbasnejad M, Denbow DM (2006) Interaction between nociceptin/orphanin FQ (N/OFQ) and GABA in response to feeding. Physiology and Behaviour 89(3): 410-413.

5. Okada T, Sugishita T, Murakami T, Murai H, Saikusa T, et al. (2000) Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. Nippon Shokuhin Kagaku KogakuKaishi 47(8): 596-603.

6. Park JH, Kim IH (2015) Effects of dietary gamma-aminobutyric acid on egg production, egg quality, and blood profiles in layer hens. Veterinární medicína 60(11): 629-634.

7. Dai SF, Gao F, Zhang WH, Song SX, Xu XL, et al. (2011) Effects of dietary glutamine and gamma-amino butyric acid on performance, carcass characteristics and serum parameters in broilers under circular heat stress. Animal Feed Science and Technology 168(1-2): 51-60.

8. Freeman BA, Crapo JD (1982) Biology of disease: Free radicals and tissue injury. Laboratory Investigation 47(5): 412-426.

9. Slater TF (1984) Free-radical mechanisms in tissue injury. Biochemical Journal 222(1): 1-15.

10. Natt MP, Herrick CA (1952) A New Blood Diluent for Counting the Erythrocytes and Leucocytes of the Chicken. Poultry Science 31(4): 735-738.

11. Armour FE, Blood FR, Belden DA (1965) The manual for laboratory work in mammalian physiology. 3rd (Edn.), Illinois Chicago. University of Chicago Press, pp: 4-6.

12. Coles HE (1986) Veterinary Clinical Pathology. WB Saunders Company Philadelphia; 4th (Edn.), pp: 17-19.

13. Jain CN, OW Schalm (1986) Schalm's veterinary haematology, 4th (Edn.), Lea and Febiger, Philadelphia, pp: 42.

14. Schalm OW, Jain NC, Carroll EJ (1975) Veterinary Hematology 3rd (Edn.), Lea and Febiger, Philadelphia USA.

15. Richmond W (1973) Preparation and properties of a bacterial cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clinical Chemistry 19: 1350.

16. Wieland H, Seidel D (1983) A simple specific method for precipitation of low-density lipoproteins. Journal of Lipid Research 24: 904.

17. Lopes Virella MF, Stone P, Ellis S, Colwell JA (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. Clinical Chemistry 23(5): 882-884.

18. Fossati P, Prencipe L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry 28(10): 2077-2080.

19. Mason HL, Myers SC, Kendall EC. (1936) Chemical Studies of the Suprarenal Cortex: II. The Identification of a Substance which Possess the Qualitative Action of Cortin; Its Conversion into a Diketone Closely Related to Androstenedione. Journal of Biological Chemistry 116: 267-276.

20. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine 70(1): 158-169.

21. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47: 469-474.

22. Okhawa H, Ohisi N, Yagi K (1979) Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95(2): 351-358.

23. Zamocky M, Koller F (1999) Understanding the function of catalases: Clues from molecular evolution and *in vitro* mutagenesis. Progress in Biophysics and Molecular Biology 72(1): 19-66.

24. Thaxton P, Siegel HS (1970) Immunodepression in young chickens by high environmental temperature. Poultry Science 49: 202-205.

25. Mashaly MM, Hendricks GL, Kalama MA, Gehad AE, Abbas AO, et al. (2004) Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Science 83: 889-894.

26. Nathan DB, Heller ED, Perek M (1976) The effect of short heat stress upon leucocyte count, plasma corticosterone level, plasma and leukocyte ascorbic acid content. British Poultry Science 17(5): 481-485.

27. Mogenet LY, Youbicier Simo BJ (1998) Determination of reliable biochemical parameters of heat stress, and application to the evaluation of medications: example of erythromycin E. Proceedings of 10th European Poultry Conference, Jerusalem, Israel, pp: 538-541.

28. Attia YA, Hassan RA, Qota EMA (2009) Recovery from adverse effects of heat stress on slow-growing chicks in the tropics 1: Effect of ascorbic acid and different levels of betaine. Tropical Animal Health and Production 41(5): 807-818.

29. Wei XJ, Wu J, Ni YD, Lu LZ, Zhao RQ (2011) Antioxidant effect of a phytoestrogen equol on cultured muscle cells of embryonic broilers. In Vitro Cellular and Developmental Biology-Animal 47(10): 735-741.

30. Chen Z, Tang J, Sun YQ, Xie J (2013) Protective effect of γ-aminobutyric acid on antioxidation function in intestinal mucosa of Wenchang chicken induced by heat stress. Journal of Animal and Plant Sciences 23(6): 1634-1641.

31. Huang HL, Zhao WJ, Zou XT, Li H, Zhang M, et al. (2011) Effect of γ-aminobutyric acid on incubation, immunity and antioxidant activity in pigeon. Chinese Journal of Veterinary Science 9: 1327-1331.

32. Williams AJ, Bautista CC, Chen RW, Dave JR, Lu X, et al. (2006) Evaluation of gabapentin and ethosuximide for treatment of acute nonconvulsive seizures following ischemic brain injury in rats. Journal of Pharmacology and Experimental Therapeutics 318(3): 947-955.

33. Zhu YZ, Cheng JL, Ren M, Yin L, Piao XS (2015) Effect of γ-aminobutyric acid-producing lactobacillus strain on laying performance, egg quality and serum enzyme activity in Hy-line Brown hens under heat stress. Asian-Austalian Journal of Animal Science 28: 1006-1013.

34. Rosmond R, Bouchard C, Bjorntorp P (2002) Allelic variants in the GABAA a6 receptor subunit gene (GABRA6) is associated with abdominal obesity and cortisol secretion. International journal of obesity and related metabolic disorders 26: 938-941.

35. McCorry LK (2007) Physiology of the autonomic nervous system. American Journal of Pharmaceutical Education 71(4): 78.

36. Jin Z, Mendu SK, Birnir B (2013) GABA is an effective immunomodulatory molecule. Amino Acids 45(1): 87-94.

37. Zhang M, Zou XT, Li H, Dong XY, Zhao W (2012) Effect of dietary γ-aminobutyric acid on laying performance, egg quality, immune activity and endocrine hormone in heat-stressed Roman hens. Animal Science Journal 83: 141-147.

38. Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, et al. (2016) Regulation of the hypothalamic-pituitary-adrenocortical stress response. Comprehensive Physiology 6(2): 603-621.

39. Habibian M, Ghazi S, Moeini MM, Abdolmohammadi A (2014) Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. International Journal of Biometeorology 58: 741-752.

40. Rivest S (2010) Interactions between the immune and neuroendocrine systems. Progress in Brain Research 181: 43-53.