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Effect of Gingko Biloba, Dry Peppermint and Vitamin C as Anti-Stress on Broiler Welfare During Summer Heat Stress

[1]K.G. El Iraqi, [1]E.M. Abdelgawad, [2]H.M. Ibrahim and [3]A.E. El Sawe

[1]Department of Veterinary Hygiene and Management,
Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt
[3]Animal Health Research Institute, Doki, Giza, Egypt

Abstract: This study was conducted to evaluate the effects of anti-stress herbs(GingkoBiloba and Dry Peppermint) and vitamin C as feed additives, on behavioral parameters, productive performance, Histopathology and Hematology of broiler chickens during the summer heat stress. A total of 300 one day-old chicks of Cobb species were the subject of this study, chicks were divided into 5dietary treatments, each consisting of 2 replication (n=10). First treatment (Control group) the chicks were fed on commercial basal diet, second group (T1), fed on the same diet enriched with 0.2 %dry peppermint,third group (T2) fed on the same diet enriched with 0.06 % Gingko biloba (GB), fourth group (T3) fed on the same diet enriched with 0.2 % dry peppermint plus 0.06% Gingko biloba (GB) and fifth group (T4) fed on the same diet supplemented with 0.05% Vitamin C. During 5 weeks experimental period, the behavioural measurements as frequency of feeding, drinking, comfort behaviour; including leg and wing stretch, preening, ground scratch, body shaking and resting behaviour were observed and recorded. Broiler performance including feed intake and body weight gain, final body weight, feed conversion ratio, dressing percentage, mortality rate,internal organs weight (gizzard, liver, heart, spleen and bursa). Hematological and Immunity parameters including antibodies titer for infectious bronchitis, Newcastle and Infectious Bursal were measured also Total erythrocyte count, Total leucocyte count, lymphocyte percentage, Hemoglobin concentration and packed cell volume.Histopathology section for bursa, spleen small and large intestine were examined. Significant differences were observed between different herbal treatments and vitamin C in ingestive behavior, comfort behaviour, feed intake, final body weight, food conversion ratio, dressing weight, dressing percentage, mortality rate, Internal organs weight and immunity. It can be concluded that Broiler welfare, productive performance and immune response of broilers against Newcastle disease, Infectious Bursal disease and Infectious Bronchitis improved through supplying broiler with Anti-stress plants like dry peppermint, Gingko Biloba as management practices during summer.

Key words: Broiler · Heat Stress · Herbal · Behavior

INTRODUCTION

Heat stress is a common problem world wide in poultry production. High ambient temperatures coupled with high humidity can be devastating to commercial broilers and layers [1].

The expression of heat stress in poultry production can be described as acute or chronic; acute heat stress refers to short and sudden periods of extremely high temperature, whereas chronic heat stress refers to extended periods of elevated temperature [2].

Chronic heat stress has detrimental effects on the performance of broiler birds reared in the open sided poultry houses; principally through reducing feed intake, growth rate, negatively affect feed efficiency and carcass quality as well as health. In addition, prolonged periods of elevated ambient temperature increase the time to reach market weight and increase mortality [3-7].

Corresponding Author: Kassem G. El Iraqi, Department of Veterinary Hygiene and Management,
Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
Fax: +202 35730865, E-mail: kassemgabr@gmail.com.

The effects of heat stress on avian species were reported as: decreases in the feed consumption, body weight gain, as well as the total white blood cell count and antibody production [2, 8]. Decreases in the number of peripheral blood lymphocytes and induction of an electrolyte imbalance [3,9], decreases in the blood lymphocytes and spleen weight [4, 10] decreases in in macrophage activity [5, 11] and disseases in the intestinal villi heights, [12].

Herbs or products containing plant extracts are among the alternative growth promoters and anti-stress that are already being used in practice [13-16].

The present study was conducted to describe the effects of dietary supplementation of Gingko Biloba, Dry peppermint and vitamin C as anti-stress agents on behaviour, growth performance, immunological response and gastrointestinal tract characteristics in broiler chickens.

MATERIALS AND METHODS

Birds and Housing: A total of 300 one day-old Cobb chicks were used in this study. All chicks were housed in a broiler house belonging to Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. On arrival, chicks were randomly housed in experimental pens (1 m x 2 m x 3 m). With stocking density 15/ m^2. Continuous lighting was provided throughout the experiment; the ambient temperature during brooding was 35°C at one day old and then left on the environmental temperature range 32°C – 40°C and relative humidity range 65 % - 75 %. Feed and water were provided adlebtum via trough feeders and bell drinker. The birds were vaccinated against Newcastle disease and infectious bronchitis on day 6 of age and against Gumboro on day 12 of age.

Experimental Design: The study was conducted on one control group and 4 treatment groups, 2 replicates for each of them. The replicate was 30 chicks so each treatment consists of 60 chicks. All diets used were in mash form and formulated to meet the nutrient requirement of the broiler chickens during a five weeks experimental period (35 days) according to recommendations of the national research council [17]. Table 1 presents the groups treatments and table 2 indicates the composition and nutritive value of the basal diet used in the experiment.

Table 1: The experimental groups treated with Different anti-stress agents as feed additives

Groups	Treatment
Group one (control)	Chicks fed on Basal diet without any additives
Group two (treated)	Chicks fed on Basal diet enriched with 0.2% dry peppermint powder according toOcak *et al*. [18]
Group three (treated)	Chicks fed on Basal diet enriched with 0.06 %Gingko biloba powder Agricultural Science [19].
Group four (treated)	Chicks fed on Basal diet enriched with0.2 % dry peppermint powder and 0.06 % Gingko biloba powder.
Group five (treated)	Chicks fed on Basal diet enriched with Vitamin C 0.05 %

Table 2: Composition and nutritive value of starter and grower diet used

	Components	Starter Kg/100 kg	Grower Kg/100 kg
Ingredients use in the diet	Yellow Corn	60.28	64.3
	Soya bean meal (47%)	34.25	29.28
	Safflower oil	1.51	2.49
	Na bicarbonate	0.18	0.07
	Sodium chloride	0.33	0.33
	DL-Methionine	0.16	0.15
	Lysine	0.18	0.21
	Di calcium phosphate	1.49	1.52
	Lime stone	1.33	1.34
	Premix	0.3	0.3
Total	----	100	100
Chemical analysis of diet	Metabolisable Energy (Kcal/kg)	2988	3083
	Crude Protein (%)	21	19
	Crude Fat (%)	4.83	5.79
	Fiber (%)	3.7	3.42
	Calcium (%)	0.9	0.9
	Phosphorus (total) (%)	0.73	0.7
	P. Available (%)	0.40	0.4

Measurements: Behavioral measurements, the following behavioral parameters were observed and measured throughout the experiment; ingestive behaviour (feeding and drinking); comfort behaviour (wing and leg stretch, preening, Leg scratch and Body shaking. All behavioral measurements were measured according toAlt mann [20] through; Daily Scan sampling for 2 times daily /group, 10 minutes / for each time; so the total scan observation period was 20 minutes for each group/day.

Productive Performance: Body weight and body gain (gram/bird): At 5-weeks of age the average body weight gain was calculated by subtracting the initial weight at the beginning of the experiment from the final weight at the end of the experiment

Feed Intake (gram/bird): Feed intake is calculated for each treatment. At the end of the experiment, the residual amount of feed was weighed and subtracted from the known weight of feed at the beginning of experiment.

Feed Conversion Ratio (gram feed/gram gain): Feed conversion ratio is calculated at the end of experiment as the amount of feed consumed per unit of body gain.

Mortality Rate (%): Accumulated mortality rate is calculated by subtracting the number of live birds at the end of the experiment from the total number of birds at the beginning of the experiment and the product is multiplied by 100 toob tain the percentage of mortality rate.

Internal Organsweight: From each group 10 birds were picked for eviscerating to calculate the Heart, spleen, Bursa, Liver and Gizzard weight.

Hematological and Immunological Parameters: After the end of the experimental period two birds from each replicate(four for each group) were slaughtered and blood samples were collected in test tubes with an anticoagulant to determine the Total Erythrocyte count (RBC), Total leucocyte count (WBC) Hemoglobin concentration (Hb), PCV and Humeral immune response against Newcastle disease, infectious bronchitis and infectious Bursal disease vaccines was measured using HI test (Haemagglutination inhibition) and ELISA.

Histopathology Changes: Every 10 days, 4 birds scarified from each group, for histopathological tissue samples of duodenum, jejunum and ileum were obtained. That issues were then fixed in 10% neutral buffered formalin. Following histological fixation, the tissues were processed through a standard alcohol dehydration-xylen sequence and embedded in paraffin. Transversal serial sections (5 µm) were cut at 50 µm intervals from the tissues according to Samanya and Yamauchi, Trarachai and Yamauchi [21, 22]. The sections were stained by the Crossman's stain for general histopathological examination according to Baum *et al*. [23].

Statistical Analysis: The data were subjected to statistical analysis using SPSS program (statistical Package for Social Science) for windows 17 (SPSS 17.1 2005), according to Dytham [24]. Descriptive statistics were used for the analysis of the data result as follow, means and stander error. The analytical tests which used to compare between the different groups where T test, One way ANOVA, Post Hoc Tests and Kruskal-Wallis Test. Statistical significant level was at $P \leq 0.05$.

RESULTS AND DISCUSSION

Behavioral Measurements: During thermal stress, birds alter their behaviour to help maintain body temperature within the normal limits. As shown in Table 3 the behavioral parameters observed and measured throughout the experiment; were ingestive behaviour including (feeding and, drinking); comfort behaviour including (wing and leg stretch, Leg scratch and preening).

As shown in Table 3, the results indicated that feeding behaviour was affected by the addition of anti-stress feed additives the higher feeding rate 4.79±0.454 birds/minute and drinking rate 2.24±0.314 birds/minute were recorded in T4 group that feed on basal diet enriched with both dry Peppermint 0.2% and Gingko Biloba 0.06 %. Mashaly *et al*. [8] mentioned that chickens during exposure to high temperature, consume less feed and more water, but using combination of dry peppermint and Gingko Biloba have synergistic effect on both feeding and drinking behaviour [26]that both raw plant materials may contain mixtures of organic chemicals, which may include fatty acids, sterols, flavonoids, alkaloids and glycosides of different pharmacological activities, including anti-inflammatory, vasodilator, antimicrobial, anticonvulsant, sedative and antipyretic effects so enhancing feed intake and increase in water consumption to meets the immediate demands of evaporative cooling from respiratory surfaces during summer stress.

Table 3: Effect of Dry Peppermint, Gingko Bilob aand Vitamin C on Broiler Behaviour

	Control Mean±SE	T1 Mean±SE	T2 Mean±SE	T3 Mean±SE	T4 Mean±SE
Feeding behaviour	4.715±0.602	4.755±0.346	4.62±0.326	3.41±0.4*	4.79±0.454*
Drinking behaviour	1.83±0.270	1.96±0.158	1.99±0.174	1.875±0.184	2.24±0.314*
Leg and wing stretch	0.72±0.11	0.99±0.136	1.165±0.131*	0.920±0.136	1.185±0.196*
Leg scratch	0.27±0.035*	0.44±0.033*	0.37±0.035	0.39±0.035	0.36±0.03
Preening	1.8±0.3*	1.9±0.3	2±0.3	2.2±0.3	3±0.3*

T1: means group treated with dry peppermint

T2: means group treated with dry Gingko Biloba

T3: means group treated with vitamin C

T4: means group treated with both dry peppermint and Gingko Biloba

*Means significance difference at P ≤ 0.01

Table 4: Effect of Dry Peppermint, Gingko Biloba and Vitamin C on Performance of Broiler

	Control Mean±SE	T1 Mean±SE	T2 Mean±SE	T3 Mean±SE	T4 Mean±SE
Initial Body weight (gm)	45	45	45	45	45
Final Body weight (gm	1740±0.093	1835±0.019	1875±0.04	1805±0.07	1920±0.003 *
Body weight increase(gm)	1695	1790	1830	1760	1875
Post slaughter weight (gm)	1484±0.05	1587±0.028	1575±0.01	1599±0.07	1635±0.1
Dressing %	85.3	86.5	84	85.2	88.6
Total feed Intake (gm)	3532	3376	3543	3168	3429
FCR	2.03*	1.84	1.89	1.9	1.65*
Mortality rate %	15*	8	6	10	5*

T1: means group treated with dry peppermint

T2: means group treated with dry Gingko Biloba

T3: means group treated with vitamin C

T4: means group treated with both dry peppermint and Gingko Biloba

Means significance difference at P≤ 0.01

The comfort behaviour patterns used as indicator for degree of stress, it include Leg and wing stretch, Leg scratch and Preening, it was observed that the minimal comfort behaviour recorded in control group according to McFarlane, *et al.* [27], who concluded that when the ambient temperature increases above the comfort zone, chickens devote less time to walking and standing and keep their wings drooped and lifted slightly from the body to maximize sensible heat loss. But when anti-stress feed additives added, the rate of comfort behaviour increased and the higher rate of the Leg and wing stretch was 1.185±0.196 birds / minute and Preening was 3±0.3 birds / minute; both recorded in T4 group that feed on basal diet enriched with both dry peppermint and Gingko Biloba.

This may be related to the Ginkgo Biloba mode of action that which appears to work by promoting vasodilation in the brain, thus improving oxygenation to brain tissue plus it act at the hypothalamic level and are able to reduce corticotrophic releasing hormone expression and secretion [28] and in the same time the peppermint is one of cool herbs that stimulate perspiration, improve circulation, dispel fevers and chills and cooling to the skin and mucosa because it stimulates cold receptors [1] so the combination between both dry peppermint and Gingko Biloba promote a synergistic effect to stimulate the bird comfort behaviour to maximize the bird welfare and minimize the suffering and stress during summer.

Productive Performance: As shown in Table 4 there was a highly statistical significance difference betweenT4 group and other groups

The T4 group achieve the higher final body weight 1920±0.003 gm/bird, followed by T2, T1 groups and T3 respectively while control group given the lowest final body weight 1740±0.093 gm/ bird. T3 recorded the lowest total feed intake3168 gmthrough the experiment while control group and T2 give the highest feed intake 3532 gm and 3543 gm respectively. The food conversion rate for T4 was 1.65 followed by T1, T2, T3 and control was 2.03, also the dressing percentage was much higher in T4 88.6 % followed by 86.5 % in T1. This

may be related to the active principles of both peppermint and Gingko Biloba from essential oils act as a digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry [29].

Therefore, the main compound of peppermint may probably improve the digestibility of diet as a digestion stimulant and hence increase the nutrient entry rate at an early stage of bird's life without affecting feed conversion [16, 30].

The mortality rate was much higher in control group 15±0.07, this agree [31] who mentioned that whenever ambient temperatures exceed 35°C, mortality can reach 25–30% in a poorly managed house. While in T3 group mortality was 10±0.016 then T1 group 8±0.025 and finally T4 group 5±0.033, it was clear that the groups supplied anti-stress feed additives showing lower mortality than control group as the additives from peppermint, Gingko Biloba or vitamin C minimized the degree of stress of birds and so the side effects of heat stress specially in T4 group that supplied by both dry peppermint and showed low mortality rate than control group and this may related to the effect of peppermint as the rich source for electrolytes and vitamins to the bird and Gingko Biloba is heart tonic so increase the resistance of birds to heart attacks that frequently occur during heat stress. This according to Darre and Harrison andWang *et al*. [32, 33] they observed that the birds after heat exposure showing an increase in heart rate and it has contributed to increased mortality due to heart attacks.

Internal Organs Weight: From table 5 it can be observed that there was no any significance difference between different groups in the internal organs weight and all organs within normal weight, only bursa in T4 group was slightly higher than other groups. This explains that the anti-stress feed additives either dry peppermint, Gingko Biloba or vitamin C; has no any adverse effect in internal organs [18].

Hematological and Immunological Parameters: From Table 6 the hematological data revealed that chicks in control group subjected to chronic heat stress so it had significantly decreased number circulating RBCs, lymphocyte percentage, hemoglobin concentration and packed cell volume, This may be due to the impact of chronic stress on iron (Fe) in broilers [34]. Hemoglobin concentration was reduced due to increased water loss due to panting and the birds were not able to adapt [7]. In the same time the WBCs count was increased as a normal response of body to stress.

Table 5: Effect of Dry Peppermint, Gingko Biloba and Vitamin C on Gizzard wt., liver wt., heart wt. and bursa wt. of Broiler

	Control Mean±SE	T1 Mean±SE	T2 Mean±SE	T3 Mean±SE	T4 Mean±SE
Gizzard wt.	32.64±3.08	25.36±1.14	31.94±2.24	28.76±1.58	30.68±1.47
Liver wt.	40.76±3.96	35.56±1.34	36.34±2.04	36.25±1.26	32.76±2.52
Heart wt.	6.16±0.29	5.44±0.27	6.72±0.19	5.88±0.30	6.62±0.34
Spleen wt.	1.28±0.19	1.38±0.09	1.24±0.10	1.28±0.12	1.26±0.21
Bursa wt.	1.4±0.25	1.2±0.13	1.12±0.146	1.22±0.13	2.12±0.35

T1: means group treated with dry peppermint

T2: means group treated with dry Gingko Biloba

T3: means group treated with dry Vitamin C

T4: means group treated with both dry peppermint and Gingko Biloba

Means significance difference at P≤ 0.01

Table 6: Effect of different Anti-stress agent on the Hematological parameters of Broiler

	Control Mean rank	T1 Mean rank	T2 Mean rank	T3 Mean rank	T4 Mean rank	Chi square	Sign.
RBC Count (no x 10^6cell/µl)	13.56*	20.33	28	24	25.06	10.87	0.02
WBCs (no x 10^6cell/µl)	29.72*	22.61	25.11	23.72	20.83	12.44	0.01
Lymphocyte %	18.61*	27.17	26.44	23.44	29.33	7.83	0.02
Hb (gm/dl)	18.67*	24.61	25.28	25.22	26.22	6.28	0.17
PCV %	16.06*	20.78	27.33	23.17	20.67	16.64	0.002

RBC: means red blood cell count

WBCs: means white blood cell count

Hb: Hemoglobin concentration PCV: packed cell volume

*Means significance difference at P ≤ 0.01

Kruskal Wallis Test used to obtain mean rank

Table 7: Effect of Dry Peppermint, Gingko Biloba and Vitamin C on the immune response of broiler to NDV, IBD and I.B. vaccines

	Control Mean	T1 Mean	T2 Mean	T3 Mean	T4 Mean
NDV "HI"	10^4	10^5	10^5	10^4	10^8
IBD AB titer	317.27 -ve	194.98 -ve	2089.25 +ve	1058.89 +ve	1602.415 +ve
I.B. AB titer	3897.79 +ve	3780.53 +ve	4666.03+ve	3792.665 +ve	3905.875 +ve

T1: means group treated with dry peppermint

T2: means group treated with dry Gingko Biloba

T3: means group treated with dry Vitamin C

T4: means group treated with both dry peppermint and Gingko biloba

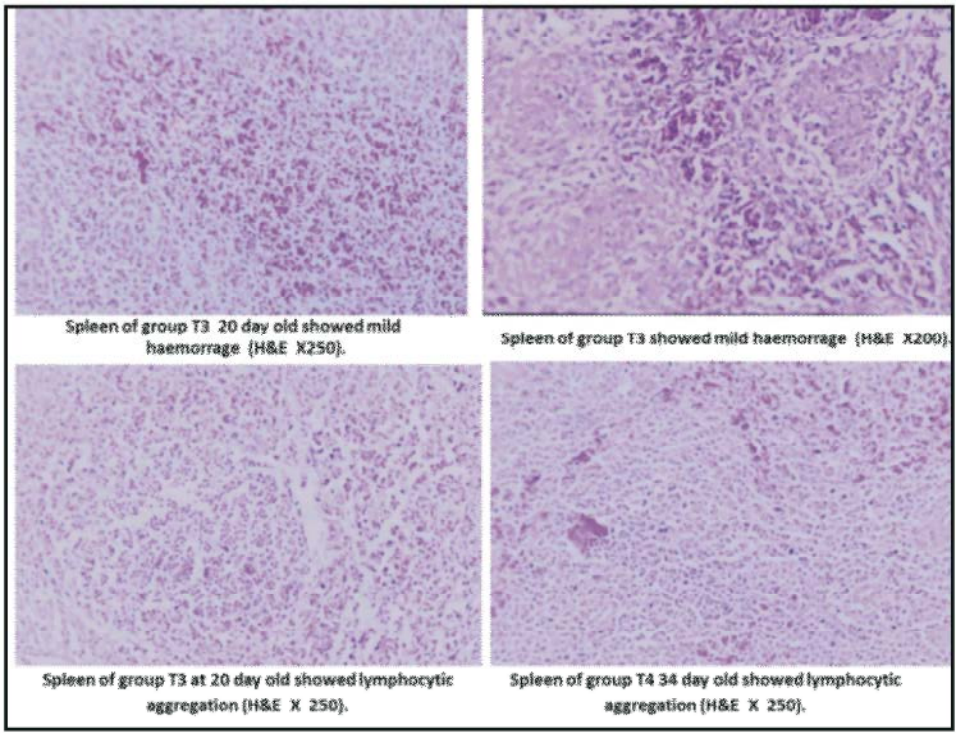

Plate 1: Effect different Anti-stress agents on histopathology of spleen.

While in treatment groups in which we add the anti-stress feed additives showing no statistical significance difference in the blood parameters but the number circulating RBCs, lymphocyte percentage, hemoglobin concentration and packed cell volume were increased compared with control group and WBCs count was less than control group.

In table 7 the vaccination immunological response for Newcastle disease, infectious bronchitis and infectious Bursal disease, High ambient temperature has been known to affect immune response of birds according to El-Gendy *et al*. [35] and Zhang *et al*. [36]. The highest values of antibodies titer for Newcastle Disease vaccine were observed in the birds feed on basal diet fortified with both dry peppermint and Gingko Biloba, while the IBD vaccination antibodies titer recorded in T2 group that supplied with Gingko Biloba, in T3 group supplied with Vitamin C and in T4 group that feed on basal diet fortified with both dry peppermint and Gingko Biloba. Finally Infectious bronchitis vaccination antibodies titer, all groups are positive, showing high antibodies titer and no differences between groups.

Histopathology Changes: From plate 1, 2 and 3 histopathological changes were not observed in all groups in the small intestine, large intestine, cecal tonsils, spleen and bursa of fabricious, only mild changes in spleen and cecal tonsils were observed in group T3 that supplied with vitamin C in form of lymphocytic activation and aggregations, also in T4 group that feed on basal diet fortified with both dry peppermint and Gingko Biloba, bursa of fabricious showing proliferation of heterophiles and mild depletion of lymphocytes and cecal tonsils showing lymphocyte activation.

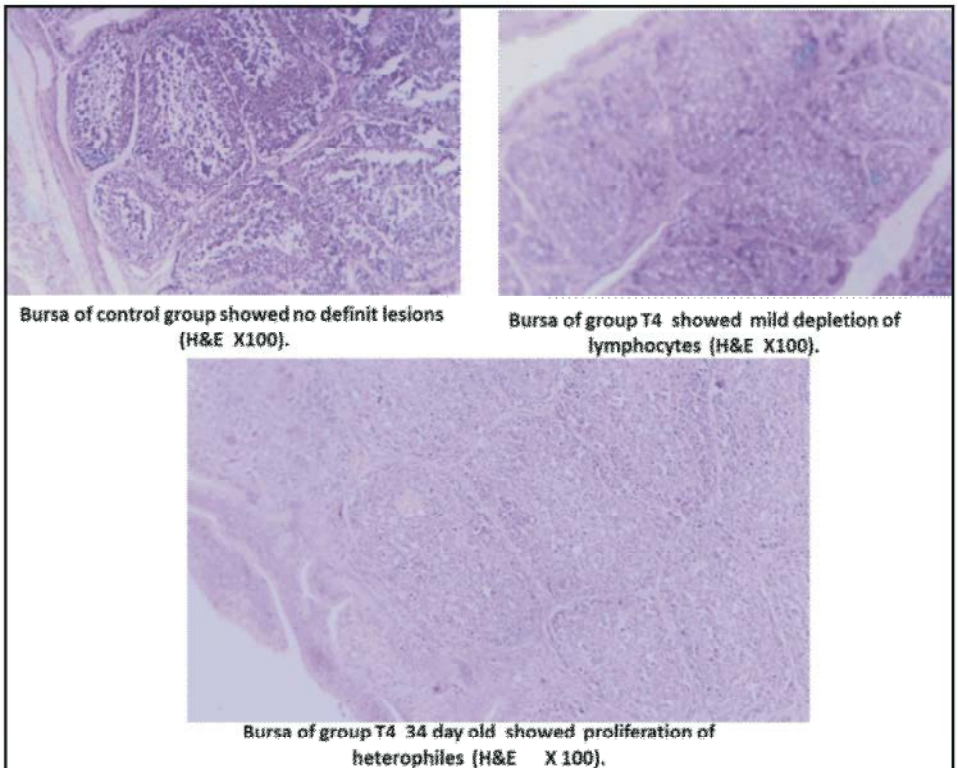

Plate 2: Effect different Anti-stress agents on histopathology of Bursa of fabricious

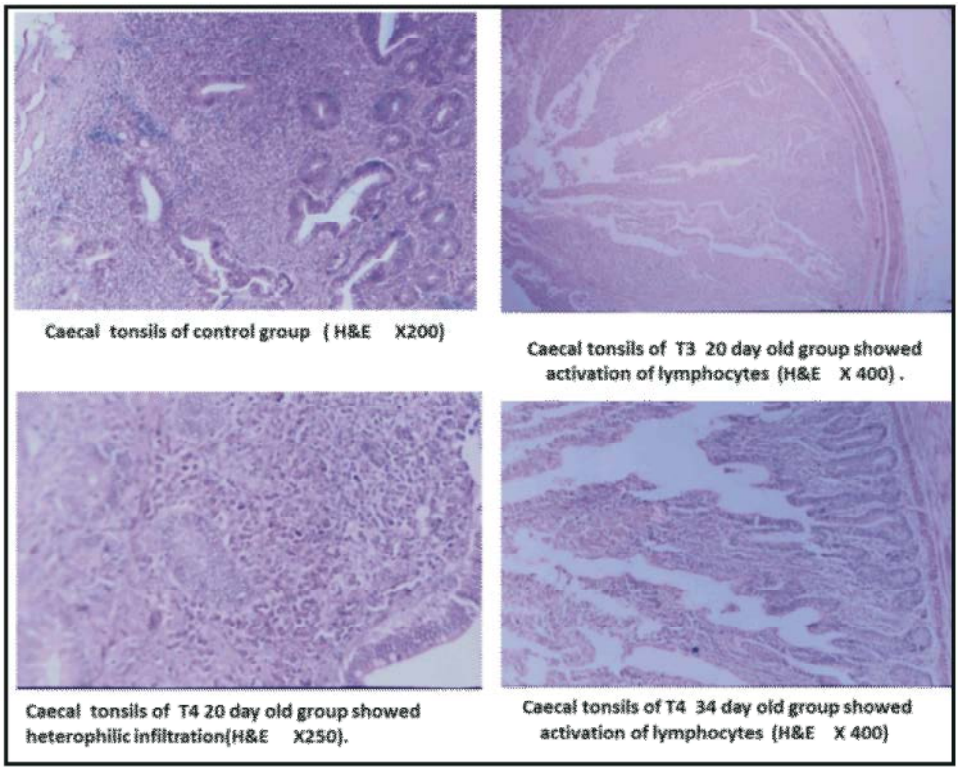

Plate 3: Effect different Anti-stress agents on histopathology of cecal tonsils

CONCLUSION

Based upon findings of the study, it may be concluded that Boiler welfare, comfort behaviour, production performance and immune response of broilers against Newcastle disease, Infectious Bursal disease and Infectious Bronchitis may be improved through supplying broiler with Anti-stress plants like dry peppermint, Gingko Biloba as management practices during summer.

REFERENCES

1. Viola Holik, 2009. Management of laying hens to minimize heat stress. Lohmann information, 44(1): 16.
2. Griggs, J.P. and J.P. Jacob, 2005. Alternatives to antibiotics for organic poultry production J. Appl. Poult. Res., 14: 750-756.
3. Carmen, A., M. France, G. Macleod and E. Julie, 1991. Alleviation of acute heat stress by food withdrawal or darkness. Br. Poult. Sci., 32: 219-225.
4. Har, L., D. Rong and Z.A. Zhang, 2000. The effect of thermal environment on the digestion of broilers. Anim. Physiol., 83: 75-61.
5. Howlider, M.A.R. and S.P. Rose, 1989. Rearing temperature and the meat Yield of broilers. Br. Poult. Sci., 30: 61-67.
6. Temim, S., A. Chagneau, M. Peresson and S. Tesseraud, 2000. Chronic heat exposure alters protein turnover of three different skeletal muscles in finishing broiler chickens Fed 20 or 25% protein diets1, 2. J. Nutr., 130: 813-819.
7. Yahav, S., A. Straschnow, I. Plavnik and S. Hurwitz, 1996. Effects of diurnal cycling versus constant temperatures on chicken growth and food intake. Br.Poult. Sci., 37: 43-54.
8. Mashaly, M.M., G.L. Hendricks, M.A. Kalama, A.E. Gehad, A.O. Abbas and P.H. Patterson, 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci., 83: 889-894.
9. Borges, S.A., A.V. Fischer da Silva, A. Majorka, D.M. Hooge and K.R. Cummings, 2004. Physiological responses of broiler chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, mille equivalents per kilogram). Poult. Sci., 83: 1551-1558.
10. Trout, J.M. and M.M. Mashaly, 1994. The effects of adrenocorticotropic hormone and heat stress on the distribution of lymphocyte populations in immature male chickens. Poult. Sci., 73: 1694-1698.
11. Bartlett, J.R. and M.O. Smith, 2003. Effects of different levels of zinc on the performance and immune competence of broilers under heat stress. Poult. Sci., 82: 1580-1588.
12. Mitchell, M.A. and A.J. Carlisle, 1992. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (Gallus domesticus). Comp. Biochem. Physiol. A Comp. Physiol., 101: 137-142.
13. Acamovic, T. and J.D. Brooker, 2005. Biochemistry of plant secondary metabolites and their effects in animals Proc. Nutr. Soc., 64: 403-412.
14. Bampidis, V.A., V. Christodoulou, P. Florou-Paneri, E. Christaki, P.S. Chatzopoulou, T. Tsiligianni and A.B. Spais, 2005. Effect of dietary dried oregano leaves on growth performance, carcase characteristics and serum cholesterol of female early maturing turkeys. Brit. Poult. Sci., 46: 595-601.
15. Lee, K.W., H. Everts, H.J. Kappert, M. Frehner, R. Losa and A.C. Beynen, 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in femalebroiler chickens. Br.Poult. Sci., 44: 450-457.
16. Williams, P.and R. Losa, 2001. The use of essential oils and their compounds in poultry nutrition. Worlds Poult., 17: 14-15.
17. National Research Council (NRC), 1994. Nutrient requirements of Poultry, 7th revised edition, National academy press, Washington. D.C.
18. Ocak, N., G. Erener, F. Burak Ak, M. Sungu, A. Altop and A. Ozmen, 2008. Performance of broilers fed diets supplemented with dry peppermint (Mentha piperita L.) or thyme (Thymusvulgaris L.) leaves as growth promoter source, Czech J. Anim. Sci., 53(4): 169-175.
19. Agricultural Science, 2012. the Study of Extract of Ginkgo Biloba on the Productivity and Plasma Index of Broilerson: May 19, 2012.
20. Altmann, J., 1974. Observational study of behaviour: sampling methods. Behaviour, 49: 227-267.
21. Samanya, A.M. and K. Yamauchi, 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. Comp.Biochem. Physiol. Part A., 133: 95-104.
22. Trarachai, P. and K. Yamauchi, 2000. Effects of luminal nutrient absorption, intraluminal physical stimulation and intravenous parenteral alimentation on the recovery responses of duodenal villus morphology following feed withdrawal in chickens. Poult. Sci., 79: 1578-1585.

23. Baum, B., Liebler, E.M. Tenorio, M.L. Enb, J.F. Pohlenz and G. Breves, 2002. Saccharomyces boulardii and Bacillus cereus var. Influence the morphology and the mucins of the intestine of pigs. Z. Gastroenterol., 40: 277-284.
24. Dytham, C., 2003. Choosing and Using Statistics: A Biologist's Guide. 2nd edition. By Blackwell, Oxford.
25. National Research Council (NRC, 1994. Nutrient requirements of Poultry, 7th revised edition, National academy press, Washington. D.C.
26. Rotblatt, M. and I. Ziment, 2002. Evidence-Based Herbal Medicine. Philadelphia, Pa: Hanley and Belfus, pp: 388.
27. McFarlane, J.M., S.E. Curtis, R.D. Shanks and S.G. Carmer, 1989. Multiple concurrent stressors in chicks. 1. Effect on weight gain, feed intake and behaviour. Poultry Science, 68: 501-509.
28. Marcilhac, A., N. Dakine, N. Bourhim, *et al*., 1998. Effect of chronic administration of Ginkgo biloba extract or Ginkgolide on the hypothalamic-pituitary-adrenal axis in the rat. Life Sci., 62: 2329-2340.
29. Lovkova, M.Y., G.N. Buzuk, S.M. Sokolova and N.I. Kliment'eva, 2001. Chemical features of medicinal plants (a review). Appl. Biochem. Microbiol., 37: 229-237.
30. Cross, D.E., R.M. McDevitt, K. Hillman and T. Acamovic, 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Brit. Poult. Sci., 48: 496-506.
31. Nuhad, J. and Daghir, 2008. Poultry production in hot climates, 2nd edition, CABI North American Office, Cambridge, MA 02139, USA.
32. Darre, M.J. and P.C. Harrison, 1987. Heart rate, blood pressure, cardiac output and total peripheral resistance of single comb white leghorn hens during an acute exposure to 35°C ambient temperature. Poultry Science, 66: 541-547.
33. Wang, S., W.G. Bottje, S. Kinzler, H.L. Neldon and T.I. Koike, 1989. Effect of heat stress on plasma levels of arginine vasotocin and mesotocin in domestic fowl (Gallus domesticus). Comparative Biochemistry and Physiology, 93A(4): 721-724.
34. Jamadar, S.J. and B.W.V. Jalnapurkar, 1995. Effect of high ambient temperature on iron status of broilers. Indian Vet. J., 72: 577-579.
35. El-Gendy, E.A., A.A. Atallah, F.R. Mohamed, A.M. Atta and N.E. Goher, 1995. Strain variation in young chickens in response to chronic heat stress conditions. Egypt J. Anim. Prod., 32: 237-251 Emery, 2004.
36. Zhang, L.C., S.H. Liu, S.B. Wang, S.Z. Hou, Y.Q. Liu, L.C. Zhang, S.H. Liu, S.B. Wang, S.Z. Hou and Y.Q. Liu, 1998. Study on the effect of heat stress on the morphology of immune organs of broilers. Chinese J. Vet. Med., 24: 24-5.