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RESEARCH ARTICLE

EFFECT OF DRINKING WATER SUPPLEMENTED WITH GAMMA-AMINOBUTYRIC ACID (GABA) ON LAYING PERFORMANCE AND EGG QUALITY PARAMETERS IN LAYING HENS

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Abstract

The laying performance and egg quality parameters were studied after Gamma-aminobutyric acid (GABA) supplementation in laying hens. One hundred and fifty Lohmann Brown laying hens (32 weeks old) were randomly allotted to 3 treatments: control (without drug), GABA (40 mg/kg b.wt) and GABA (80 mg/kg b.wt). Each group consisted of 5 replicates of 10 hens and given the medication orally, daily for 4 weeks. The laying performance parameters (egg production, egg weight, egg mass, and feed conversion ratio) were improved after oral administration of GABA (80 mg/kg b.wt) compared with control. Higher egg shape index, shell weight, and shell thickness were recorded in the GABA (80 mg/kg b.wt) supplemented group compared with control. The egg yolk quality parameters (yolk weight, yolk index, and yolk color) and albumin quality parameters (albumin weight, albumin index and albumin haugh unit) were improved by using 80 mg GABA/kg b.wt. The egg yolk cholesterol and triglycerides concentrations were decreased in both GABA treated groups when compared with control. In conclusion, the best laying performance and egg quality parameters were obtained by oral medication of a higher dose of GABA in drinking water of layers. The two doses of GABA induced low-cholesterol and triglycerides concentrations in egg yolk and this experiment showed that GABA is a valuable supplementation for layers in drinking water.

Introduction:-

GABA is an inhibitory neurotransmitter not only in the CNS but also in the peripheral nervous system which acting through the GABA receptors and acts also as a nutritive factor (Watanabe et al., 2002; Elkomy et al., 2019). Injecting GABA into different regions of the brain can promote animal's feed intake in a dose-dependent manner (Pu et al., 1999). An important role of GABA in central thermoregulation in mammals as a result of its higher concentrations in various hypothalamic nuclei (Jha et al., 2001; Frosini et al., 2002). GABA exhibits various nutritional and pharmacological functions, such as the promotion of the absorption of metal ions, and immunomodulatory effects (Jin et al. 2013). It exerted stress-relaxation functions, helping to prevent heat stress-related symptoms in growth performance in broilers (Dai et al., 2011).

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Heat stress in laying hens (Zhang et al., 2011) and broilers (Dai et al., 2011; Wang et al., 2011) which leads to depression in the performance could be prevented by GABA. Furthermore, it has been reported that GABA had widely varying effects on animals, and played a great role in alleviating stress, improving nutrition utilization efficiency and regulating appetite (Cherubini et al., 1991). No available information on the effect of GABA supplemented as oral medication, thus the objective of our study was to investigate the effects of oral GABA supplementation on laying performance, external egg quality, and egg yolk and albumin quality in layers.

Materials and Methods:-

Experimental birds and design:

One hundred and fifty commercial Lohmann Brown Lite laying hens at 32 weeks old were divided to 3 equal groups (each group with 5 replicate and 10 hens per replicate); control (without drug), GABA (40 mg/kg b.wt) and GABA (80 mg/kg b.wt), the chosen doses were according to Xie et al., (2013). The medication was given once daily for 4 weeks. All groups were given a corn-soybean-basal diet without any additives. The chemical composition and ingredients in the diet were illustrated in Table 1. Diet formulations were according to the breeder recommendations (Lohmann Brown Lite layers, Germany). Body weights of group 1 were (1500-1700 gm); Group 2 (1550-1750 gm) and group 3 (1600-1800 gm) at the time of experiment.

During the experiment, water was provided ad libitum. The experiment was carried out between 32 and 36 weeks of age (4 weeks experimental period). Hens were housed in battery cages supplied with feeders and nipple drinkers. Continuous light per day (16 h) from 6 AM to 10 PM. The temperature was 29ºc and relative humidity 72%. Laying hens were fed 115 gm per hen per day were according to the breeder recommendations (Lohmann Brown Lite layers, Germany). The GABA was provided by AMECO-BIOS (USA) under a trading name (GABA-L)® as water-soluble solution and each ml contains 100 mg GABA. Ethics Committee of the Faculty of Veterinary Medicine, Benha University, approved the use of laying hens and study protocols.

Collection and egg quality parameters analyses:

All hens weighed individually at the 32nd and 36th week of age. Hen per day egg production (%) was measured. In 7-d intervals, feed consumption and feed conversion ratio were recorded (Singh and Kumar, 1994). By multiplying egg weight by egg production, egg mass was obtained.

Internal and external egg quality parameters were studied after GABA supplying. Albumen and yolk weights, albumin and yolk indexes, and haugh unit were measured as internal egg quality parameters. Egg shape, egg yolk, and albumen indexes were recorded (Romanoff and Romanoff, 1949). Yolk index (%) = [Yolk height (cm)/ Yolk diameter(cm)] x 100 & Albumen index (%) = Albumen height (mm)/ Average of albumen length and width) mm x100. Egg yolk color was determined and a tripod micrometer was used for the estimation of haugh units.

Egg shell thickness was determined by using a special micrometer and albumen height and yolk color were determined using an egg multi tester (Touhoku Rhythm Co., Tokyo, Japan). Egg quality determinator (NABEL Co., Ltd, Kyoto, Japan) was used for the determination of shell weight.

The cholesterol and triglycerides concentrations (mg /g) in the egg yolk were measured by an ultraviolet spectrophotometer (Unico Co., Dayton, USA) using commercial kits (Biodiagnostic, Egypt) (Rotenberg and Christensen ,1976).

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:-

From the results in Table 2, layers orally administered GABA (80 mg/kg b.wt) during weeks 32 to 36 showed significant increases in egg production, egg mass, heavier egg weight, and better feed conversion ratio compared with the control treatment. However, there was no significant difference between the two groups administered GABA.

The external egg quality parameters (egg shape index, shell weight, and shell thickness) were significantly increased by supplementation of the drinking water by GABA (80 mg/kg b.wt) as shown in table (3). The effect on egg yolk quality was presented in table (4); a significant increase in yolk weight, yolk color score, and higher yolk shape index were recorded in laying hens supplemented with oral GABA (80 mg/kg b.wt) compared with the control group. Also, egg yolk cholesterol and triglycerides were decreased in both GABA (40 mg/kg b.wt) and GABA (80 mg/kg b.wt). As shown in Table 5, the effects on albumen quality (albumen weight, albumin %, and albumen haugh unit) were improved by the addition of GABA (80 mg/kg b.wt) to drinking water.

Discussion:-

Eggs are the second widely consumed food all over the world (Surai and Sparks, 2001) and the economic income in the poultry industry was affected by egg quality (Hussein et al., 2018). Chicken's eggs contain minimum fat with high-quality protein.

Overall, production, egg weight, egg mass and, FCR were increased with the high dose of orally administered GABA in drinking water of laying hens. The increased laying performance evoked by GABA supplementation in this study might relate to neuroendocrine system-mediated effects on nutrient metabolism (Zhang et al., 2012). It was recorded that, the addition of GABA in the layer diet not only alleviated stress in hens but also played a major role in the improvement of nutrient utilization, appetite regulation and increasing the utilization of dietary calcium and phosphorus, resulting in improving egg laying performance and quality (Zhu et al., 2015). Dietary GABA could increase lipase; amylase and trypsin activities in the gastrointestinal tract, increasing egg production and average egg weight (Zhang et al., 2012). The improved feed intake, electrolyte balance, immune function and antioxidation enzyme following GABA supplementation resulted in the improvement of laying performance and egg quality (Zhu et al., 2015). Also, GABA resulted in increases in egg production, egg weight, and egg quality (Park and Kim, 2015**)**. Moreover, GABA possesses many anti-oxidant activities, which may result in improved performance (Chen et al. 2013).

The basic composition of egg includes protein and yolk. Following GABA administration at a dose of 80 mg/kg b.wt, egg quality parameters were significantly increased. Similar results were recorded by Zhang et al., (2012) who mentioned an increase in yolk color, yolk weight and haugh unit in hens by dietary GABA supplementation. Moreover, improvement in egg quality index, shell thickness and weight by dietary GABA were reported (Zhu et al., 2015). GABA increased the absorption and utilization of dietary minerals (calcium and phosphorus) which considered important components of eggshells and improved eggshell characters as GABA modulates the electrolyte balance (Zhang et al., 2012). Electrolytes (calcium, phosphorus, potassium, and sodium) involved in the synthesis of eggshells and play an important role in modulating serum electrolyte balance (Viveros et al., 2002). GABA helps the transportation and absorption of the mineral elements, especially calcium and phosphorus (Zhu et al., 2015).

GABA had a direct beneficial effect on laying performance and egg quality, which manifested as a significant effect on egg production, egg mass, eggshell thickness, and egg weight and albumin height. 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 (Park and Kim (2015). The diet supplemented with GABA improved the laying performance, egg quality and physical condition of hens, which had a significant effect on egg production, average egg weight, egg strength, hen immune activity, anti-oxidation activity and hormone levels (Zhang et al., 2012). GABA improved egg production and egg quality by increasing the total protein (TP) concentration (protein is the basic composition of the egg) and modulating the electrolyte balance (Calcium and phosphorus are very important for egg quality) (Zhang et al., 2012).

Cholesterol, triglycerides, phospholipids, lipoproteins are most of the lipids in eggs, which are concentrated in the yolk (Hussein et al., 2018). The cholesterol and triglycerides content of egg were reduced by the supplementation of GABA.

Environmental condition, management, nutrition, type and dosage of additive as well as birds' related factors (age, species and production stage) may be partly responsible for these contradictory results (Torki et al., 2018).

Table 1:- Ingredients and nutrients composition of the basal diet of laying hens.

Ingredient %	Treatments	
	1st+2nd week	3rd +4th week
Corn, grain	62.5 kg	63 kg
Soybean meal 48%	22 kg	22 kg
Corn gluten meal 60%	4 kg	4 kg
Soya oil	900 gm	500 gm
Dicalcium phosphate	1.6 kg	1.6 kg
Limestone	8 kg	8 kg
Salt	200 gm	250 gm
Methionine	100 gm	150 gm
Choline	100 gm	100 gm
Premix	300 gm	300 gm
$NaHCO_3$	300 gm	100 gm
Chemical composition		
Kcal ME /kg diet	2910.9	2926
Crude protein %	15.34	15.34
Lysine %	0.69	0.69
Methionine %	0.32	0.32
Crude fat %	3.28	3.2
Linoleic acid %	1.82	1.66
EPA, DHA %	-	0.25
Calcium %	3.3	3.3
Available phosphorus %	0.26	0.26
Sodium %	0.15	0.15
Chloride %	0.27	0.27
Potassium %	0.53	0.53

Table (2):- Effect of GABA on laying performance in laying hens during the experimental period.
Values are means ± SE. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Parameters	Experimental groups			
	Weeks	Group (1)	Group (2)	Group (3)
Egg Production (%)	1st wk	57.00±1.06^{a}	67.85±3.71^{a}	77.28±3.09^{a}
	2nd wk	55.85±0.40^{ab}	75.28±3.85^{b}	84.14±1.33^{a}
	3rd wk	59.42±0.71^{ab}	78.42±2.21^{b}	89.85±1.84^{a}
	4th wk	63.00±1.11^{c}	80.14±2.42^{b}	92.01±1.46^{a}
Egg weight (g)	1st wk	54.42±0.57^{a}	57.00±1.06^{a}	58.14±1.99^{a}
	2nd wk	55.14±0.50^{b}	55.85±0.40^{ab}	57.14±0.50^{a}
	3rd wk	57.28±0.42^{b}	59.42±0.71^{ab}	62.28±1.71^{a}
	4th wk	59.28±0.47^{b}	63.00±1.11^{ab}	65.14±2.97^{a}
Egg mass (g/ hen/ d)	1st wk	36.84±1.80^{b}	41.10±0.42^{a}	42.62±0.48^{a}
	2nd wk	41.56±2.33^{b}	47.07±1.35^{a}	50.97±0.79^{a}
	3rd wk	45.04±1.59^{b}	48.08±2.88^{ab}	53.73±1.16^{a}
	4th wk	49.80±1.65^{b}	51.98±0.68^{b}	58.84±0.79^{a}
FCR	1st wk	2.10±0.11^{a}	2.02±0.29^{a}	1.90±0.18^{a}
	2nd wk	2.44±0.13^{a}	2.07±0.06^{a}	2.04±0.18^{a}
	3rd wk	2.40±0.07^{a}	2.16±0.07^{b}	1.90±0.04^{c}
	4th wk	2.31±0.08^{a}	2.12±0.07^{ab}	2.00±0.08^{b}

Table (3): Effect of GABA supplementation on external egg quality parameters in laying hens during the experimental period.
Values are means ± SE. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Parameters	Experimental groups			
	Weeks	**Group (1)**	**Group (2)**	**Group (3)**
Egg shape index (%)	1st wk	0.76±0.007[a]	0.77±0.004[a]	0.78±0.016[a]
	2nd wk	0.75±0.010[b]	0.77±0.007[a]	0.79±0.015[a]
	3rd wk	0.75±0.010[b]	0.77±0.008[b]	0.78±0.005[a]
	4th wk	0.76±0.021[b]	0.78±0.005[ab]	0.80±0.004[a]
Shell weight (gm)	1st wk	6.89±0.35[a]	7.11±0.10[a]	6.93±0.017[a]
	2nd wk	7.12±0.19[b]	7.25±0.14[b]	7.80±0.16[a]
	3rd wk	8.01±0.13[b]	8.19±0.20[b]	8.65±0.09[a]
	4th wk	7.81±0.29[b]	7.99±0.14[b]	8.76±0.08[a]
Shell thickness (mm)	1st wk	0.42±0.011[a]	0.43±0.009[a]	0.45±0.019[a]
	2nd wk	0.40±0.003[b]	0.43±0.017[ab]	0.45±0.009[a]
	3rd wk	0.40±0.006[b]	0.44±0.014[a]	0.45±0.010[a]
	4th wk	0.46±0.011[b]	0.48±0.013[ab]	0.51±0.020[a]

Table (4):- Effect of GABA supplementation on egg yolk quality in laying hens during the experimental period.

Parameters	Experimental groups			
	Weeks	**Group (1)**	**Group (2)**	**Group (3)**
Yolk weight (gm)	1st wk	18.78±1.43[a]	20.07±0.60[a]	19.84±0.45[a]
	2nd wk	19.27±0.95[b]	20.37±1.22[b]	26.14±1.44[a]
	3rd wk	21.33±0.43[b]	22.45±0.10[ab]	23.89±0.84[a]
	4th wk	22.21±0.57[b]	24.01±0.44[a]	26.36±0.66[a]
Yolk index%	1st wk	33.93±2.98[a]	35.11±1.60[a]	38.01±0.76[a]
	2nd wk	35.91±2.13[b]	39.80±2.86[ab]	45.33±1.84[a]
	3rd wk	39.66±4.59[b]	41.42±3.89[ab]	51.18±1.37[a]
	4th wk	40.30±3.25[b]	44.38±3.34[ab]	49.29±0.82[a]
Yolk color	1st wk	9.42±0.42[a]	9.85±0.34[a]	10.28±0.81[a]
	2nd wk	9.00±0.48[b]	9.57±0.52[b]	11.00±0.37[a]
	3rd wk	9.85±0.34[b]	10.14±0.34[ab]	11.14±0.35[a]
	4th wk	8.42±0.20[b]	9.14±0.51[ab]	9.71±0.42[a]
Yolk cholesterol (mg/whole yolk)	1st wk	249.41±19.22[a]	205.57±10.60[b]	168.21±4.28[b]
	2nd wk	247.69±9.61[a]	206.31±5.30[b]	164.05±2.10[c]
	3rd wk	140.28±3.74[a]	123.44±5.41[b]	115.48±0.68[b]
	4th wk	138.85±2.35[a]	120.58±2.71[b]	114.05±1.57[b]
Triglycerides (mg/whole yolk)	1st wk	233.85±15.03[a]	191.68±9.17[b]	153.00±4.19[c]
	2nd wk	230.99±6.45[a]	190.25±5.58[b]	151.57±2.18[c]
	3rd wk	127.43±3.75[a]	109±4.57[b]	105.84±1.46[b]
	4th wk	125.36±2.32[a]	110.36±1.97[b]	106.55±0.71[b]

Values are means ± SE. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Table (5): Effect of GABA on albumen quality of laying hens during the experimental period.

Parameters	Experimental groups			
	Weeks	Group (1)	Group (2)	Group (3)
Albumen weight (gm)	1st wk	29.59±2.22[a]	30.86±0.94[a]	32.65±1.36[a]
	2nd wk	36.79±1.58[b]	38.66±1.05[ab]	41.50±1.31[a]
	3rd wk	38.24±0.67[b]	41.92±2.26[ab]	44.14±1.93[a]
	4th wk	38.43±1.63[b]	38.78±1.39[b]	43.82±1.05[a]
Albumen %	1st wk	52.88±3.12[a]	54.28±0.52[a]	58.03±1.11[a]
	2nd wk	54.04±3.25[b]	55.76±3.10[b]	64.25±1.62[a]
	3rd wk	60.87±0.61[b]	61.51±1.28[b]	66.95±1.29[a]
	4th wk	61.11±2.12[b]	62.36±2.73[b]	70.65±1.73[a]
Albumen haugh unit	1st wk	106.12±1.09[b]	107.86±1.31[ab]	115.15±1.52[a]
	2nd wk	109.67±0.97[b]	110.75±2.07[b]	117.35±1.67[a]
	3rd wk	110.99±1.63[b]	113.34±2.69[b]	120.58±2.15[a]
	4th wk	113.88±2.33[b]	115.51±3.74[b]	124.62±2.70[a]

Values are means ± SE. Means within the same row of different letters are significantly different at ($P \leq 0.05$).

Conclusion:-

This study showed that GABA is a valuable supplementation for layers, and in particular, exerts beneficial effects concerning laying performance, egg quality, as well as a lowering of egg yolk cholesterol and triglycerides concentrations as demanded by health-conscious consumers. However, the action of oral GABA in laying hens still needs additional investigation to determine the effective treatment duration, perfect dosage, and mode of action.

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