ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2021.76.86

Research Article
Evaluation of Antioxidant and Antistress Effects of Essential Oils (Phyto-Plus) Supplementation in Broiler Chickens

[1]Mahmoud, K. Eldeib, [1]Hoda M.L. AbdAllah, [2]Abd-Allah S. Hamed, [2]Sahar, N. Mohamedy, [1]Magda, N.A. AbdElall and [1]EmanI. M. Ismail

[1]Department of Toxicology and Biochemical, Animal Health Research Institue, Zagazig Branch, Agriculture Research Centre, Sharkia, Egypt
[2]Department of Pathology, Animal Health Research Institue, Zagazig Branch, Agriculture Research Centre, Sharkia, Egypt

Abstract

Objective: This study was conducted to evaluate the effect of thyme, oregano, peppermint, garlic, and rosemary combination (Phyto-plus) on the growth performance of broiler chickens in addition to their potential to be used as antistress and antioxidant. **Materials and Methods:** A total of 180 broiler chicks were divided into three equal groups with three replicates in each group: group1 (GP1) was served as a control given normal diet without any supplement. Group 2 (GP2) and group 3 (GP3) were supplemented with phyto-plus at doses of 100 and 400 g t^{-1} of ration respectively for 6 weeks. At the end of the 3rd and 6th week of experiment, live body weight and body weight gain of broiler chickens were calculated. Blood samples were taken to determine the levels of heat shock protein 70 (HSP70) and corticosterone hormone, in addition to enzymatic activity of superoxide dismutase (SOD), glutathione peroxidase (GST PX), catalase (CAT) and malondialdehyde (MDA). Side effects of phyto-plus were detected by estimation of liver and kidney markers. The histopathological examination of the liver, kidney and intestine were recorded. **Results:** The present data revealed improved growth performance and intestinal histomorphology in both two treated groups (GP2 and GP3) of broiler chickens, which were more pronounced with low dose than high dose of phyto-plus treated groups. There were a significant elevation in the level of HSP70, superoxide dismutase (SOD), glutathione peroxidase (GST PX) and catalase (CAT), while decreasing levels of corticosterone was detected with two doses of phyto-plus. No clinically significant elevation in liver and kidney markers (AST, ALT, Uric acid and creatinine) were seen as well as there were a normal liver and kidney pictures in broiler chickens given the low dose of phyto-plus. **Conclusion:** The low dose of phyto-plus (100 g t^{-1} of ration) was an optimum supplementation dose compared to the high dose (400 g t^{-1} of ration).

Key words: Phyto-plus, antistress, antioxidant, growth performance, intestinal histomorphology

Citation: Mahmoud, K. Eldeib, Hoda M.L. AbdAllah, Abd-Allah S. Hamed, Sahar, N. Mohamedy. Magda, N.A. AbdElall and EmanI. M. Ismail, 2021. Evaluation of antioxidant and antistress effects of essential oils (phyto-plus) supplementation in broiler chickens. Int. J. Poult. Sci., 20: 76-86.

Corresponding Author: Mahmoud, K. Eldeib, Department of Toxicology and Biochemical, Animal Health Research Institue, Zagazig Branch, Agriculture Research Centre, Sharkia, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

During intensive poultry breeding, birds subject to diverse stressors as vaccination, hypoxia, starvation and infection; these stress conditions, activate the hypothalamic-pituitary-adrenal (HPA) axis, leading to an increase in concentration of circulating corticosterone and altered cellular immune responses[1-3]. Interestingly, animals have defense systems which protect them from various stresses, one of which is expression of heat shock protein (HSP). Heat shock protein70 (HSP70) is one of the most well-known HSPs. It is a 70-kDa protein expressed in almost all organs[4] such as the gastrointestinal tract[5] and the nervous system[6]. When the organism is subjected to highly stressful conditions, it could systemically enhance the induction of heat shock protein (HSPs) to protect the body[7]. It has been reported that, HSP70 shows apoptosis-suppressive effects[8] and anti-inflammatory activity[9], leading to cytoprotective functions from various stresses. Dietary modulations are one of the proven approaches to minimize the negative effects of stress on production and anti-oxidant variables in chicken. Feed additives from plant origin are gaining wider acceptability in recent times both in human health practices and livestock production owing to several benefits associated with the natural compounds. Essential oils (phyto-plus powder) is a mixture of herbal extracts, including, Thyme, Oregano, peppermint, Garlic, and Rosemary. Origanum and Thymus, are regarded as oil-rich on the basis of their essential oil content >2%[10]. The major constituents of the oils of thyme and oregano species have been reported to be thymol, carvacrol and c-terpinene[11,12].

In general, thymol [5-methyl-2-(1-methylethyl) phenol], is a main component of thyme essential oil, and its isomer and carvacrol [2-methyl-5-(1-methylethyl)phenol], is a main component of oregano essential oil[13]. Thyme ingredients seemed to protect against peroxide- and mutagen-induced DNA damage in human lymphocytes[14,15]. Carvacrol dietary supplementation has pharmacological effects and beneficial health effects, such as antimicrobial, antioxidant, anticancer, antiplatelet, antiviral, anti-inflammatory, antifungal, anti-Alzheimer disease, anti-obesity activity and growth promoting properties[16]. Wieten *et al.*[17] recorded that Carvacrol, had a notable capacity to co induce cellular HSP70 expression *in vitro* and upon intra gastric administration, in Payer's patches of mice *in vivo*. Murakami *et al.*[18] reported that adding essential oil to the broiler's ration increased villus height in the jejunum on day 14. Phytogenic feed additive may also reduce mucosal thickness, thus contributing to the diffusion of nutrients to the apical surface of epithelial cells and increased absorption and feed efficiency[19]. Furthermore, in the *C. perfringens* challenged broiler chickens, Du *et al.*[20] reported that the dietary supplementation of essential oils, which contained 25% thymol and 25% carvacrol as active components could alleviate the intestinal injury by improving intestinal integrity and modulating immune responses. In contrast, it was reported that supplementing essential oils to the broiler's diets had no effect on villus height in the jejunum statistically, but it increased villus height numerically[21].

Aim of the study: This study aimed to evaluate the effect of essential oils (Phyto-plus) as antioxidants and antistresser in broiler chicken by measuring, corticosterone, Heat shock protein (Hsp70), superoxide dismutase, glutathione peroxidase, catalase (CAT) and malondialdehyde (MDA), in addition to employment histopathological examination of the liver, kidney and intestine .

MATERIALS AND METHODS

Phyto-plus powder is a dry stabilized preparation manufactured by (Kanzy Medipharm, Canada), it is a mixture of herbal extracts (Garlic, Thyme, Oregano, Peppermint, Rosemary) with Calcium carbonate as carrier.

Experimental design: The trial was carried out at Poultry Production Farm, Feed Research House, Orabi Community, Qalyubia Governorate. A total of 180 one-day old Ross 308 broiler chicks (as hatched) with an initial body weight around 44 g were obtained from a local commercial hatchery. The chicks were weighed and randomly distributed in 3 pens 60 chicks each, to examine the effect of 2 experimental treatments comprising three feeding regimens: (GP1) Basal diet without Phyto-plus powder supplement (control group) and basal diet supplemented with either 100 or 400 g Phyto-plus powder per ton of ration (GP2 and GP3, respectively) for 6 weeks.

All the experimental birds were reared in well ventilated shed on soft wood shaving litter used as bedding material in pens. Basal starter (0-14 day), grower (15-24 day) and finisher (25- end) diets were formulated according to the nutritional recommendation of National Research Council[22] for broiler. Feed and water were available ad-libitum during the experiments.

Growth performance: Chicks of all groups were weighed individually before the start of the experiment and at the end of the 3rd and 6th week of experiment.

Blood sampling: Blood samples were collected from each bird via heart puncture at the end of the 3rd and 6th week. Blood samples were taken without anticoagulant in a clean and dry centrifuged tube, left to clot at room temperature and then centrifuged at 3000 rpm for 15 min. The sera were collected for analysis of all biochemical parameters and Oxidant/antioxidant markers.

Tissue samples: Specimens from liver, kidney and intestine were collected from slaughtered birds for histopathological examination.

Biochemical analysis: The biochemical assays of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated according to Reitman and Frankel[23]. Total Protein was measured according to Doumas, *et al.*[24], Uric acid was measured according to Yound[25] and serum creatinine was measured according to Kroll[26].

Oxidant/antioxidant markers including Malondialdehyde (MDA)[27], Catalase (CAT)[28] and Superoxide dismutase (SOD)[29] were calorimetrically assayed using chemical kits (Biomed Egypt) and Shimadzu UV 240. Glutathione peroxidase (GST PX) was measured by using ELISA Kits cat. No. MBS 1600242. Heat shock protein 70 (HSP70) was estimated by using ELISA Kit cat. No. MBS035085. Corticosterone hormone was determined by using ELISA Kit cat. No: MBS761865.

Histopathological examination: The intestine, liver and kidney samples from birds were taken for each treatment after 3 and 6 weeks of phyto-plus supplementation. Samples were cleaned with distilled water to remove the intestinal contents and fixed in 10% neutral buffered formalin solution then dehydrated, cleared and embedded in paraffin wax, then sectioned to 4-5 micron thickness, prepared and stained with Hematoxylin and Eosin stain (H&E) and examined microscopically[30]. Morphometric measurements of the intestinal villi were performed including villus length and villus width. Measurements of 10 complete villi for villus length and width per sample of each bird intestinal section were performed at 40 magnifications using Image J Analyzer software[31] and the average of these values were used for statistical analysis.

Statistical analysis: The obtained data were computerized and analyzed for significance, standard error and variance was calculated using SPSS 14[32].

RESULTS

The effects of two doses of Phyto-plus Powder on the live body weight and body weight gain of broiler chicken are presented in Table 1. There were no significant differences in the live body weight of chickens among groups (control group, Phyto-plus 100 and Phyto-plus 400) after 3 weeks. However, after 6 weeks of Phyto-plus Powder supplementation the birds showed a significantly increased live body weight in GP2 as compared with the control group, while the increase in the live body weights were non-significant in GP3 compared to control group. Chicken fed diet supplemented with 100 or 400 mg of Phyto-plus t^{-1} of ration exhibited a higher body weight gain after 3 and 6 weeks of Phyto-plus supplementation, that was not statistically different in birds with high dose of Phyto-plus compared to the control group.

Table 2 exhibits the level of heat shock protein 70 (Hsp70) and corticosterone hormone in chickens fed on phyto-plus at dose of 100 or 400 g t^{-1} of ration for 6 weeks. The result demonstrated elevation in HSP70 levels significantly in two treated groups of chickens after 3 weeks and in chickens with low dose (GP2) of phyto-plus, whereas, the corticosterone hormone lowered significantly in the two treated groups of chickens (GP2 and GP3) as compared to the control one.

Concerning, antioxidant enzymes activity, the results revealed a significant elevation in the activities of CAT, SOD and GH-PX levels of birds in Group 2 after 3 and 6 weeks of phyto-plus supplementation, while these enzymes increased significantly in chickens fed diet supplemented with high dose of phyto-plus only after 6 weeks as compared to the control group. There were no significant changes in MDA level in chickens of the two treated groups, although its level decreased non-significantly with low dose of phyto-plus compared to the control while Gp3 (400 mg phyto-plus t^{-1} of ration) revealed significant increase in AST activity and creatinine concentration as compared to other groups indicating that the low dose of phyto-plus (100 g t^{-1} of ration) is more safe than its high dose (Table 3).

Table 1: Growth performance in chickens supplemented with 100 and 400 g of phyto-plus ton^{-1} of ration for 6 weeks (n = 5)

Time of sampling	Groups	Average weight gain (g)	Life body weight (g)
3rd week	Group 1 (Control)	568±6.58^{b}	1653±45.75^{a}
	Group 2(Phyto-plus 100 g)	588±5.26^{a}	1669±18.34^{a}
	Group 3 (Phyto-plus 400 g)	585±5.00^{ab}	1644±19.84^{a}
6th week	Group 1 (Control)	1987±7.34^{b}	2032±33.65^{b}
	Group 2 (Phyto-plus 100 g)	2091±9.98^{a}	2138±11.51^{a}
	Groups 3 (Phyto-plus 400 g)	2028±37.07^{ab}	2098±17.99^{ab}

Data presented as Mean±SE (standard error) followed by different letters in the same column were significantly different at $p \leq 0.05$

Table 2: Mean values of heat shock protein 70 and corticosterone in chickens supplemented with 100 and 400 g of Phyto-plus t^{-1} of ration for 6 weeks (n = 5)

Time of sampling	Groups	HSP70 (Pg mL^{-1})	Corticosterone (ng mL^{-1})
3rd week	Group 1 (Control)	69.60±2.60^{c}	28.80±1.36^{a}
	Group 2 (Phyto-plus 100 g)	83.40±1.29^{a}	22.00±0.95^{b}
	Group 3 (Phyto-plus 400 g)	76.40±1.29^{b}	21.00±1.30^{b}
6th week	Group 1 (Control)	71.00±2.02^{b}	25.40±2.11^{a}
	Group 2 (Phyto-plus 100 g)	85.60±3.19^{a}	18.00±0.45^{b}
	Group 3 (Phyto-plus 400 g)	75.06±1.69^{b}	19.86±0.71^{b}

Data presented as Mean±SE (standard error) followed by different letters in the same column were significantly different at $p \leq 0.05$

Table 3: Mean values of some antioxidant enzymes and malondialdehyde in chickens supplemented with 100 and 400g of phyto plus t^{-1} of ration for 6 weeks (n = 5)

Time of sampling	Groups	MDA (nmol mL^{-1})	CAT (u mL^{-1})	SOD (u mL^{-1})	GSH-px ng mL^{-1}
3rd week	Group 1 (Control)	23.00±0.005^{a}	30±0.05^{b}	20±0.01^{b}	32.40±1.36^{b}
	Group 2 (Phyto-plus 100 g)	0.19±0.03^{a}	42±0.01^{a}	28±0.02^{a}	43.40±3.23^{a}
	Group 3 (Phyto-plus 400 g)	21.00±0.01^{a}	39±0.03^{ab}	25±0.02^{ab}	35.20±2.05^{b}
6th week	Group 1 (Control)	24.00±0.01^{a}	28±0.02^{b}	31±0.011^{b}	35.00±1.00^{b}
	Group 2 (Phyto-plus 100 g)	22.00±0.01	34±0.01^{a}	35±0.016^{a}	46.20±3.31^{a}
	Group 3 (Phyto-plus 400 g)	26.00±0.02^{a}	36±0.03^{a}	36±0.012^{a}	42.80±2.39^{a}

MDA: Malondialdehyde, CAT: Catalase, SOD: Superoxide dismutase, GHS-px: Glutathione peroxidase, Tp: Total protein

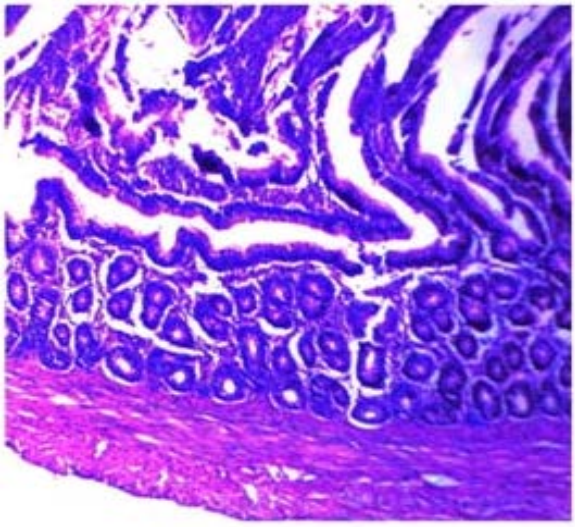

Fig. 1: Intestinal villi of chickens in Gp2 after 3 weeks fed with basal diet and 100 g t^{-1} phyto-plus showing proliferation in glandular lining epithelial cells. (H&E100)

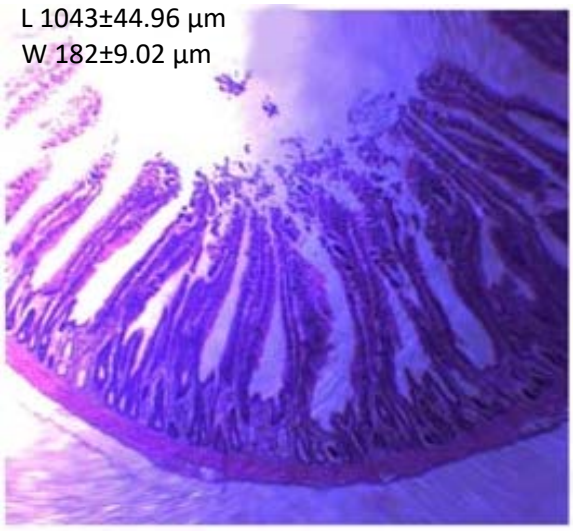

Fig. 2: Intestinal villi of chickens in Gp2 after 3 weeks fed with basal diet and 100 g t^{-1} phyto-plus showing increasing length (1043±44.96 µm) and width (182±9.02 µm) of villi (H&E40)

Liver and kidney markers were measured for the analysis of the safety use of phyto-plus (Table 4). The results demonstrated that birds fed on phyto-plus at dose of 100 g t^{-1} of ration (Gp2) showed normal values of ALT, AST, uric acid and creatinine with a significant increase in total protein level after 3 and 6 weeks of supplementation as compared to the control, while Gp3 (400 g phyto-plus t^{-1} of ration) revealed significant increase in AST activity and creatinine concentration as compared to other groups. Indicating that the low dose of phyto-plus (100 g t^{-1} of ration) is safer than its high dose.

The results of villus height and width of the control and experimental groups are presented in Table 5. Results showed that supplementation of broilers diets with 100 g t^{-1} ration in group two after 3 weeks revealed proliferation in glandular lining epithelial cells (Fig. 1) and increased the length (1043±44.96) and width (182±9.02) of the intestinal villi (Fig. 2). Histological analysis of liver and kidney did not indicate any pathological changes but appear normal (Fig. 3 and 4). After 6 weeks the villi showed hyperplasia of the goblet cells in addition to increase the length (1057.10±13.74) and width (163.90±5.09) of villi compared to 3 weeks (Fig. 5 and 6), the villi were found to be very long in this group.

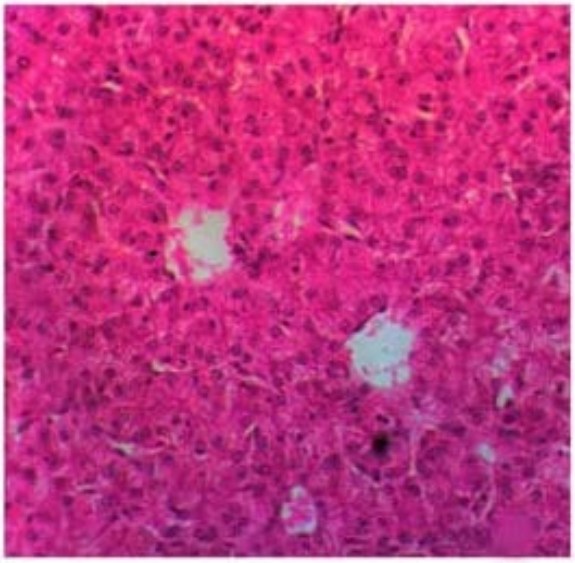

Fig. 3: Liver of chickens in Gp2 after 3 weeks feed with basal diet and 100 g t^{-1} phyto-plus showing apparently normal hepatocytes. (H&E100)

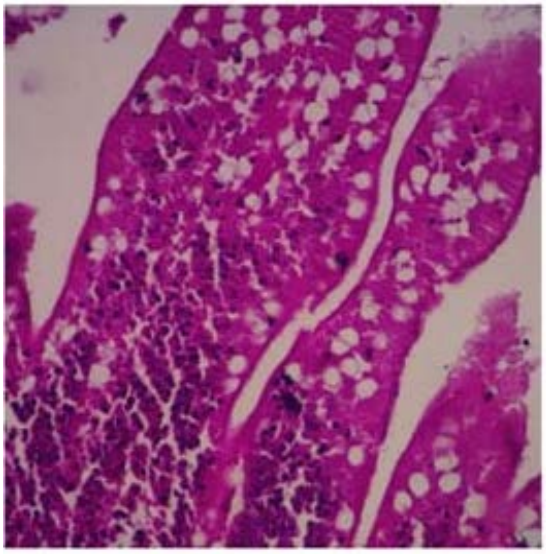

Fig. 5: Intestine of Gp2 after 6 weeks fed with basal diet and 100 g t^{-1} phyto-plus showing hyperplasia of the goblet cells. (H&E100)

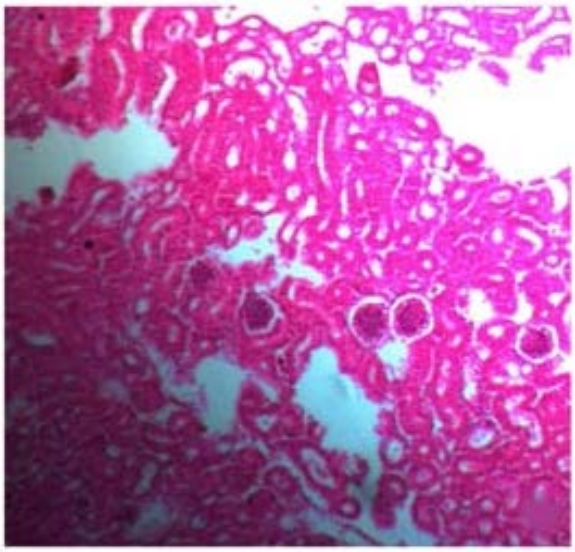

Fig. 4: Kidney of chickens Gp2 after 3 weeks fed with basal diet and 100 g t^{-1} phyto-plus showing apparently normal glomeruli and renal tubules. (H&E100)

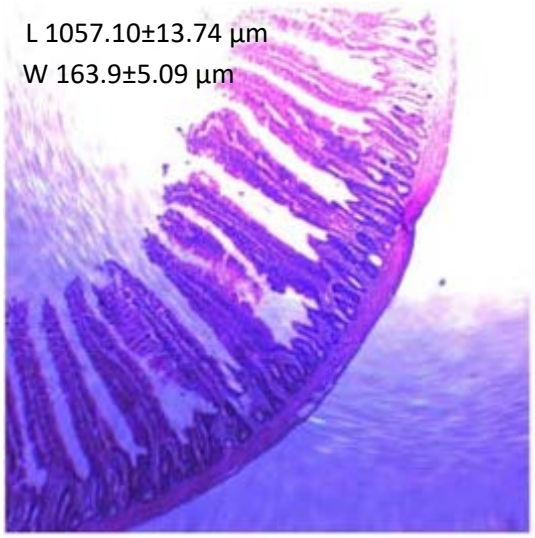

Fig. 6: Intestinal villi of chickens in Gp2 after 6 weeks fed with basal diet and 100 g t^{-1} phyto-plus showing increasing length (1057.10±13.74 µm) and width (163.90±5.09 µm) of villi. (H&E40)

Concerning the intestine, liver and kidney of chickens in group 3 which fed diet supplemented with phyto-plus at dose of 400 g t^{-1} of ration for 3 weeks, the liver revealed widely dilated central vein (Fig. 7) and kidney showed hydropic degeneration in the epithelial cell lining of renal tubules (Fig. 8) but after 6 weeks the intestinal villi revealed slightly decrease in the length (996.50±24.52) and width (149.30±9.32) compared to control group (Fig. 9) while n liver and kidney the lesions progressed to fatty degeneration (signet ring appearance of hepatocytes) (Fig. 10) and tubular nephrosis with cystic dilatation of some renal tubules (Fig. 11).

Table 4: Mean values 0f some biochemical parameters in chickens supplemented with 100 and 400 g of phyto plus t^{-1} of ration for 6 weeks (n=5)

Time of sampling	Groups	AST (u L^{-1})	ALT (u L^{-1})	TP (g dL^{-1})	Uric acid (mg dL^{-1})	Creatinine (mg dL^{-1})
3rd week	Group 1 (Control)	30.6±2.32^{a}	19.00±4.3^{a}	3.60±0.19^{b}	5.98±0.52^{a}	0.70±0.08^{a}
	Group 2 (Phyto-plus 100 g)	32.0±1.95^{a}	17.00±0.72^{a}	4.40±0.17^{a}	4.50±0.45^{a}	0.74±0.07^{a}
	Group 3 (Phyto-plus 400 g)	36.4±2.11^{a}	18.14±0.62^{a}	3.80±0.16^{b}	4.96±1.16^{a}	0.92±0.057^{a}
6th week	Group 1 (Control)	32.2±1.9^{b}	16.00±0.71^{a}	3.12±0.14^{b}	6.36±0.69^{ab}	0.66±0.040^{b}
	Group 2 (Phyto-plus 100 g)	33.6±1.33^{b}	16.00±0.6^{a}	4.02±0.17^{a}	5.26±0.37^{b}	0.73±0.073^{b}
	Group 3 (Phyto-plus 400 g)	38.6±0.93^{a}	17.00±0.58^{a}	3.28±0.26^{b}	7.72±0.32^{a}	1.16±0.19^{a}

Data presented as Mean±SE (standard error) followed by different letters in the same column were significantly different at $p \leq 0.05$. AST: Aspartate transferase ALT: Alanine transferase

Table 5: Means (μm) of intestinal villi height and width of chickens supplemented with 100 and 400 g of phyto plus t^{-1} of ration for 6 weeks

Time of sampling	Groups	Villi height (μm)	Villi (μm)
3rd week	Group 1 (Control)	979±55.53^{a}	148±21.60^{ab}
	Group 2 (Phyto-plus 100 g)	1043±44.96^{a}	182±9.02^{a}
	Group 3 (Phyto-plus 400 g)	919±53.51^{a}	131±11.26^{b}
6th week	Group 1 (Control)	1003.90±9.56^{b}	179.90±7.92^{a}
	Group 2 (Phyto-plus 100 g)	1057.10±13.74^{a}	163.90±5.09^{ab}
	Group 3 (Phyto-plus 400 g)	996.50±24.52^{b}	149.30±9.32^{b}

Data presented as Mean±SE (standard error) followed by different letters in the same column were significantly different at $p \leq 0.05$

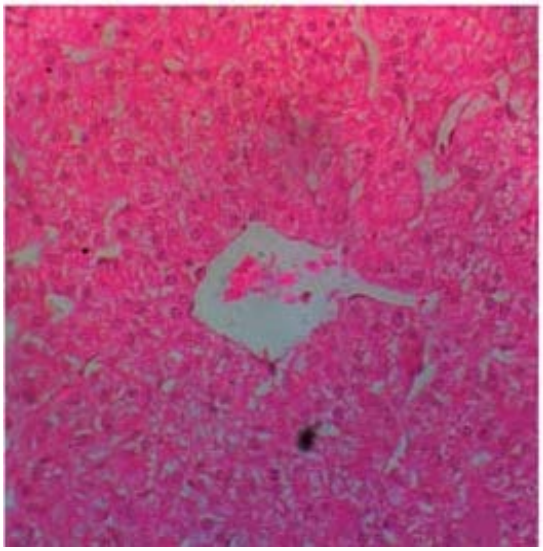

Fig. 7: Liver of chickens in Gp3 after 6 weeks fed with basal diet and 400 g t^{-1} phyto-plus showing widely dilated central vein. (H&E100)

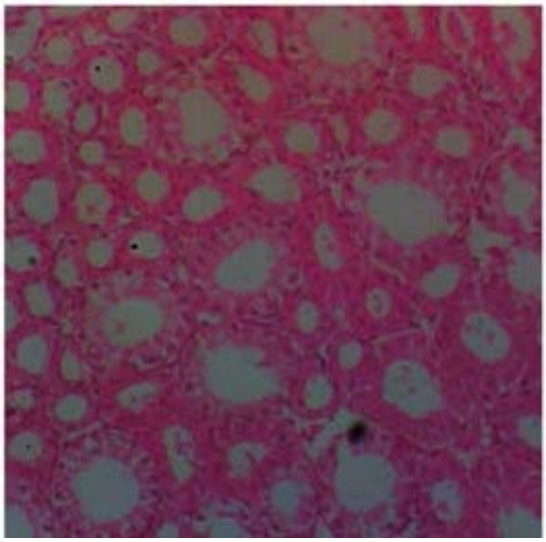

Fig. 8: Kidney of Gp3 after 6 weeks fed with basal diet and 400 g t^{-1} phyto-plus showing hydropic degeneration in the epithelial cells lining of renal tubules. (H&E100)

DISCUSSION

Thymol and carvacrol are believed to exhibit a range of beneficial physiological effects. The obtained results showed that chickens fed diets supplemented with low dose of Phyto-plus Powder (Gp2) exhibited a significant heavier live body weight (LBW) at 6 weeks and body weight gain (BWG) at 3 and 6 weeks of ages. In consistent with these results, Ocak *et al.*[33] recorded a significant higher LBW at 21 and 42 day of ages as well as higher BWG from 7-35 day of age in broilers fed peppermint and thyme. Moreover Al-Kassie[34] and Abid[35] showed that chickens fed with thyme supplement had significantly heavier live body weight and body weight gain. Furthermore, using oregano herbs tested in broiler diets improved body weight and feed intake at 1,000 ppm[36], 500 ppm[37], 100 and 250 ppm[38]. These may be due to the fact that thyme improves diet palatability as well as stimulates and improves the appetite and the digestive process[39]. On the another hand, Cross, *et al.*[40] reported that using 5 g kg^{-1} thyme caused a substantial decrease in BWG approaching almost a level of significance.

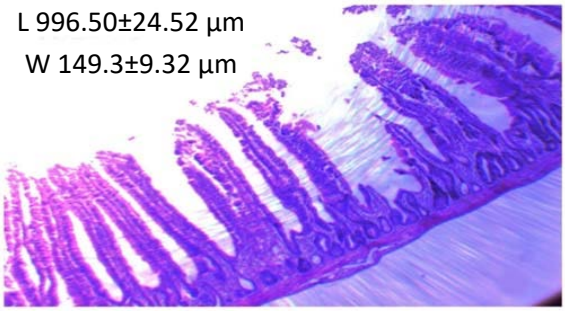

Fig. 9: Intestinal villi of chickens in Gp3 after 6 weeks fed with basal diet and 400 g t^{-1} phyto-plus showing slightly decrease in length (996.50±24.52 µm) and width (149.30±9.32 µm) of villi. (H&E40)

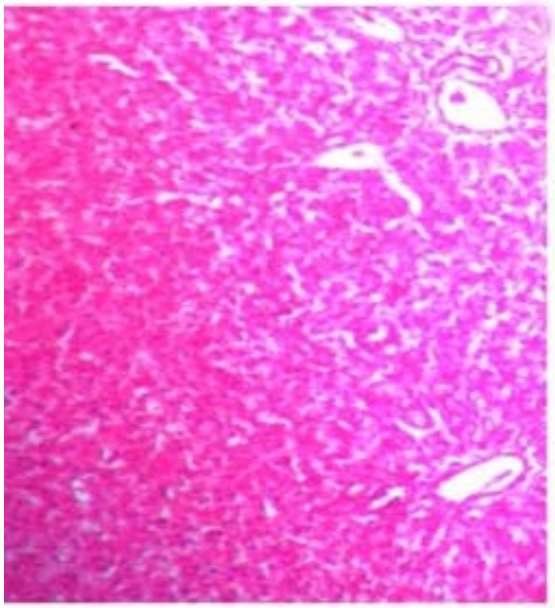

Fig. 10: Liver of chickens in Gp3 after 6 weeks fed with basal diet and 400 g t^{-1} phyto-plus showing fatty degeneration in some hepatocytes. (H&E100)

It is well known that heat shock protein 70 (HSP70) provides protection against free radical toxicity therefore; it is involved in the protection of stressed cells and organisms due to its prominent response to diverse stressors[41]. The current study was performed to identify the enhanced HSP70 in response to phyto-plus and its antistress effect. The obtained results showed a significant increase in the levels of HSP70 with a significant decrease in the levels of corticosterone hormone in groups of birds fed diet supplemented with phyto-plus at dose of 100 g t^{-1} of ration for 3 and 6 weeks.

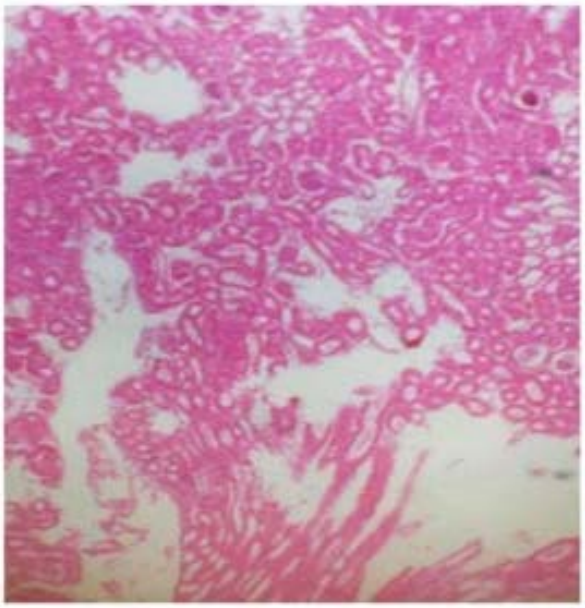

Fig. 11: Kidney of GP3 after 6 weeks, fed with basal diet and 400 g t^{-1} phyto plus showing tubular nephrosis and cystic dilatation of some renal tubules. (H&E 100)

In accordance with these results Wieten *et al.*[17] found enhanced cellular expression of HSP70 in Payer's patches of mice after intragastric administration of carvacrol, attributing to boosting of cellular HSP70 levels in Payer's patches which led to increase in presentation of HSP70 peptides on antigen presenting cells. Furthermore, Ito *et al.*[42] recorded that human daily consumption of enzyme-treated asparagus extract for 7 days increased expression of HSP70 mRNA. It has been reported that HSP70 mRNA expression were higher in chicken hearts under heat stress[43] and in quails exposed to cold stress[41], indicating that HSP70 plays a critical role in cellular homeostasis[44] and protects from stress sensitivity through the oxidant defensive anti-inflammatory and other mechanism[42]. Intriguing, the increased HSP70 has been shown to protect skeletal muscle cells against oxidative stress and free radical toxicity[45]. Since elevation of HSP70 levels leads to cell protection and results in protecting organs against various stresses, the antistress activity of phyto-plus was established. Additionally, lowering the levels of stress hormone as corticosterone hormone in phyto-plus treated groups in the present study is considered another hypothesis sustained the antistress effect of phyto-plus. This may be due to the fact that the autonomic nervous, endocrine, and immune systems interact with complicated biological defense reactions (as heat shock proteins) against various stress[42]. Therefore, it may be due to the fact that enhanced HSPp70 by phyto-plus had

direct inhibitory effect on endocrine gland (adrenal cortex) secreting corticosterone. Further studies were warranted to elucidate validity of this hypothesis through the effect of phyto-plus on HSP70 and corticosterone in stressed boiler chickens.

The present data exhibited increased antioxidant enzymes activities (superoxide dismutase, catalase and glutathione peroxidase) in birds fed diet supplemented with phyto-plus for 6 weeks. The results are in agreement with the results of Hashemipour, *et al.*[46] who showed that thymol plus carvacrol supplementation linearly increased superoxide dismutase and glutathione peroxidase activities and decreased malondialdehyde level in thigh muscle of chickens at d 42 and serum and liver at d 24 and 42, suggesting that the high antioxidant activity of these herbs are due to the presence of phenolic OH groups that act as hydrogen donors to the proxy radicals produced during the first step in lipid oxidation, thus retarding the hydroxyl peroxide formation. Based on these findings, the authors recorded that thymol and carvacrol might play an important role as an exogenous antioxidant and in turn confirms the antioxidant activity of phyto-plus. Moreover, Silveira *et al.*[47] recorded that, herbs rich in flavonoids such as thymol and carvacrol could improve the immune functions through acting as antioxidants and extending the activity of vitamin C. Furthermore, luna *et al.*[48] showed that antioxidant activity of both thymol and carvacrol feed supplementation have similar effectiveness to retard lipid oxidation compared to the supplementation with the antioxidant butylated hydroxyl toluene, indicating that supplementation with natural antioxidant thymol and carvacrol could be applied to improve poultry meat quality.

Morphological changes in the gastrointestinal tract caused by phytogenic feed additive (PFA) may provide further information on possible benefits to the digestive tract. In general, PFA revealed significantly increased in the villus height (VH) across the small intestine. In the absence of any inflammation, this may be due to increase absorptive surface area and efficiency of digestion and absorption[49]. A similar effect of PFA on VH has been reported by Namkung *et al.*[50]. In the present experiment Supplementation of broilers diets with 100 g phyto-plus t^{-1} of ration for 3 weeks increase the length and width of the villus in the duodenum and proliferation of glandular lining epithelial cells. The increase of villi length is an indicator of increased intestinal absorption surface area. Similar to this result, Murakami *et al.*[18] reported that adding essential oil to the broiler's ration increased villus height in the jejunum on day 14. In contrast, Jamroz *et al.*[21] found that supplementing essential oils to the broiler's diets had no effect on villus height in the jejunum statistically but it increased villus height numerically. Increasing the villus height in the duodenum villi could increase the efficiency of absorptive process considering the fact that the majority of absorption occurs in the duodenum. Furthermore, greater villus height increases the activities of mucosal digestive enzymes, resulting in improved digestibility[49,51]. As intestinal crypts are the sources of epithelial cells for villi.

Histological analysis of liver, kidney did not indicate any pathological changes but appear apparently normal with low dose of phyto-plus, indicating that thymol as a potential antioxidant, protects cells against damage by lowering/preventing the loss of mitochondrial membrane potential and inhibition of reactive oxygen species overproduction[52]. Increased length and width of villi with numerous proliferation of goblet cells after 6 weeks of phyto-plus supplementation, in this study, are in agreement with the results obtained by Murugesan *et al.*[49] who reported that villus height, width and goblet cell increased with increase of age of the broiler chicken. This shows a promising effect of phyto-plus on gut health of broilers. The increased villus height and villus width are linked with increased surface area which, in turn, leads to better digestion and absorption. Similarly, Chacher *et al.*[53] observed that a higher number of goblet cell per villus indicates higher production of mucins and glycoprotein compounds that bind with the pathogenic bacteria thus preventing their attachment with the intestinal mucosa. It is worth to mention that essential oils have the ability to reduce the growth of pathogenic bacteria and increase the numbers of benefit one when fed to birds[54]. On the contrary, Vieira *et al.*[55] reported that inclusion of some organic acids did not significantly influence the villus height at any age of broiler's life.

Regarding to pathological changes in intestine, liver and kidney of chickens fed diet supplemented with phyto-plus at dose of 400 g t^{-1} of ration, showed slightly decrease in length and width of the intestinal villi, in addition to the lesion of liver and kidney progress after 6 weeks to fatty change and coagulative necrosis in epithelial cells lining tubules. The results of the present study were in line with the findings of Yigit *et al.*[56] who reported that high level of oregano oil exerted slightly negative effects on organ histology as slightly degeneration and necrosis of liver and kidney of fish, this may be related to toxic constituents, excessive doses, or allergic conditions but they generally have no effects on health when used in the proper doses. Additionally, Fernandez *et al.*[57] found sinusoidal dilatation, vascular congestion and fatty degeneration which observed in all liver of quail fed diet supplemented with 4-5 g thymol per kg of feed, indicating to over feeding. The obtained result in Table 4 and 5 also confirmed that the low dose of phyto-plus (100 g t^{-1} of ration) is more effective and safe than its high dose (400 g t^{-1} of ration).

CONCLUSION

Phyto-plus play an important role as antioxidant and antistress. It also improved bird growth performance and the intestinal histomorphology. Based on the results obtained in this study it can be concluded that the low dose of phyto-plus (100 g t^{-1} of ration) was an optimum supplementation dose compared to the high dose (400 g t^{-1} of ration). More studies are needed to explore its capabilities to support this study.

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