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Physiological Panel of Some Feed Additives for Turkey Toms

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Abstract

A tolerance test was conducted with total 48 male turkeys (strain, Big 6, of three months old) over a period of 70 days, to find the effect of basil and thyme (medicinal plants) and some enzymes (kemzyme and zymogen) as feed additives on turkey toms' performances. Forty-eight male turkeys (3 months old) were randomly divided into eight groups (6 toms/each). The control group (C) was fed a basal diet without feed supplementation. Group (B) was supplemented with basil (3 g basil/kg diet) group (T) was fed thyme (2 g thyme/kg diet), group (TB) was fed a mixture of basil and thyme (3 g basil + 2 g thyme kg diet), group (Z) was fed zymogen (1 ml/4 liter water); and group (K) was supplemented with kemzyme (0.5 g/kg diet), group (BTK) was fed a mixture of basil, thyme and kemzyme, the last group was fed with diet supplemented with basil, thyme and zymogen. Body weight gain, feed conversion ratio was measured at the end of 10^{th} week of experiment. Blood samples were taken to measure some biochemical parameters, at the same time some tissues for morphological and molecular studies were prepared. Toms fed with either basil, thyme or kemzyme (BT) had significantly ($p < 0.05$) heaviest body gain than the control, (Z) or (BTZ). Significant increase occurred of the dressing percent (DP%) in group (B) and (BT) compared to the groups (T, Z, BTK and BTZ).

Supplementation with (K) significantly decreases the serum total lipids than the (C) group and all supplemented groups at $P < 0.05$. Serum cholesterol levels of the groups (T), (BT) and (Z) recorded a significant increase than those of groups (C), (K), (BTK) and (BTZ). Meanwhile; triglyceride levels revealed significant decrease in control group at ($p < 0.05$) than all other groups. All experimental groups recorded no differences in both serum protein and albumin. Although there were an increased levels of serum AST of groups (T) and (Z) compared to control and other groups, meanwhile; (T) and (Z) groups revealed the lowest level of serum ALT.

Concerning the antioxidant parameters, results reviewed that (T), (BT), (BTK) and (BTZ) had a higher level of MDA activity than the (B), (K) and (Z) supplemented groups illustrate no difference versus the serum (MDA) activity of the (C) group. Serum SOD activity revealed no differences within all groups. Serum TAC recorded significant increase in all supplemented groups compared to their level in control group.

Studies of intestinal integrity; morphometry studies indicated that the villous height increased in groups (T), (Z) and (BTK) with a higher villous to crypt ratio and goblet cell numbers of group supplemented with (BTK). Moreover, the villous width revealed a significant increase in the (C) and (Z) groups. The measurement of total DNA of duodenal tissues which reflect the cell mitosis was higher in (T), (Z) and (BTK), (BTZ) at $P < 0.05$; the recorded results of DNA/protein of the same segment of the duodenal tissues revealed higher ratio (higher ribosomal activity) of (T), (Z), and (BTK) groups. Cell size of the duodenal tissues as indicated by (protein/DNA) was increased by (K) and decrease by (T) and (Z) supplementation at $P < 0.05$.

Keywords: basil, kemzyme, thyme, turkey, zymogen

1. Introduction

The use of some antibiotics as growth promoters creates a huge problem for environmental condition and health of consumers around the world (Houshmand et al., 2011; Toghyani et al., 2010). Therefore, there has been growing interest in developing natural alternatives to antibiotic growth promotersin order to maintain both

performance and health (Khan et al., 2012). Consequently, Peric et al. (2010) reported that some natural substances which would influence improvement of health and production traits of broiler chicken can be used such as herbs, enzymes, antioxidants, plant extracts and medicinal and aromatic plants.

The herbs and plant extracts used as feed additives include many different bioactive ingredients such as alkaloids, bitters, flavonoids, glucosides, mucilage, saponin and tannins (Wenk, 2000; Zheng & Wang, 2001). Therefore, the expected effects of herbs and plant extracts are also various, the herbs and plant extracts act on appetite and on intestinal microflora, stimulate pancreatic secretions to increase endogenous enzyme activity and immune system. Many plant products and their constituents have a broad antimicrobial and antioxidant properties. Besides scientists discovered that appetizing and stimulating activity of herbs and plant extracts on animal digestive and immune system could benefit performance and health of poultry (Galinish & Halle, 2000; Tucker 2002; Osman et al., 2010) they postulated the effect could be due to improvement of absorption and utilization of digestive products. Moreover, Abdel-Azeem (2006) and Osman et al. (2005) reported that using medicinal and aromatic plants in broiler diets improved body weight, body weight gain and reduce the cost of feed. Other scientific evidence proved that many herbs and their bioactive constituent possess broad antimicrobial activities which in turn minimize pathogenic bacteria activity in the gastrointestinal tract (Laughout, 2000; Lewis et al., 2003).

The use of enzymes in the feed of animals particularly poultry has become more common (Saleh et al., 2010; Ahmed et al., 2013). Exogenous feed enzymes have been shown to improve performance and nutrient digestibility (Taylor-Pickard & Spring, 2008). In addition Olukosi et al. (2007) found enzymes to be effective in improving growth performance especially at early age. The benefits of adding commercial enzymes preparation to poultry feed have been researched extensively in broiler. Odetallah et al. (2002) reported that adverse effects induced as a result of NSPS complex including the formation of viscous mucus, when fully hydrated and the consequent impedance of nutrient absorption from chyme which cause reduced growth performance, can be alleviated by appropriate enzyme supplementation. Supplementing endoxylanse and β glucanase enzyme mixture to wheat-based diet, significantly improved body weight gain and reduce viscosity of digesta of small intestine, increase growth performance in growing turkey toms (Mathlouthi et al., 2003). Little information is available about the benefits of adding herbs and multienzymes to turkey tom breeder diets. Therefore the present study was designed to investigate the benefits of adding basil, thyme and their combination on some productive performances, integrity and functionality of gastrointestinal tract, Evaluate enzymes as additives (amylase, protease and xylanase) in the form of kemzyme/ration or zymogen/water and compare between a mixture of (basil, thyme and kemzyme) (BTK) and (basil, thyme and zymogen) (BTZ).

2. Material and Methods

2.1 Birds and Location

The present study was carried out at Animal and Poultry Management center, Faculty of Veterinary Medicine, Cairo University, Egypt. The experiment lasted for 10 weeks, during the period from March till May 2015.

Forty eight male turkey toms of Big six breed three months old, with an average body weight 5.5 kg were divided into eight groups of six toms each, they were treated as follow: the control group (C) was fed a basal diet without supplementation, group (B) was supplemented with basil (3 g/kg diet), group (T) was fed Thyme (2 g/kg diet), group (BT) was fed a mixture of basil and thyme (3 g basil + 2 g thyme/kg diet), group (Z) was fed zymogen (1 ml/4 L water), group (K) was supplemented with kemzyme (0.5 g/kg diet), group (BTK) was fed a mixture of basil, thyme and kemzyme and the last group (BTZ) was fed diet supplemented with basil, thyme and zymogen. Each group was kept individual cage with lighting regimen 23 hours light daily (Prescott et al., 2003).

2.2 Feed Additives

Basil: Natural feed additive, its main constituents are methyl chavicol, eugenol, linalool, camphor and methyl cinnamate. It is obtained from *Haraz spice shop Egypt*.

Thyme: Natural feed additives, its main constituents are thymol, carvacrol, linalool and Caffeic acid. It is obtained from *Haraz spice shop Egypt*.

Zymogen: Amultienzyme feed additive composed of amylase, protease, lipase, cellulase, xylanase and pectinase it was a gift from United Biomed Company-Egypt.

Kemzyme: A multienzyme feed additive containing protease, α-amylase, β-glucanase, cellulose, amylase and lipase produced by Kemin industry and provided as a gift from United Biomed Company-Egypt.

2.3 Feed

Basal ration were formulated to cover the nutrient requirements of growing turkey according to National Research Center (NRC) (1994).

The composition of the formulated diet is shown in Table 1, then the additives were added according to the group:

Table 1. Composition percentage and calculated nutrients profile of the basal diets

Ingredients %	Age (weeks)			
	8-12	13-17	18-21	22-24
Yellow corn	49.336	59.262	68.766	75.353
gluten meal	5.657	4.358	3.227	2.467
Soybean meal (44% CP)	38.426	29.598	21.917	16.758
Soy oil	2.176	2.326	2.10	1.169
Dicalcium phosphate	2.273	2.074	2.153	2.21
Limestone	1.162	1.383	0.788	0.795
Common salt	0.298	0.30	0.30	0.30
DL Methionine	0.142	0.148	0.196	0.229
L-Lysin	0.230	0.251	0.253	0.419
Vitamin & mineral premix*	0.30	0.30	0.30	0.30
Calculated Analysis**				
ME (Kcal/kg)	2906.43	3000	3085	3100.62
Crude Protein%	24.02	20.00	17.00	15.01
Calcium%	1.12	1.13	0.90	0.90
Non-phytate phosphorus%	0.55	0.50	0.50	0.50
Methionine%	0.55	0.50	0.50	0.50
Lysine%	1.40	1.00	1.00	1.00
Meth. + Cyst.%	0.95	0.85	0.80	0.77

Note. * Per kg premix: 10 000 000 IU vit. A, 1 000 000 IU vit. D_3, 50 000 mg vit. E, 7 000 mg vit. K_3, 2000 mg vit. B_1, 6000 mg vit. B_2, 2000 mg vit. B_6, 25 mg vit. B_{12}, 50000 mg niacin, 220 mg biotin, 15000 mg folic acid, 400000 mg choline, 2000 mg pantothenic acid, 400000 mg magnesium, 70000 mg zinc, 30000 mg manganese, 75000 mg iron, 5000 mg copper, 750 mg iodine and 250 mg cobalt.

** Calculated analysis was based on National Research Center (NRC) (1994).

2.4 Measured Parameters

2.4.1 Growth Parameter

The live body weight of turkey toms was recorded at the beginning of the experiment (3 month age), he weekly changes and daily feed consumption calculated till the end of the experiment (at the end of the 10^{th} week) to compute the following: Average daily feed intake, average daily body weight gain (g/week), feed efficiency and feed conversion ratio.

2.4.2 Serum Biochemical Parameter

All tests were performed using kits purchased from Spectrum Company, Dokki, Giza, Egypt. Cholesterol was estimated according to Ellefson and Carawy (1976), triglycerides according to (Bucolo & David, 1973), Total lipids estimated according to (Zollner & Kirsch, 1962), total protein method by (Kaplan and Szalbo, 1983), Albumin was measured according to method of (Grant et al., 1987), alanine amino transferase and asprtate amino transferase were estimated according to (Breuer, 1996).

Antioxidant parameters such as malonaldehyde (MDA), serum oxide dismutase (SOD) were performed using kits purchased from Biodiagnostic Company, Dokki, Egypt according to method by Ohkawa et al. (1979), (Nishikimi et al., 1972) respectively and total antioxidant capacity (TAC) according to (Koracevic et al., 2001).

2.4.3 Intestinal Morphometry

At the end of experiment samples from duodenum taken for measuring the length and width of intestinal villi, depth of crypts and number of goblet cells using light microscope according to (Brancroft et al., 1996).

2.4.4 Intestinal Integrity

DNA and RNA purification from tissues (QIA amp DNA, RNA min kit) were applied according to (Fisher & Suttle, 2011).

2.5 Statistical Analysis

Data are represented as means and analyzed by one-way ANOVA using Microsoft office excel computer program excel version 2007 according to (Snedecor & Cochran, 1980). The groups were compared by pooled standard error (SE pooled) at P value ≤ 0.05) (Bret Larget, 2003).

3. Results and Discussion

The present study was conducted to clear the turkey gastrointestinal tract integrity and functionality using feed additives basil (B), thyme (T), kemzyme (K) and zymogen (Z). Most of published research has dealt with growth performance without looking to the integrity of intestinal tract and metabolic parameters. Moreover, the panel of combination of natural medicinal plants and some enzyme endorse little attention.

The herbs and plant extract act on appetite, intestinal microflora, stimulate pancreatic secretions to increase endogenous enzymes activity and immune system. Many plant products have a broad antimicrobial activity, antioxidant and sedative properties (Demir et al., 2005). They postulated that, effect could be due to increase production of digestive enzymes and improved utilization of digestive products (Laughout, 2002).

3.1 Effect of Basil, Thyme, (Basil +Thyme), Zymogen, Kemzyme, (Basil + Tyme + Kemzyme) and (Basil + Thyme + Zymogen on Body Weight (BWt/Kg), Body Weight Gain (BWG/g), Feed Efficiency (FE) and Feed Conversion Ratio (FCR) of Turkey Toms

The results presented in Table 2 indicates that, the body weight (BWt), dressing percentage (D.P.) and both feed efficiency (FE) and feed conversion ratio (FCR) at the end of the experimental period (10^{th} week). Data identify shows a significant increase in (BWt) of toms supplemented with (B) or (T) or (BT) and (K) as compared to that of (C) or (BTK) or (BTZ) groups. The former results is proud with that of Wenk (2000) and Zheng and Wang (2001) who cleared the benefits of herbs and plant like basil and thyme which include many different bioactive ingredients such as alkaloids, bitters, flavinoids, glucosoids, mucilage, saponines and tannins. As well as the results of the growth performance and feed efficiency confirm the finding of Abbas et al. (2010), Alloui et al. (2012), Iji et al. (1999), Feizi et al. (2013), Khan et al. (2012), Mamoun et al. (2014) and Osman et al. (2010), who reported that, broilers fed basil diet had significantly the heaviest body weight. While the natural medicinal plant was considered alternative to antibiotics growth promoters. Also, the results are inagreement with Sharifi et al. (2013) who suggested that herbs and various plants have appetizing and antimicrobial properties. Concerning to all finding as a growth performance and feed conversion the present results in groups supplemented with either (B) or (T), it go hand by hand with the former finding of Alloui et al. (2012) and Mamoun et al. (2014) who suggest a morphological change of a gastrointestinal tissue in broiler chicken that increase the production performance (weight gain and feed conversion).

Moreover, indirectly the increase of Body weight are proud with the evidence that herbs and plant extract stimulate the growth of beneficial bacteria and minimize pathogenic bacteria activity in the gastrointestinal tract (Laughout, 2000; Wenk, 2000). Besides that, scientists discovered appetizing and stimulating activity of herbs and plant extracts on animal digestive and immune system could benefit performance and health of poultry (Abdel Azeem, 2006; Ahmed, 2011; Osman et al., 2005; Tucker, 2002). Thus the obtained results assured the former recorded data. At the same time, the improved body weight could be attributed to the presence of thyme oil which stimulate secretion of digesting enzymes (Aji et al., 2011; Khan et al., 2010; Feizi et al., 2013). Additionally, enzyme supplementation (K) was associated with assured the finding of (Falcao-e-Cunha et al., 2007; Saleh et al., 2006, 2008) While enzyme can partially hydrolyze the non-starch polysaccharide (NSP) reduce the viscosity of gut contents and result in improvements of nutrient absorption.

Santos et al. (2004) and Zhou et al. (2009) who declared that the negative effects of intestinal viscosity due to NSP can be alleviated by exogenous enzyme like kemzyme and enhance metabolizable energy value of the diet and improved body gain. Those results are in agreement with our results concerning (K) supplementation for (BWG) and (FE).

Additionally, the increase of BWt in group supplemented by enzyme (k) declared and confirmed the finding that, the enzyme supplementation improve, digestibility and absorbability of dietary elements (Mathlouthi et al., 2003). Moreover, Margovi et al. (2001) pointed that the exogenous enzymes can survive in the intestine together with the endogenous enzymes and exert their action on available substrates. These activities could be favorable change in pH of gut mediated by exogenous enzyme (Abdl-Rahman et al., 2010). While Douglas et al. (2000)

recorded that broiler performance was not affected by enzyme supplementation. Zakaria et al. (2010) recorded that, exogenous enzyme was not capable of modifying gastrointestinal environment to improve efficiency of feed utilization. Meanwhile Hajati (2010) found that adding enzymes to broilers diet significantly decreased body weight gain and feed intake.

Table 2. Effect of basil, thyme, (basil + thyme), zymogen, kemzyme, (basil +thyme + kemzyme) and (basil + thyme + zymogen) on body weight (BW/kg), body weight gain (BWG/g), Feed Efficiency (FE) and feed conversion ratio (FCR) of turkey toms

Growth	Group								
	C	B	T	BT	Z	K	BTK	BTZ	SE pooled
BWt (kg)	14.91	16.49	16.03	16.26	14.25	16.09	15.87	14.31	0.8
BG (gm)	1.06	1.19	1.26	1.42	1.03	1.32	1.56	1.12	0.5
DP (%)	85.30	86.30	84.30	86.50	82.80	85.00	83.30	81.71	2.1
FE	2.82	2.54	2.75	2.68	2.48	2.68	2.88	2.98	1.2
FCR	0.35	0.71	0.66	0.40	0.42	0.49	0.43	0.72	0.5

Note. Data indicate mean, n = 6/group. Control (C), basil (B), thyme (T), basil + thyme (BT), zymogen (Z), kemzyme (K), basil + thyme + kemzyme (BTK), basil + thyme + zymogen (BTZ), body weight (BWt), body weight gain (BWG), feed efficiency (FE), feed conversion (FCR), pooled standard error (SE pooled).

3.2 Effect of Basil, Thyme, (Basil + Thyme), Zymogen, Kemzyme, (Basil + Tyme + Kemzyme) and (Basil+ Thyme + Zymogen) on Some Serum Metabolic Parameters

Data presented in Table 3 records that serum cholesterol levels were increased by supplementation with (T) or (BT) or (Z) versus to those (C), (B), (K), (BTK) or (BTZ). Moreover, the serum triglyceride was observed to be significantly increased in all experimental groups compared to that level. The former results are in contrast to the previous records of Al-Kassie et al. (2009), Darshana and Thyagaraja (2014), Khan et al. (2011), Isa (2011), and Toghyani et al. (2010) who attributed the reduction of serum cholesterol and triglyceride to the presence of high fiber level in thyme which increased the bile excretion and thus decrease serum cholesterol level. Moreover, Mansoub et al. (2011a, 2011b) found that 1 gm/kg thyme fed to broilers resulted in improved cholesterol profiles. Also, Lee et al. (2003) found a reduction of serum cholesterol and triglyceride levels as results of thyme supplementation was due to the lowering effect of thymol and carvacrol on HMG-COH reductase which is the rate limiting enzyme of cholesterol synthesis. Meanwhile, Sengül et al. (2008) found no effect of thyme on blood cholesterol level. Also, Abbas et al. (2010) reported that basil as a feed additive for broilers resulted in a reduction for cholesterol level. Moreover, increased level of both serum triglycerides and cholesterol levels in groups supplemented with enzyme (K) or (Z) confirm the former reported data of Ahmed et al. (2013), Hajati (2010), Saleh et al. (2006), and Saleh et al. (2010).

The same table shows a significant increase of aspartate amino transferase (AST) serum activities in groups supplemented with either (T) or (Z). Meanwhile, both groups (T) and (Z) recorded a significant decrease of serum alanine amino transferase (ALT) than all other experimental groups in contrast to the results obtained by Ahmed et al. (2013) who found that supplementation of broilers ration with polyzymes did not produce changes in the activities of ALP, AST and ALT. But, these results go hand by hand with (K) group indicating that multi enzyme (K) is safe does not alter hepatic function of turkey toms (Abaza & Omara, 2011).

Table 3. Effect of basil, thyme, (basil + thyme), zymogen, kemzyme, (basil + thyme + kemzyme) and (basil + thyme + zymogen) on some serum metabolic parameters

Parameters	Group								
	C	B	T	BT	Z	K	BTK	BTZ	SE pooled
Cholesterol (mg/dl)	105	99	121	125	114	107	98	94	6.7
Triglyceride (mg/dl)	77	93	92	87	108	108	94	99	6.3
Total lipid (mg/dl)	454	480	545	686	575	419	582	702	5.6
Total protein (g/dl)	6.5	6.5	6.8	6.9	6.5	6.7	6.7	6.9	0.5
Albumin (g/dl)	3.1	2.9	3.0	3.1	3.2	3.1	3.1	2.8	0.2
AST (RFU/ml)	62.3	50.0	72.7	56.3	77.7	53.3	51.7	59.3	6.9
ALT (RFU/ml)	32.3	22.3	21.7	35.0	23.7	28.3	29.0	31.7	5.6

Note. Data indicate mean, n = 3/group. Control (C), basil (B), thyme (T), basil + thyme (BT), zymogen (Z), kemzyme (K), basil + thyme + kemzyme (BTK), basil + thyme + zymogen (BTZ), pooled standard error (SE pooled).

3.3 Effect of Basil, Thyme, (Basil + Thyme), Zymogen, Kemzyme, (Basil + Tyme + Kemzyme) and (Basil + Thyme + Zymogen) on Serum Superoxide Dismutase (SOD) U/ml, Total Antioxidant Capacity (TAC) mM/L and Lipid Peroxidation Malondialdehyde (MDA) nmol/ml

The illustrated results of serum antioxidant activity in Table 4 of turkey toms at the end of experimental period (10 weeks), recorded that there was a significant decrease in activity of lipid peroxide (Malondialdehyde) in groups of toms supplemented with (B) while those toms supplemented with (T) or (BT) or (BTK) or (BTZ) revealed significant activities of lipid peroxidase (MDA) Moreover the same table shows the (MDA) activity in serum of toms supplemented with (K) or (B) or (Z) had no change versus control group. The present results are proud with those obtained by Hamada et al. (2015) which reported that broilers supplemented with basil showed a significant increase of SOD and TAC. While the result of MDA in all groups contradict the former recorded data by the same author. Moreover, Dusgupta et al. (2004) reported that O. basilica increase activity antioxidant enzyme responses by significantly increasing activities of the hepatic glutathione reductase, superoxide dismutase and catalase activity in liver. Also, Hussain et al. (2008), and Meera et al. (2009) added that basil extract showed significant anti-lipid peroxidation effect in vitro, in addition to exhibiting significant activity in scavenging superoxide radical and nitric oxide radicals indicating potent antioxidant effect.

MDA is one of the most frequently used biomarkers providing an indicator of the overall lipid peroxidation and may be a potential biomarker for oxidative stress (Killie et al., 2003). The decrease of (MDA) activities may be due to antioxidant activities of basil (Seung-Jool et al., 2005; Hussein et al., 2008; Zhang et al., 2009; Meera et al., 2009) on the other hand values of superoxide dismutase (SOD) illustrate no differences in all groups. Meanwhile, the serum total antioxidant capacity (TAC) recorded significant higher values in all experimental groups compared to that level of control. At the same time, the present values of MDA for groups supplemented with (T) is opposite to the finding of Bolukbasi et al. (2006) and Schiavone et al. (2007) who considered thyme leaves and their contents of biphenylic as well as flavinoid compounds have been found to exhibit antioxidant capacity. As well as Rahim et al. (2011) assured that thyme and oregano contain large amount of monotrepens thymol, carvacrol and flavinoids are exerting antioxidant properties. Concerning the increase of SOD in group supplemented with thyme (Shwartiz et al., 1996; Yanishlieva et al., 1999) recorded that, Phenolic components like carvacrol and thymol were found to be responsible for the stabilization of thyme oil due to their antioxidant activities Tymol and carvacrol are reported to inhibit lipid peroxidation. Moreover, the phenolic trepens improved stability of poultry production by decreasing lipid peroxidation. (Schiavone et al., 2007) suggested that high antioxidant activity of the thymol is due to the presence of phenolic hydroxy (OH) group that act as hydrogen donors to the proxy radicals which are produced during the first step of lipid oxidation which in turn retard the hydroxyl peroxide formation.

Moreover, Hashimpour (2012) found that chicken supplemented with thymol caused an increase in SOD activity while MDA was reduced in the liver and serum by these inclusions thus the active substance of these phytogenic products may improve the antioxidant status due to antioxidant properties of thymol and carvacrol by elevating the activity of antioxidant enzymes.

Concerning the total antioxidant capacity (TAC) measures the overall antioxidant capacity (Sharma & Kaur, 2015) Assured the antioxidant properties of all used feed additives ((T), (B), (K), (Z), (BTK) and (BTZ)) by the obtained higher values of (TAC) of all groups. Sometimes the present data of superoxide dismutase (SOD)

which contribute to the first line of antioxidant pathway as it plays a role in the defense of cell against the toxic effects of oxygen radicals (Chakraborty et al., 2009).

Table 4. Effect of basil, thyme, (basil + thyme), zymogen, kemzyme, (basil + thyme + kemzyme) and (basil + thyme + zymogen) on serum superoxide dismutase (SOD) (U/ml), total antioxidant capacity (TAC) (mM/L) and lipid peroxidation (malonaldehyde) MDA (nmol/ml)

Anti-Oxidants	Group								
	C	B	T	BT	Z	K	BTK	BTZ	SE pooled
MDA (nmol/ml)	34	30	39	47	32	37	39	42	3.0
SOD (U/ml)	200.8	202.1	202.1	203.4	199.9	201.7	200.9	201.5	2.1
TAC (mM/L)	0.6	1.6	0.8	2.1	1.7	0.9	1.5	1.9	0.1

Note. Data indicate mean, n = 3/group. Control (C), basil (B), thyme (T), basil + thyme (BT), zymogen (Z) kemzyme (K), basil + thyme + kemzyme (BTK), basil + thyme + zymogen (BTZ), pooled standard error (SE pooled).

3.4 Effect of Basil, Thyme, (Basil + Thyme), Zymogen, Kemzyme, (Basil + Thyme + Kemzyme) and (Basil + Thyme + Zymogen) on Serum Thyroid Hormones (T_3 & T_4)

Turkeys supplemented with B and BTZ recorded a significant increase in serum T_4 level compared to C while group supplemented with Z, BTK and BTZ showed significantly higher T_3 level compared to control.

Iqbal et al. (1990) and Yahav et al. (1998) pointed the relation between ambient temperature, feed intake, growth rate and plasma T_3 levels may be associated with changes in structural and function of intestinal tract. Supplementation of turkey ration in the current study with (B), (BT) and enzymes exhibited significant increase in the concentration of serum T_3 this increase in T_3 level after treatment with herbs and enzymes may be due to enhancement of the digestion of food and absorption of nutrients, similar results obtained by Adams (2001), Ahmed et al. (2013) ,Collin et al. (2003), Hajati et al. (2009), and Saleh et al. (2006) who revealed that blood level of T_3 was elevated in broiler supplemented with enzymes while T_4 level was not significantly affected. Mousa (2008) found that no difference was observed in serum TSH and serum T_4 in broilers supplemented with polyzyme. This might be attributed to the direct or indirect stimulatory effect of the de-iodinaze activity in liver and kidney tissue promoting the transformation of T_4 to T_3 as described. A positive linear correlation has been found in chicken and turkeys between plasma T_3 level and feed intake and growth rate (Yahav et al., 1998).

Table 5. Effect of basil, thyme, basil + thyme, zymogen, kemzyme, basil + thyme + zymogen and basil + thyme + kemzyme on serum thyroxine level (T_4) (ug/dl) and tri-iodothyronine level (T_3) (nmol/L)

Hormone	Group								
	C	B	T	BT	Z	K	BTK	BTZ	SE pooled
T_4	1.4	2.1	1.4	1.6	1.2	1.5	1.6	2.0	0.8
T_3	4.7	8.6	4.2	8.0	7.2	9.4	6.9	7.6	0.5

Note. Data indicate mean, n = 3/group. Control (C), basil (B), thyme (T), basil + thyme (BT), zymogen (Z) kemzyme (K), basil + thyme + kemzyme (BTK), basil + thyme + zymogen (BTZ), pooled standard error (SE pooled).

3.5 Effect of Basil, Thyme, (Basil +Thyme), Zymogen, Kemzyme, (Basil + Thyme + Kemzyme) and (Basil + Thyme + Zymogen) on Intestinal Villous Length, Crypt Depth and Number of Goblet Cells

It is well known that the intestinal lumen is protected with developed healthy integrity of intestinal wall. Continuous layer of epithelial cells which is formed as a proliferation of crypt cells, that differentiate into mature cells (absorptive, goblet, and enteroendocrine cells). Also, part of the epithelial differentiation the so called villous structure (the main absorptive area). The villous height and crypt depth are studied by many authors (Ferrer et al., 1995; Uni et al., 1995, 1996, 1998). The functional integrity of the intestinal mucosal epithelial cells depends on coordinated regulation of the mucus layer which is main secretory product of goblet cells which serve as the front line of innate host defense mechanism (Kim & Samuel, 2010).

The results obtained from the present study (Table 6, Figures 1 and 2) revealed that, dietary supplementation of (T), (Z) or (BTK) groups induced higher values of villous height in comparison of those supplemented with (B),

(BT), (K), (BTZ) or (C) groups. At the same time, all groups supplemented with either herbs or enzymes recorded a higher significant ratio of villous height to crypt depth (V/C) in comparisons to those of control group. Similar results were obtained by Loodi et al. (2004) and Mathalothi et al. (2002) who concluded that the exogenous enzymes improved nutrient digestibility leading to increasing the villous surface and concentration of conjugated bile salts. Moreover, Ahmed et al. (2013), Bala murugan et al. (2011), Neto et al. (2012), and Zikic et al. (2008) stated that enzymes are able to increase the villi height and width and reduce height and width of crypts. These changes were represented by elongation of villi and a higher villi/crypt depth ratio which indicate lower rate of enterocytes migration from crypts to the villous.

Moreover, the increase in villous height of turkey toms supplemented with thyme (T) in their diet in the present study showed that intestinal mucosal architecture can reveal information on intestinal function, increasing the villous height suggests an increased surface area for greater absorption of available nutrients as recommended by (Awad et al., 2009). Also, the present results revealed that (V/C) was higher in thyme (T), thyme plus basil (BT) treated groups versus control. This increase is related to increase digestion& absorption (Montague et al., 2003; Plusk et al., 1996).

Contradictory results obtained by Guo et al. (2004), and Garcia et al. (2007) who found no differences in intestinal morphology among groups receiving either medicinal plants or a blend of plant extract. Moreover, Peric et al. (2010), and Shams Sharph (2012) pointed that, phytogenic blend did not affect villous height, but caused the highest crypt depth and lower villous height/crypt depth (V/C). Consent the previous finding of Xu et al. (2003) that antimicrobial agents such as essential oils or their active components are known to reduce the intestinal microbial load which in turn reduces the presence of toxins that associated with the changes in intestinal histomorphology such as villous height and crypt depth. Moreover, Ocak et al. (2008) suggests that thyme leaves as growth promotors in broilers feed had no effect on relative weights of whole gut but, with other mechanism. Thus, indicate that the improved production results obtained in broilers fed phytogenic additives are not directly connected the improved gut morphology,

The obtained results revealed that the highest number of goblet cells was recorded in basil and basil plus thyme supplementation groups. Similar results were obtained by Jamaroz et al. (2006) who observed quantitative increases in the number of goblet cells and mucin secretion at the surface of the villi when feeding broilers, a mixture of plant extract contain carvacrol.

Although the density of goblet cells may result in an increase in mucin secretion, the number of goblet cells cannot be used to quantify this mucin secretion. The mucus layer plays key roles in the establishment of the commensal intestinal microbiota and the protection from colonization and invasion by the pathogenic microbiota (Kim & Samuel, 2010).

Table 6. Effect of basil, thyme, (basil + thyme), zymogen, kemzyme, (basil + thyme + kemzyme) and (basil + thyme + zymogen) on intestinal villus length (µm), villus width (µm), crypt depth (µm), villus length to crypt depth ratio and number of goblet cells/villus in turkey duodenum

Parameter	Group								
	C	B	T	BT	Z	K	BTK	BTZ	SE Pooled
VL (mean)	978	643	1187	682	1317	1028	605	1290	67.4
Min	876	611	1125	603	1300	960	519	1225	
Max	1055	688	1322	822	1350	1106	672	1340	
CD (mean)	388	85	208	96	277	200	72	107	48.6
Min	322	79	106	80	147	157	54	92	
Max	489	96	284	111	342	273	96	127	
V/C Ratio	3.00	8.00	6.00	7.00	5.00	5.00	8.60	12.00	1.0
NGC (mean)	74	121	79	106	27	69	179	91	8.6
Min	68	112	72	98	23	65	86	162	
Max	82	130	85	117	32	75	95	192	
VW (mean)	12.4	11.0	10.3	9.3	14.6	10.1	10.6	11.7	1.2
Min	9.2	9.2	9.6	8.1	12.8	9.1	9.6	9.8	
Max	13.9	13.8	11.5	10.3	16.5	11.3	11.5	13.5	

Note. Data indicate mean, n = 4/group. Control (C), basil (B), thyme (T), basil + thyme (BT), zymogen (Z) kemzyme (K), basil + thyme + kemzyme (BTK), basil + thyme + zymogen (BTZ), VL (villus length), VW (villus width), CD (crypts depth), NGC (number of goblet cells).

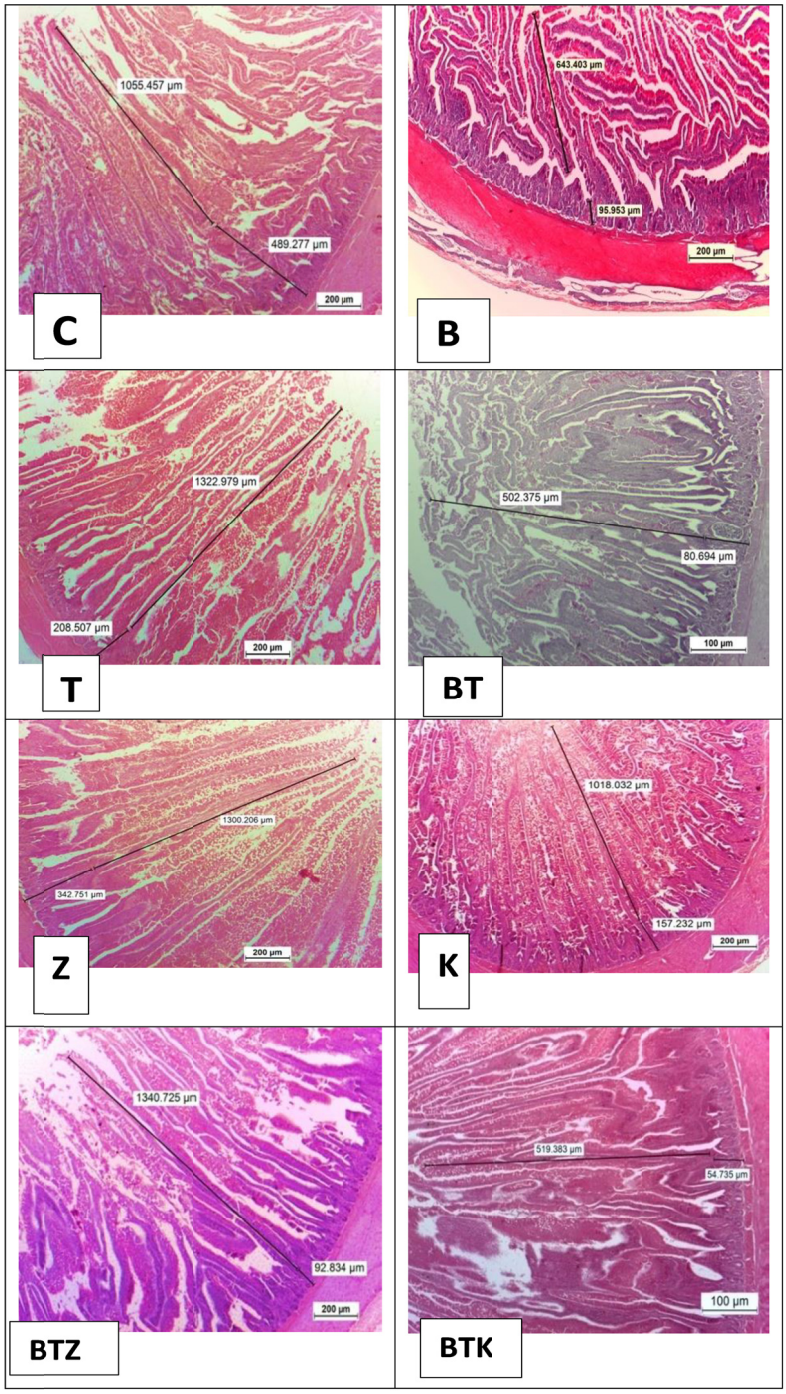

Figure 1. Photomicrographs of the small intestine (duodenum) from male turkey chicken showing the villous heights, crypt depths (H&E X40)

Note. Control (C), basil (B), thyme (T), basil + thyme (BT), zymogen (Z), kemzyme (K), basil + thyme + kemzyme (BTK), basil + thyme + zymogen (BTZ).

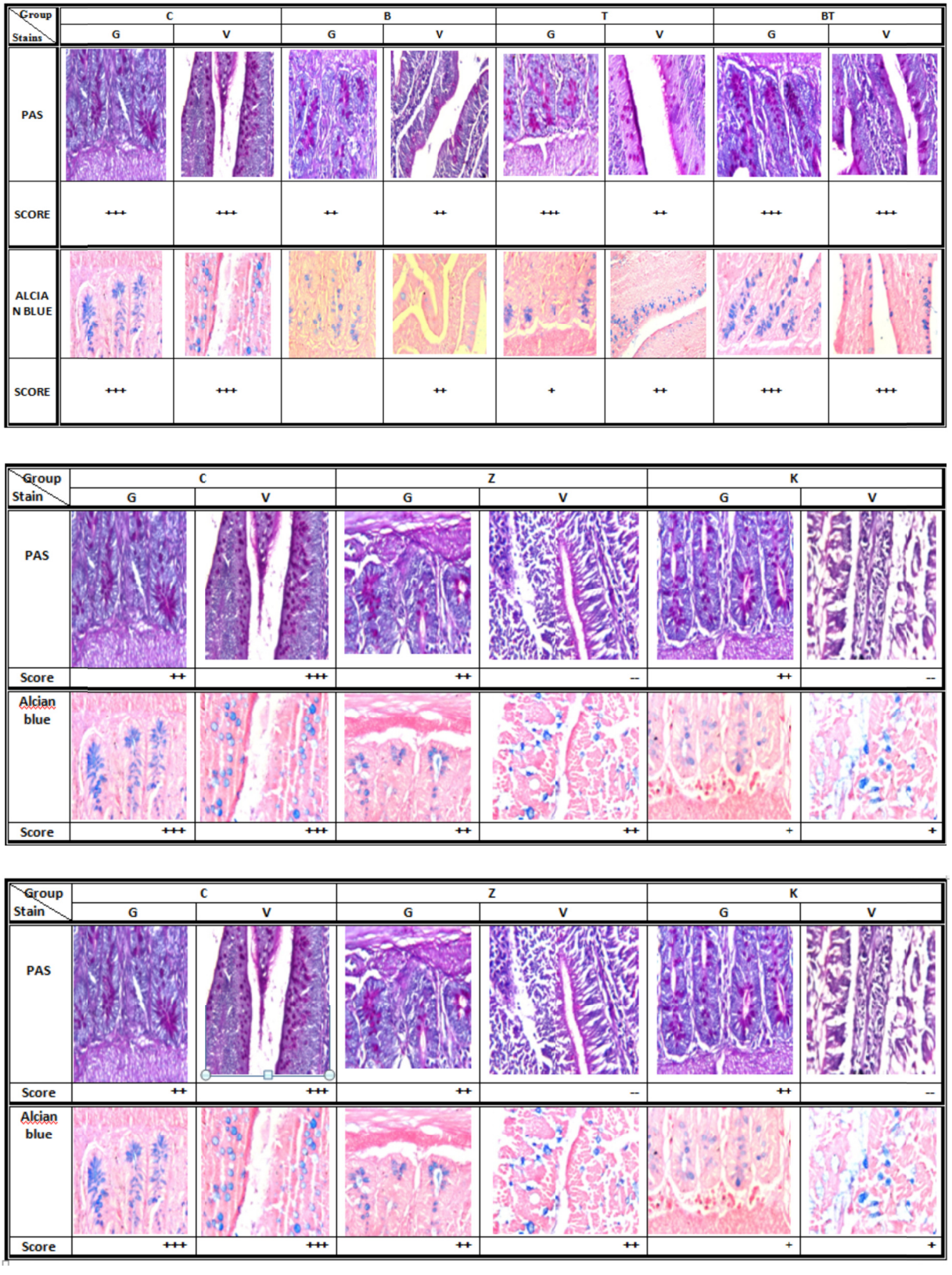

Figure 2. Showing the histochemical alteration in mucin of the intestinal mucosa of different experimental groups (H&E X40)

Note. G = gland, V = Villi mucosa. +++ severe, ++ moderate, + mild, -- nil.

3.6 Effect of Basil, Thyme, (Basil + Thyme), Zymogen, Kemzyme, (Basil + Thyme + Kemzyme) and (Basil + Thyme + Zymogen) on Intestinal DNA, RNA Concentration and Protein Synthesis

The determination of DNA, RNA and protein concentration in of intestinal tissue, together with morphological measurements, has provided novel knowledge about intestinal development and integrity such as, DNA/RNA ratio, DNA/Protein ratio (Uni & Saklan, 1999). The DNA concentration in a tissue reflects its rate of mitosis in a cell population, with the protein to DNA ratio indicating the cell size (Jin et al., 1998).

The present data recorded (Table 7) a significant increase in the total DNA of duodenal tissue of the groups supplemented with (B), (T), (Z), (BTK) or (BTZ). Moreover, the revealed results of DNA/protein of the duodenal tissues indicate higher ratio. These results increase DNA concentration in the duodenal tissue and reflect its mitoses in cell population which consent of Jin et al. (1998). The same table revealed a decrease of RNA concentration of most experimental groups (T), (K) and (BTK). The former obtained results go hand by hand with the previous suggestions of Jin et al. (1998), Uni et al. (1998), and Iji (1999) indicated that the concentration of DNA, RNA and their ratio to protein content, undergo variation and these variation not always consistent and differ from species or breed to others.

Taking the concepts of the all former authors in consideration of the development and cell size the present results recorded that supplementation of (T) and (BTK) increase the DNA/RNA, DNA/Protein and RNA/Protein. Meanwhile, RNA/Protein was increased by supplementation of most of the used feed additives (T), (B), (BT), (Z) and (BTZ). Also, the ratio of Protein/DNA increased only in group supplemented with (K). The variable results may be due to individual variation or the more short duration and late time (age of toms) of experimental application.

Herbs and botanicals contain many different antioxidants with a high potential for the protection of nutrient against oxidation and digestive tract in metabolism as well as in the products Several phytochemicals like essential oils or dietary fiber can contribute a balanced microflora (Eubiosis) and optimal precondition for an effective production against pathogenic microorganisms and intact immune system (Casper, 2003).

Table 7. Effect of basil, thyme, (basil + thyme), zymogen, kemzyme, (basil + thyme + kemzyme) and (basil + thyme + zymogen) on intestinal DNA, RNA concentration and protein synthesis

Parameter	Group							
	C	B	T	BT	Z	K	BTK	BTZ
DNA (ng/µl)	124	254	532	337	674	158	1440	685
RNA (ng/µl)	1530	728	415	1045	1105	410	181	835
Protein (ng/µl)	0.92	0.96	0.98	1.49	0.97	1.49	1.51	0.93
DNA/RNA	0.08	0.35	1.28	0.32	0.61	0.39	7.96	0.82
DNA/Protein	135.4	264.3	541.8	226.2	695.6	105.8	955.	738.1
RNA/Protein	1663	758.3	423.5	701.3	1139	275.2	119.9	897.9
Protein/DNA	0.007	0.004	0.002	0.004	0.001	0.009	0.001	0.001

Note. Data indicate mean, n = 3/group. Control (C), basil (B), thyme (T), basil + thyme (BT), zymogen (Z), kemzyme (K), basil + thyme + kemzyme (BTK), basil + thyme + zymogen (BTZ), pooled standard error (SE pooled).

4. Conclusion

Supplementation of turkey rations with herbs (basil & thyme) and multienzymes (zymogen & kemzyme) either alone or in combination produce a significant improvement in turkeys' performance:

(1) They may regulate feed intake.

(2) Increase intestinal surface area for more absorption and utilization of feed.

(3) Contain different antioxidants activity.

All additives used in the experiment were safe and did not alter liver function and they improved all measured growth and metabolic parameters.

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