

## **EFFECT OF USING L-CARNITINE, ASCORBIC ACID AND PROBIOTICS ON PRODUCTIVE PERFORMANCE, MICROBIAL LOAD AND HISTOLOGICAL OBSERVATIONS IN LOCALLY-DEVELOPED BROILERS**

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*(Received 15/1/2015, accepted 3/3/2015)*

### **SUMMARY**

**A**n experiment was conducted to investigate effects of supplemented L-carnitine, ascorbic acid and probiotics on productive performance, carcass characteristics and some bacterial and histological aspects in locally-developed broiler strain. 252 unsexed chicks 2 weeks of age were reared up to 8 weeks of age were distributed into 7 treatments in 6 replicates of 6 birds each. The experimental groups were as follows:

T1: Control group fed basal diet.

T2: Basal diet supplemented with 500 mg ascorbic acid / Kg diet.

T3: Basal diet supplemented with 1000 mg ascorbic acid / Kg diet.

T4: Basal diet supplemented with 75 mg L-carnitine / Kg diet.

T5: Basal diet supplemented with 150 mg L-carnitine / Kg diet.

T6: Basal diet supplemented with 0.5 g Probiotic / Kg diet.

T7: Basal diet supplemented with 1.0 g Probiotic / Kg diet.

The results showed that live body weight, daily weight gain, daily feed consumption or feed conversion ratio were insignificantly different during the first 2 weeks of studied period among all groups. Birds fed T2 diet gained significantly higher weight compared with other groups at overall period. While feed conversion ratio of birds fed T4, T5, T6 and T7 were the best significantly compared with the other groups. Dressing percentage of all tested groups was increased significantly compared with control group. While birds fed T3 or T5 diets deposited significantly less abdominal fat compared with the other groups. Microbial studies showed that aerobic bacteria decreased by feeding different treatments in caecum and small intestine compared with control. Also, anaerobic (beneficial) bacteria increased in caecum and small intestine in comparison with control. As well, feeding birds on probiotics or ascorbic acid had completely eliminated *Campylobacter* from chicken meat. Probiotics treatment was the more effective in decreasing total bacterial count in carcass meat. Histological observation revealed that dietary treatments had a positive effect on the number and size of fasciculi and consequently their myofibers of muscles, normal liver and spleen structure was noticed, expect for some slight changes. Therefore, it is suggested that L-carnitine, ascorbic acid or probiotics, could be used safely as feed additives to improve performance without any adverse effect on body organs or gastro-intestinal tract microbial population.

**Keywords:** *L-carnitine, ascorbic acid, probiotic, broiler, histology, microbiology*

### **INTRODUCTION**

Since decades, with the initiation and development of synthetic and semi-synthetic antibiotics and hormones which have been used as animal feed additives. However, increasing concerns regarding over use of antibiotics has prompted extensive investigation into alternatives. Hence, research workers have been experienced throughout the last five decades and being directed to the research back to natural antimicrobial products as indispensable resources. Consequently, there is considerable research interest in the possible use of natural products, like, vita- min supplements, enzymes, probiotics, medicinal plants and herbs for the development of new additives in animal feeding. Incorporation of natural feed additives as growth promoters in some countries is not exceeding more than 10 to 20 years. Use of probiotics, as animal feed additives, in our region is still new and need more emphasize to develop its applications.

Probiotics are live microorganisms which will have beneficial effect to the host animal by improving its small intestine microbial balance through inhibiting intestinal pathogens (*E. coli*), and by growing and multiplying and competitively excluding undesirable bacteria (O'Keefe, 2005). In fact, mode of actions of probiotic is still unclear despite the suggestions given by Montes and Pugh (1993) 1) beneficial changes in gut flora with reductions in the population of *Escherichia coli*, 2) lactate production with subsequent changes in intestinal pH, 3) production of antibiotic type substances, 4) production of enzymes, 5) competition for adhesion receptors in the intestine, 6) competition for nutrients, 7) reduction of toxin release and immuno-stimulation. Cavazzoni *et al.* (1998) evaluated performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic and found that feeding probiotic supplements increase the growth rate of broilers. There have been many previous studies to evaluate probiotics on broiler and to give good reason for its impact on broiler growth and health status different mechanisms have been proposed. Kabir (2009) stated that probiotic effects on intestinal microflora and pathogen inhibition, intestinal histological changes, immuno-modulation, some haemato-biochemical parameters and subsequently improve growth performance of broilers. He also mentioned that probiotic improves sensory characteristics of dressed broiler meat and microbiological meat quality of broilers. However, it is mentioned that the main effect of probiotic is in the gastrointestinal tract and associated with its capacity to stimulate the immune response and to control the growth of pathogenic bacteria. Kabir (2009), Higgins *et al.* (2007), Huang *et al.* (2004) and Mountzouris *et al.* (2007).

The chemical name of Vitamin C, a water-soluble vitamin, is ascorbic acid and it plays an important role in capillary fragility and joint diseases. According to the fact that some metal ions play a very important role in degrading ascorbic acid, it appears that substances that form a chelate, that is to say block metal ions, have a stabilizing effect on ascorbic acid (Cemeroglu, 2004). According to Sahin *et al.* (2003) 250 mg L-ascorbic acid increased body weight gain, feed intake, feed conversion, hot and cold carcass weight in broilers.

L-carnitine as a nutritional supplement in today's commercial poultry has been considered; regarding the role of L-carnitine in fatty acid metabolism. It is expected that in addition to improve the production yield, it is important to increase the quality of meat and reduce poultry body fat gain. In this study, different levels of L-carnitine in broiler diets have been used Daskiran and Teeter (2001) and Xu *et al.* (2003). Reports about the effects of a diet filled with L-carnitine on performance, growth and composition of broilers are contradictory. Some studies have shown that L-carnitine supplementation improved body weight gain and decreased abdominal fat of broilers Lettner *et al.* (1992) and Rabie *et al.* (1997).

The objective of the present study was to examine effects of supplementation of L-carnitine, ascorbic acid (vitamin C) and probiotics on performance, histological observations of internal organs as well as some microbiological features in locally-developed broiler strain.

## **MATERIALS AND METHODS**

The present study was carried out at the Poultry Nutrition Unit, Poultry Production Department, Faculty of Agriculture, Ain Shams University.

### ***Experimental diets and birds:***

Two hundred and fifty-two local developed strain 2 weeks of age with average weight of 217g were distributed into seven treatments with six replicates of six chicks each in wire-floored batteries up to 8 weeks of age. The experimental diets were as follow:

T1: Control group fed basal diet

T2: basal diet supplemented with 500 mg ascorbic acid / Kg diet.

T3: basal diet supplemented with 1000 mg ascorbic acid / Kg diet.

T4: basal diet supplemented with 75 mg L-carnitine / Kg diet.

T5: basal diet supplemented with 150 mg L-carnitine / Kg diet.

T6: basal diet supplemented with 0.5 g Probax<sup>#</sup> / Kg diet.

T7: basal diet supplemented with 1.0 g Probax<sup>#</sup> / Kg diet.

# Probax is a probiotic for poultry containing *Lactobacillus sporogenes*.

Basal diet was formulated to provide the nutrient requirements according to guideline of NRC (1994), the composition and calculated chemical analysis was listed in (Table 1). All birds were reared under

similar environmental, managerial and hygienic conditions. Feed and water were provided *ad libitum*. Vaccination programs were applied according to the scheme of vaccination used in the laboratory.

**Table (1): Feed ingredients and chemical composition of basal diet (control diet).**

Ingredients	%
Yellow corn (grains)	66.00
Soybean meal (44%)	26.10
Corn gluten meal (60%)	1.50
Soybean oil	1.60
Calcium carbonate	1.60
Mono-calcium phosphate	1.30
Di-calcium phosphate	0.65
HCl Lysine	0.20
DL-Methionine	0.45
Salt (NaCl)	0.30
Vitamin and mineral premix <sup>#</sup>	0.30
<b>Chemical analysis (Calculated)</b>	
Crude Protein %	18.02
ME Kcal/ Kg diet	3006
Calcium%	1.05
NPP%	0.50
Methionine + cystine	1.06
Lysine	1.09

<sup>#</sup> each 3 Kg of the vitamins and minerals premix contain: Vitamins: A: 15000000 IU; Vit. D3 2000000 IU; E: 50 g; K3: 3000 mg; B1:3000 mg; B2: 8000 mg; B6:4000 mg; B12: 20 mg; Biotin: 200 mg; Coline chloride: 700 mg; Pantothenic acid: 200000 mg; Nicotinic acid: 60000 mg; Folic acid: 1500 mg; Minerals: Mn: 80 g; Zn: 80 g; Fe: 60 g; Cu: 10 g; I: 1 g; and Se: 0.2 g, and CaCo<sub>3</sub> as a carrier up to 3 Kg.

**Bird growth performance:**

Body weight and feed consumption were recorded weekly. Weight gain and feed conversion ratio (g feed/g gain) were calculated weekly.

**Bird carcass characteristics:**

At 8 weeks of age, six birds from each treatment (one from each replicate) having the average body weight of each treatment were selected and sacrificed by cervical dislocation to determine carcass characteristics.

**Microbiological studies:**

At 8 weeks of age, six hens of each experimental group were chosen for determination the total aerobic and anaerobic count in the small intestine and caecum. Determinations of total aerobic, mesophilic, psychrophilic, total coliform and faecal coliform counts in the meat were carried out too, while the contents of caecum, small intestine and meat were tested for *Salmonella*, *Staph aureus*, *Listeria* and *Campylobacter* infection.

Total aerobic, anaerobic, psychrophilic, mesophilic counts were carried out according to Berrang *et al.* (2001). Total coliform and faecal coliform counts were carried out according to Mercuri and Cox (1979). Isolation of *Salmonella* was carried out according to Ellis *et al.* (1976). The suspected colonies were sub-cultured on nutrient slope agar and incubated at 37 C° for 24 hr, biochemical tests for *Salmonella* identification attempts were made using the criteria described by Krieg and Hot (1984) using the following tests: growth on TSI, Urea, Indole, M.R, V.P and sugar fermentation. Serological tests were carried out according to Kauffmann (1973).

Isolation of *Staph aureus* was carried out according to Gouda (2002). The isolation of *Staph aureus* based on appears as black, convex and shiny colonies surrounded by a yellow zone on Vogel Johnson agar medium. Isolation of *Campylobacter* was carried out according to Oosterom *et al.* (1983). The isolation of *Campylobacter* based on appearance grey, moist, flat and spreading colonies. Isolation of *Listeria* was carried out according to USDA-FSIS (1989). The isolation of *Listeria* based on appearance dew-drop-like, dark brown or black colonies with halo.

***Tissue preparation for histological studies:***

At autopsy a representative sample (0.5×0.5cm) from the left major muscle pectoralis, spleen, and liver of six birds from each treatment at 8 weeks of age were carefully dissected during the slaughtered time and immediately fixed in adequate volume of 10% formalin solution. The paraffin technique has been used according to (Junqueira *et al.*, 1971; Al-Hussaini and Demian, 1974; Abd El-Hamid, 1981). Thin sections (4-5 micron) were cut, and mounted on glass slides (two sections/sample/slide), then stained with the ordinary hematoxyline and eosin stain procedures.

The histological technique of muscles and lymphoid organs were conducted in the Pathology Laboratory, National Cancer Institute, Cairo Univ., Egypt. The histological structures of pectoral muscles, liver and spleen were observed using light microscope (Labomed, LX 400. Labo, America, Inc. USA) endorsed with a digital camera under low ×10 and high ×40 magnification. Photography shots from the selected specimens were prepared for better illustration of the results.

***Statistical Analysis:***

Data were subjected to one-way ANOVA analysis of variance General Linear Model (GLM) procedure of SAS software SAS (1998) user's guide according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where;  $Y_{ij}$  = the obtained experimental observation,  $\mu$  = overall mean,  $T_i$  = dietary treatment,  $e_{ij}$  = experimental error. Individual effects of dietary treatments were compared using Duncan (1955) multiple range tests at  $\alpha$  level equal to 0.05 or 0.01.

## **RESULTS AND DISCUSSION**

***Productive performance:***

Data presented in (Table 2) showed the effect of L-carnitine, ascorbic acid and probiotic on productive performance traits. Birds fed basal diet supplemented with experimental treatment didn't differ with control group during first two weeks of experimental period for live body weight, daily weight gain, and daily feed consumption and feed conversion ratio.

For daily weight gain (BWG), birds fed basal diet supplemented with 1 g/ Kg probiotic (T7) gained more weight compared with control group where recorded 29.49 g/ day compared with 27.27 g/day for control group with significant differences ( $P \leq 0.01$ ).

These results were in harmony with those obtained by Kaoud (2010) who reported significant increase in BWG of broilers fed probiotic mixture (containing *Lactobacillus acidophilus*, *Lactobacillus sporogenes* and *Saccharomyces cerevisiae*) at 0.5 g/ Kg feed at 6 week of age. Similar reactions for broiler chickens fed with probiotic were also reported by Panda *et al.* (2006) and Mountzouris *et al.* (2007). The improvement in body weight gain in present study could be ascribed to improvement in digestion and absorption of nutrients in the digestive tract due to presence of amylase derived from the *Lactobacilli* and promotion of health status of broilers (Bansal *et al.*, 2011).

In contrast, probiotics supplementation did not improve the body weight gain in broilers (Midilli *et al.* (2008). On the other hand, birds fed diets supplemented with ascorbic acid (1000 mg/ Kg) gained weight more than control but without significant manner, these results were in agreement with those obtained by Sosnowka-Czajka, *et al.* (2005). In contrast to our results, El-Shafei *et al.* (2013) did not obtain any improved weight gain in the supplemental groups of ascorbic acid, and Konca *et al.* (2009) also stated that growth performance of broiler chickens was not influenced by ascorbic acid supplementation. L-carnitine supplementations (150 mg/ Kg) didn't gained significant weight compared with control (27.65 vs. 27.27), these finding were in concert with those of Rabie and Szilagyi (1998) and Buyse *et al.* (2001) who observed a positive effect of L-carnitine on the body weight of chickens on the end of fattening period but the differences were not significant ( $P < 0.05$ )

Other authors who studied the effect of L-carnitine on broiler performance found no effect on body weight (Lien and Horng, 2001; Xu *et al.*, 2003; Cevik and Ceylan, 2005). Probiotic supplementations didn't affect daily feed consumption compared with control, birds fed basal diet supplemented with 0.5 or 1 g/ Kg probiotic consumed feed (64.70 or 70.62) similar to control group (68.35) without any significant differences. These data were similar with those obtained by Chumpawadee *et al.* (2008) and Midilli *et al.*

(2008). On the contrary the finding of Balevi *et al.* (2000) indicated that supplementation with probiotic at a level of 0.5 g/ Kg diet improved feed intake.

Ascorbic acid supplementations didn't have an effect on daily feed consumption; the results were 68.35, 68.80, and 69.62 g for control, 500 and 1000 mg/ Kg respectively. These results were in agreement with those obtained by Sabah-Elkheir *et al.* (2008); Onu (2009). On the other hand, Villar-Patiño *et al.* (2002) reported that ascorbic acid supplemented diets resulted in lower feed consumption. L-carnitine supplemented diets caused lower daily feed consumption with insignificant differences. Birds fed L-carnitine consumed 65.14 and 65.07 for 75 and 150 mg/ Kg, respectively compared with 68.35 for control group. These results agreed with Leibetseder (1995) and Buyse *et al.* (2001). But it was in contrast with those of Geng *et al.* (2007) and Hrnčár *et al.* (2015).

**Table (2): Effect of different dietary treatments on productive performance.**

Items	Dietary Treatments							Sig	SEM <sup>#</sup>
	T1	T2	T3	T4	T5	T6	T7		
<b>Live body weight (g)</b>									
4 weeks	547.17	555.17	555.31	547.33	551.75	539.42	560.89	NS	49.12
6 weeks	952.47 <sup>ab</sup>	913.31 <sup>b</sup>	955.25 <sup>ab</sup>	920.78 <sup>b</sup>	967.44 <sup>a</sup>	961.36 <sup>ab</sup>	965.42 <sup>ab</sup>	*	75.25
8 weeks	1362.36 <sup>b</sup>	1335.47 <sup>b</sup>	1361.81 <sup>ab</sup>	1328.81 <sup>b</sup>	1387.50 <sup>ab</sup>	1374.44 <sup>ab</sup>	1455.67 <sup>a</sup>	**	93.21
<b>Daily weight gain (g)</b>									
2-4 weeks	23.59	24.18	24.11	23.58	23.84	23.14	24.57	NS	0.56
4-6 weeks	28.58 <sup>ab</sup>	25.58 <sup>b</sup>	28.57 <sup>ab</sup>	26.67 <sup>b</sup>	29.69 <sup>a</sup>	30.14 <sup>a</sup>	28.89 <sup>ab</sup>	*	0.95
6-8 weeks	29.28 <sup>b</sup>	30.15 <sup>b</sup>	29.04 <sup>a</sup>	29.14 <sup>b</sup>	30.03 <sup>b</sup>	29.49 <sup>b</sup>	35.02 <sup>a</sup>	*	0.60
2-8 weeks	27.27 <sup>b</sup>	26.64 <sup>b</sup>	27.24 <sup>b</sup>	26.47 <sup>b</sup>	27.65 <sup>b</sup>	27.54 <sup>b</sup>	29.49 <sup>a</sup>	**	0.89
<b>Daily feed consumption (g)</b>									
2-4 weeks	50.67	51.06	49.01	48.48	50.23	49.30	47.79	NS	5.26
4-6 weeks	69.65	71.40	73.86	68.66	69.29	66.77	73.99	NS	8.36
6-8 weeks	84.64 <sup>a</sup>	83.92 <sup>a</sup>	86.00 <sup>a</sup>	78.27 <sup>ab</sup>	75.68 <sup>b</sup>	78.03 <sup>b</sup>	90.09 <sup>a</sup>	*	12.87
2-8 weeks	68.35 <sup>ab</sup>	68.80 <sup>ab</sup>	69.62 <sup>ab</sup>	65.14 <sup>b</sup>	65.07 <sup>b</sup>	64.70 <sup>b</sup>	70.62 <sup>a</sup>	*	4.14
<b>Feed conversion ratio (g feed/ g gain)</b>									
2-4 weeks	2.14	2.11	2.03	2.06	2.10	2.13	1.95	NS	0.24
4-6 weeks	2.44 <sup>ab</sup>	2.79 <sup>a</sup>	2.58 <sup>a</sup>	2.58 <sup>a</sup>	2.33 <sup>b</sup>	2.22 <sup>b</sup>	2.57 <sup>a</sup>	*	0.33
6-8 weeks	2.87 <sup>a</sup>	2.78 <sup>a</sup>	2.96 <sup>a</sup>	2.68 <sup>ab</sup>	2.52 <sup>b</sup>	2.64 <sup>ab</sup>	2.69 <sup>ab</sup>	*	0.55
2-8 weeks	2.51 <sup>a</sup>	2.59 <sup>a</sup>	2.56 <sup>a</sup>	2.46 <sup>ab</sup>	2.35 <sup>b</sup>	2.34 <sup>b</sup>	2.39 <sup>b</sup>	*	0.74

T1: Basal diet, T2: Basal diet + Ascorbic acid 500 mg/ Kg, T3: Basal diet + Ascorbic acid 1000 mg/ Kg, T4: Basal diet + L-Carnitine 75 mg/ Kg, T5: Basal diet + L-Carnitine 150 mg/ Kg, T6: Basal diet + Probiotics 0.5 g/ Kg, T7: Basal diet + Probiotics 1.0 g/ Kg. a, b Means within the same row with different superscripts are significantly different. Sig. = Significance \*\* ( $P \leq 0.01$ ), \* ( $P \leq 0.05$ ). NS = Non Significant, # Pooled SEM.

Regarding to feed conversion ratio (FCR), birds fed basal diet supplemented with 0.5 g/Kg probiotics were the best with significant differences with control and ascorbic acid treatments but with insignificant differences with L-carnitine and 1g/ Kg probiotic treatments. Probiotic supplementation improves FCR due to its effect on daily weight gain and/ or daily feed consumption. These findings were in agreement with those obtained by Kaoud (2010) and Swain *et al.* (2012). The improvement in FCR due to the beneficial effects of probiotics represented in toxin neutralization, prevention of development and multiplication of specific bacteria, change in microbial metabolism and immunity stimulation (Fuller, 1989), in addition to the prevalence of their population against the adverse pathogens of digestive system (Bilgili and Moran, 1995).

Ascorbic acid supplementations didn't affect FCR compared with control; these were in harmony with Sosnowka-Czajka, *et al.* (2005) and Ogunwole *et al.* (2013) who found any improvement in feed conversion in broiler chickens due to ascorbic acid supplementation. However, these results contradicted with Blaha *et al.* (2000) and Onu, (2009) who found that ascorbic acid supplementation significantly improved FCR. Birds fed basal diet supplemented with L-carnitine 150 mg/ Kg recorded better FCR than control (2.35 vs. 2.51) with significant differences. It was in harmony with Xu *et al.* (2003) and Santine *et al.* (2001). But our results are not supported by the studies of Buyse *et al.* (2001) and Rezaei *et al.* (2007), according to these authors L-carnitine supplemented to chickens had no effect on FCR.

#### **Carcass characteristics:**

Data presented in Table (3) showed effects of experimental treatments on some carcass characteristics. There was an improvement in dressing percentage for all experimental groups when compared to control

group. Birds fed probiotic at level 1 g/ Kg was significantly better than control (68.13 vs. 64.27). Also, liver % was increased due to probiotic supplementation. These results were in harmony with those of Kaoud (2010) and Swain *et al.* (2012) who found that the eviscerated yield and weight of cut up parts (breast yield) were increased ( $P < 0.05$ ) in chicks fed diet supplemented with probiotic-yeast mixture 1.0 g/ Kg feed. The improvement in carcass yield may be due to better gut health and reduced pathogen load in the gastro-intestinal tract resulting in decreased post harvest loss and higher carcass yield (Swain *et al.*, 2012).

**Table (3): Effect of different dietary treatments on carcass traits, at 8 weeks of age.**

Items	Dietary Treatments							Sig	SEM <sup>#</sup>
	T1	T2	T3	T4	T5	T6	T7		
Dressing %	64.27 <sup>b</sup>	66.46 <sup>ab</sup>	68.07 <sup>a</sup>	67.69 <sup>a</sup>	67.54 <sup>a</sup>	66.37 <sup>ab</sup>	68.13 <sup>a</sup>	**	5.98
Liver %	1.66 <sup>b</sup>	1.77 <sup>a</sup>	1.40 <sup>b</sup>	1.75 <sup>a</sup>	1.81 <sup>a</sup>	1.87 <sup>a</sup>	1.79 <sup>a</sup>	**	0.08
Gizzard %	1.47	1.51	1.70	1.60	1.58	1.78	1.50	NS	0.06
Heart %	0.47	0.44	0.47	0.43	0.59	0.45	0.41	NS	0.01
Spleen %	0.19	0.19	0.16	0.17	0.18	0.19	0.18	NS	0.01
Bursa %	0.30 <sup>a</sup>	0.35 <sup>a</sup>	0.19 <sup>b</sup>	0.35 <sup>a</sup>	0.35 <sup>a</sup>	0.38 <sup>a</sup>	0.18 <sup>b</sup>	*	0.01
Thymus %	0.29	0.29	0.27	0.32	0.39	0.33	0.28	NS	0.01
Gizz. Fat %	1.18 <sup>a</sup>	0.98 <sup>b</sup>	0.91 <sup>b</sup>	1.11 <sup>a</sup>	0.80 <sup>b</sup>	0.85 <sup>b</sup>	0.71 <sup>b</sup>	*	0.01
Abd. Fat %	1.94 <sup>a</sup>	1.87 <sup>a</sup>	1.20 <sup>b</sup>	1.68 <sup>ab</sup>	1.56 <sup>b</sup>	1.97 <sup>a</sup>	1.83 <sup>ab</sup>	**	0.03
Giblets %	3.60 <sup>b</sup>	3.72 <sup>b</sup>	3.56 <sup>b</sup>	3.78 <sup>b</sup>	3.98 <sup>a</sup>	4.10 <sup>a</sup>	3.70 <sup>b</sup>	**	0.09

T1: Basal diet, T2: Basal diet + Ascorbic acid 500 mg/ Kg, T3: Basal diet + Ascorbic acid 1000 mg/ Kg, T4: Basal diet + L-Carnitine 75 mg/ Kg, T5: Basal diet + L-Carnitine 150 mg/ Kg, T6: Basal diet + Probox 0.5 g/ Kg, T7: Basal diet + Probox 1.0 g/ Kg. .a,b Means within the same row with different superscripts are significantly different. Sig. = Significance \*\* ( $P \leq 0.01$ ), \* ( $P \leq 0.05$ ). NS = Non Significant, # Pooled SEM.

Birds fed ascorbic acid at level 1000 mg/ Kg were significantly better than control (68.07 vs. 64.27). Also, abdominal fat % was decreased and liver % increased due to ascorbic acid supplementation. These findings were in agreement with Mbajjorgu *et al.* (2007) and Sahin *et al.* (2003) who recorded an increased liver, heart, spleen and empty gizzard weight in birds on dietary ascorbic acid supplementation. Ogunwole *et al.* (2013) found no significant ( $P > 0.05$ ) differences in obtained bleed, defeathered and carcass weights of broiler birds. Birds fed L-carnitine 75 and 150 mg/ Kg recorded 67.69 and 67.54 % dressing, respectively compared with 64.27% for control. Similar results were obtained by Hrnčár *et al.* (2015) who found higher carcass yield of males and females that received L-carnitine compared to the control group. Also, gizzard fat was decreased due to L-carnitine supplementation, in agreement with Wang *et al.* (2003). These authors recorded statistically significant decrease of fat content in the experimental broiler chickens supplemented with L-carnitine.

#### **Bacterial Count:**

Results showed in Table (4) indicated that aerobic bacteria in caecum and small intestine, was decreased by feeding any of dietary treatments, compared with control diet, till reached its lower count by feeding probiotic (T7), decreased from  $8 \times 10^{11}$  and  $6 \times 10^{12}$  to  $6 \times 10^3$  and  $7 \times 10^4$  cfu/ g in caecum and small intestine, respectively.

**Table (4): Effect of different dietary treatments on bacterial counts (cfu/ g).**

Items		Dietary Treatments						
		T1	T2	T3	T4	T5	T6	T7
Caecum	Aerobic	$8 \times 10^{11}$	$6 \times 10^6$	$10 \times 10^5$	$6 \times 10^8$	$15 \times 10^6$	$9 \times 10^4$	$6 \times 10^3$
	Anaerobic	$9 \times 10^8$	$8 \times 10^{12}$	$30 \times 10^{12}$	$3 \times 10^{11}$	$15 \times 10^{13}$	$6 \times 10^{14}$	$6 \times 10^{14}$
Small Intestine	Aerobic	$6 \times 10^{12}$	$16 \times 10^8$	$6 \times 10^8$	$22 \times 10^8$	$4 \times 10^5$	$7 \times 10^4$	$7 \times 10^4$
	Anaerobic	$14 \times 10^7$	$9 \times 10^8$	$11 \times 10^9$	$5 \times 10^8$	$12 \times 10^{13}$	$25 \times 10^{13}$	$25 \times 10^{13}$
Carcass Meat	Total count	$20 \times 10^4$	$12 \times 10^2$	$3 \times 10^2$	$9 \times 10^2$	$5 \times 10^2$	$9 \times 10$	$6 \times 10$
	Mesophilic	$9 \times 10^4$	$25 \times 10$	$2 \times 10^2$	$6 \times 10^3$	$12 \times 10$	$8 \times 10$	$3 \times 10$
Meat	Psychrophilic	0	0	0	0	0	0	0
	Total coliform	0	0	0	0	0	0	0
	Faecal coliform	0	0	0	0	0	0	0

T1: Basal diet, T2: Basal diet + Ascorbic acid 500 mg/ Kg, T3: Basal diet + Ascorbic acid 1000 mg/ Kg, T4: Basal diet + L-Carnitine 75 mg/ Kg, T5: Basal diet + L-Carnitine 150 mg/ Kg, T6: Basal diet + Probox 0.5 g/ Kg, T7: Basal diet + Probox 1.0 g/ Kg.

On contrary, anaerobic (beneficial) bacteria in caecum and small intestine, was increased gradually until reached its higher count by feeding probiotic (T6 or T7) in comparison with control, decreased from  $9 \times 10^8$  and  $14 \times 10^7$  to  $6 \times 10^{14}$  and  $25 \times 10^{13}$  cfu/ g in caecum and small intestine, respectively. Accordingly, probiotic treatments (T6 or T7) presents better efficacy in regard to both aerobic and anaerobic bacteria. These results assure the fact that are some probiotics provide binding sites for pathogenic bacteria that are then flushed out of the digestive tract with the faeces while others promote the growth of beneficial bacteria by acting as a food source (McCann *et al.*, 2006).

Also data presented in Table (4) showed that carcass meat samples were negative to psychrophilic, total coliform and faecal coliform. Different dietary treatments (ascorbic acid, L-carnitine or probiotic) decreased total bacterial and mesophilic bacteria count when compared to control group. Probiotic treatments were more effective in controlling total bacterial and mesophilic bacteria of carcass meat, decreased from  $20 \times 10^4$  and  $9 \times 10^4$  to  $6 \times 10$  and  $3 \times 10$  cfu/ g, respectively. Findings of bacteria count were in agreement with several authors (Ceylan *et al.*, 2003; Mountzouris *et al.*, 2007; Corduk *et al.*, 2008). Additionally, results provided in Table (5) revealed that caecum, small intestine and meat samples were free of *Salmonella* and *Listeria* but contained *Staph aureus*. While dietary application of probiotics or ascorbic acid (T2, T3, T6 or T7) completely eliminated *Campylobacter* from chicken meat.

**Table (5): Effect of different dietary treatments on incidence of pathogenic bacteria.**

Items	Dietary Treatments							
	T1	T2	T3	T4	T5	T6	T7	
Caecum	<i>Salmonella</i>	-	-	-	-	-	-	-
	<i>Staph aureus</i>	+	0	0	+	+	0	0
	<i>Listeria</i>	-	-	-	-	-	-	-
	<i>Campylobacter</i>	+	0	-	-	+	-	-
Small Intestine	<i>Salmonella</i>	-	-	-	-	-	-	-
	<i>Staph aureus</i>	+	0	0	+	+	0	0
	<i>Listeria</i>	-	-	-	-	-	-	-
	<i>Campylobacter</i>	+	0	0	+	+	-	-
Carcass Meat	<i>Salmonella</i>	-	-	-	-	-	-	-
	<i>Staph aureus</i>	+	0	0	+	+	0	0
	<i>Listeria</i>	-	-	-	-	-	-	-
	<i>Campylobacter</i>	+	-	-	+	+	-	-

T1: Basal diet, T2: Basal diet + Ascorbic acid 500 mg/ Kg, T3: Basal diet + Ascorbic acid 1000 mg/ Kg, T4: Basal diet + L-Carnitine 75 mg/ Kg, T5: Basal diet + L-Carnitine 150 mg/ Kg, T6: Basal diet + Probox 0.5 g/ Kg, T7: Basal diet + Probox 1.0 g/ Kg.

**Muscles histological examinations:**

The major components of muscles are muscle fibres which termed myofibers (m). Muscle fibers are highly specialized cells acting as the structural units of skeletal muscle tissue (Hedrick *et al.*, 1994). It is clear that within each muscle fasciculi many individual muscle fibers are spaced from each other by fine connective tissue septa called endomysium (e). And fasciculi are separated from each other by connective septa called perimysium (p). It is well known that muscle fibers number, size, and fiber-type composition are closely related to each other (Ryu *et al.*, 2004).

The performance of muscle in adult animal largely depends on muscle fiber number and type and therefore on fibre size. The muscle weight is a function of total number of myofibers, myofibre cross-section area, and length (Tumova and Teimouri, 2009). The microscopic structure of major pectoralis muscles from different treatment groups (Figure, 1) showed some changes related to our applied treatment. The myofiber size and number are increased by feeding birds both levels of ascorbic acid (T2, T3) or L-carnitine (T4, T5) or Probiotic (T6, T7). The endomysium layer was fine and delicate in bird feed both levels of L-carnitine and probiotics compared with the sections from bird of other treatments which means presence of many muscles fibers with each fasciculi. However, the perimysium was thick in birds feed both levels of ascorbic acid and L-carnitine compared with the other sections.

Regardless of different treatments levels the histological observations indicate superiority probiotic, L-carnitine and ascorbic acid, respectively, on characteristics of muscle structure. These findings reinforce the results of live body weight and dressing percent of birds obtained at 8 weeks of age, see (Tables, 2 and 3). It could be concluded that the applied treatments had a positive effect on the number

and size of fasciculi and consequently their myofibers content which may explain and support the enhanced growth of performance of the treatment groups.

***Liver histological examinations:***

Histological examination of liver sections from different treatment groups (Figure, 2) showed moderate changes. It is clear that the liver parenchyma of the control (T1) showed normal hepatocytic structure with few necrotic areas and blood sinuses. The same was also observed in (T2) liver section; however, there is moderate hypertrophy of liver cells in (T3) section accompanied with dilation of portal vein. This is the case in (T4) which may reveal hyperactivity of hepatic tissue. Moreover, liver sections of (T5, T6 and T7) groups showed dilated portal vein (T5 and T6) engorged with erythrocytes.

There are also necrotic areas and some infiltrated fluids near the portal vein especially in T6 and T7 treatment groups. In general, liver sections of all treatment groups were normal in their structure expect for some slight changes related to the hyper activity of liver cells due to the role of liver in metabolic activities.

***Spleen histological examinations:***

Spleen sections of different treatments are illustrated in (Figure, 3). In (T1) group, spleen section showed the normal structure of the splenic tissues, where a large white pulp (WP) area and dark-stained red pulp area (RP) could be seen. This is the same in all sections; expect the proportion of the two areas and the number and size of lymphocytes in all tissues.

It is clear that the number of large (LL) and small (SL) lymphocytes was greater in (T2, T3 and T6) were greater than the other treatments. Moreover, (T5 and T7) sections showed an obvious increase in the large lymphocytes than the small ones. It appears from these sections that the applied treatments had a desirable and beneficial effect on spleen tissues which indicate their importance as feed additives.

## **CONCLUSION**

After reviewing results of the present study, it might be suggested that additives used (L-carnitine, ascorbic acid or probiotics), could be applied to broiler (locally-developed) diets in order to improve bird's productive performance without any adverse effect on internal body organs or microbial population of gastro-intestinal tract.

## **ACKNOWLEDGMENTS**

The support given by Prof. F. Abdelazeem for financing this research project was acknowledged. We also thank Prof. I. El-Wardani for his effort given during interpretation of histological examinations.

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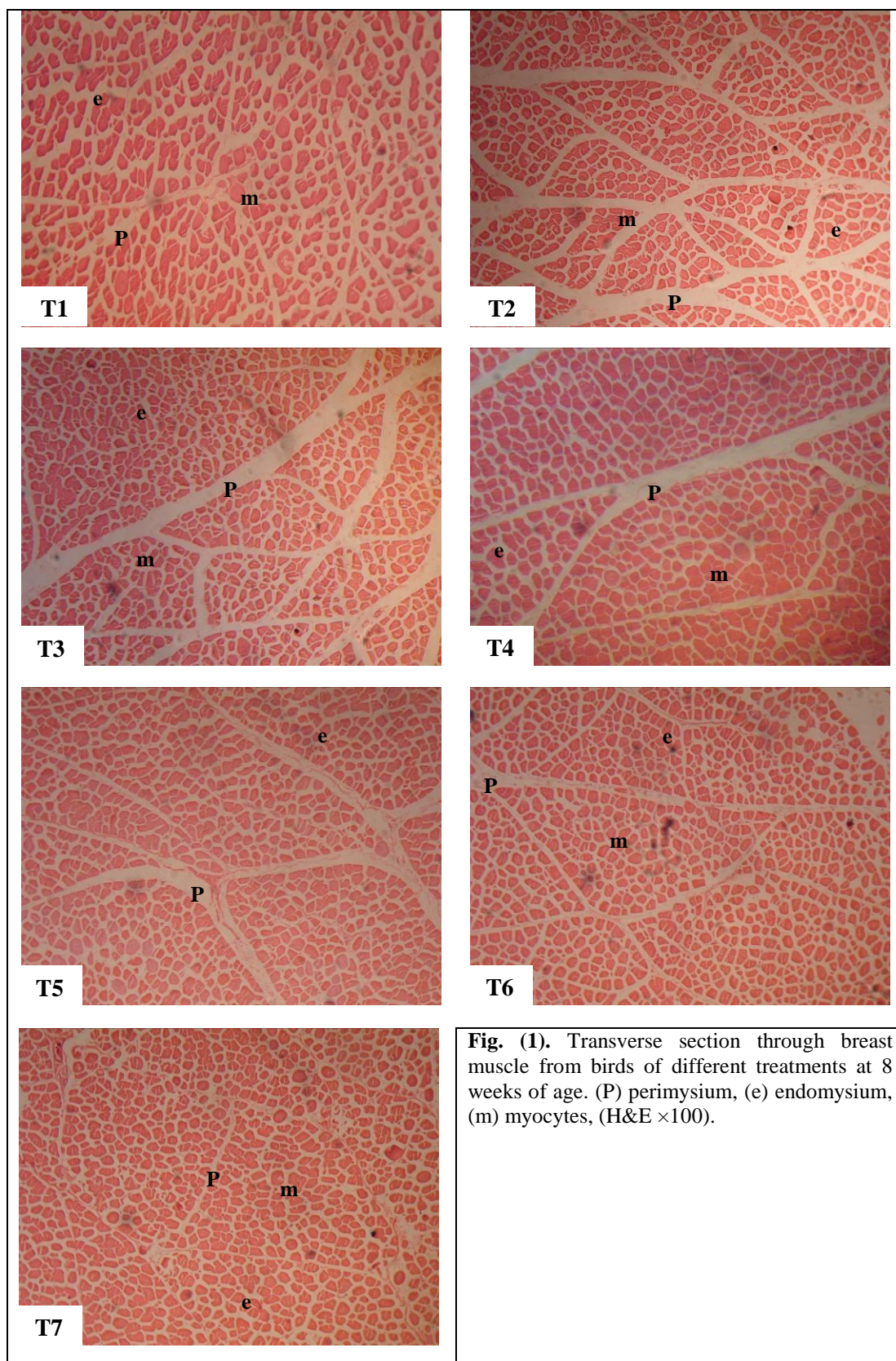
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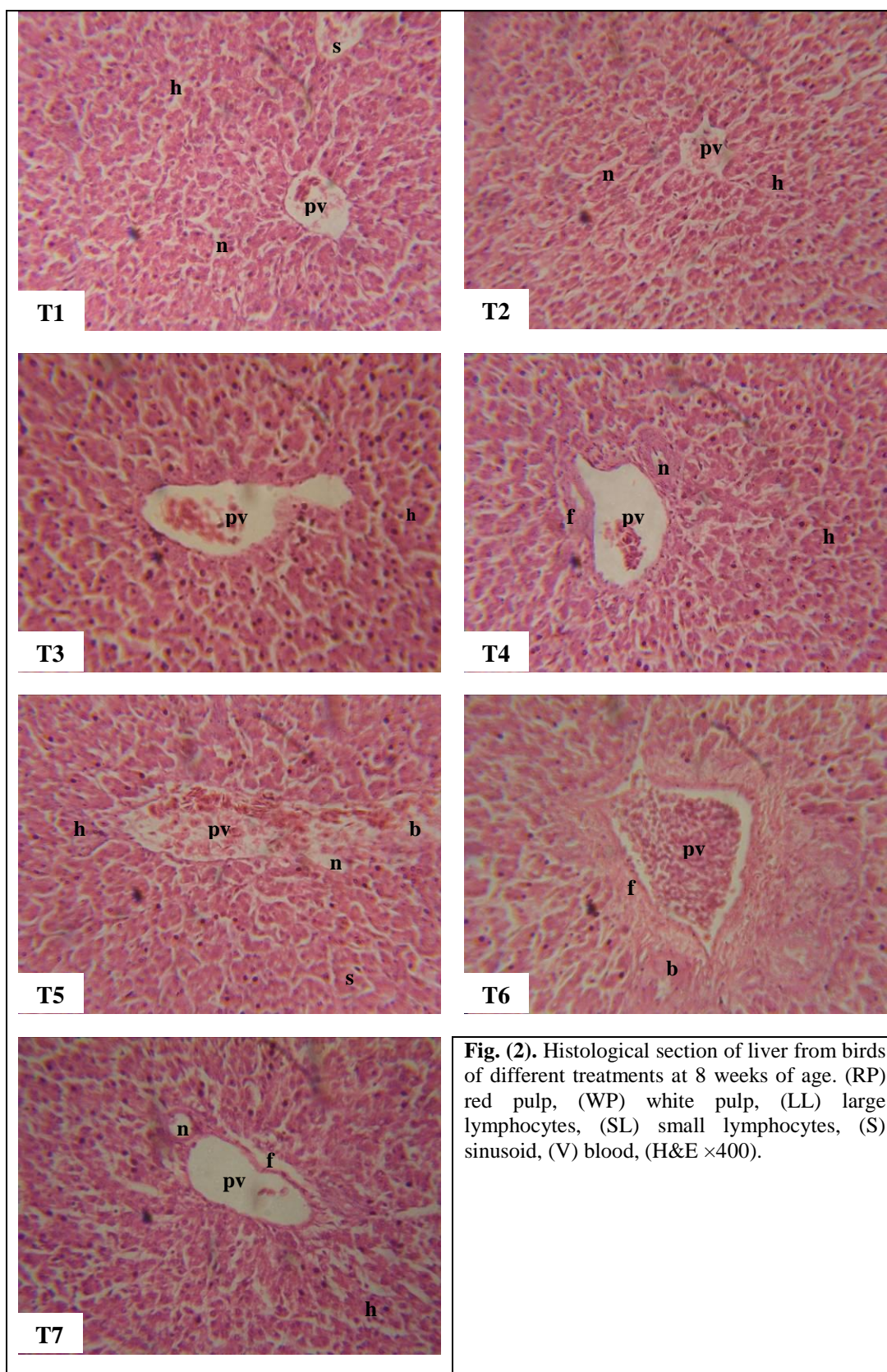
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**Fig. (1).** Transverse section through breast muscle from birds of different treatments at 8 weeks of age. (P) perimysium, (e) endomysium, (m) myocytes, (H&E  $\times 100$ ).

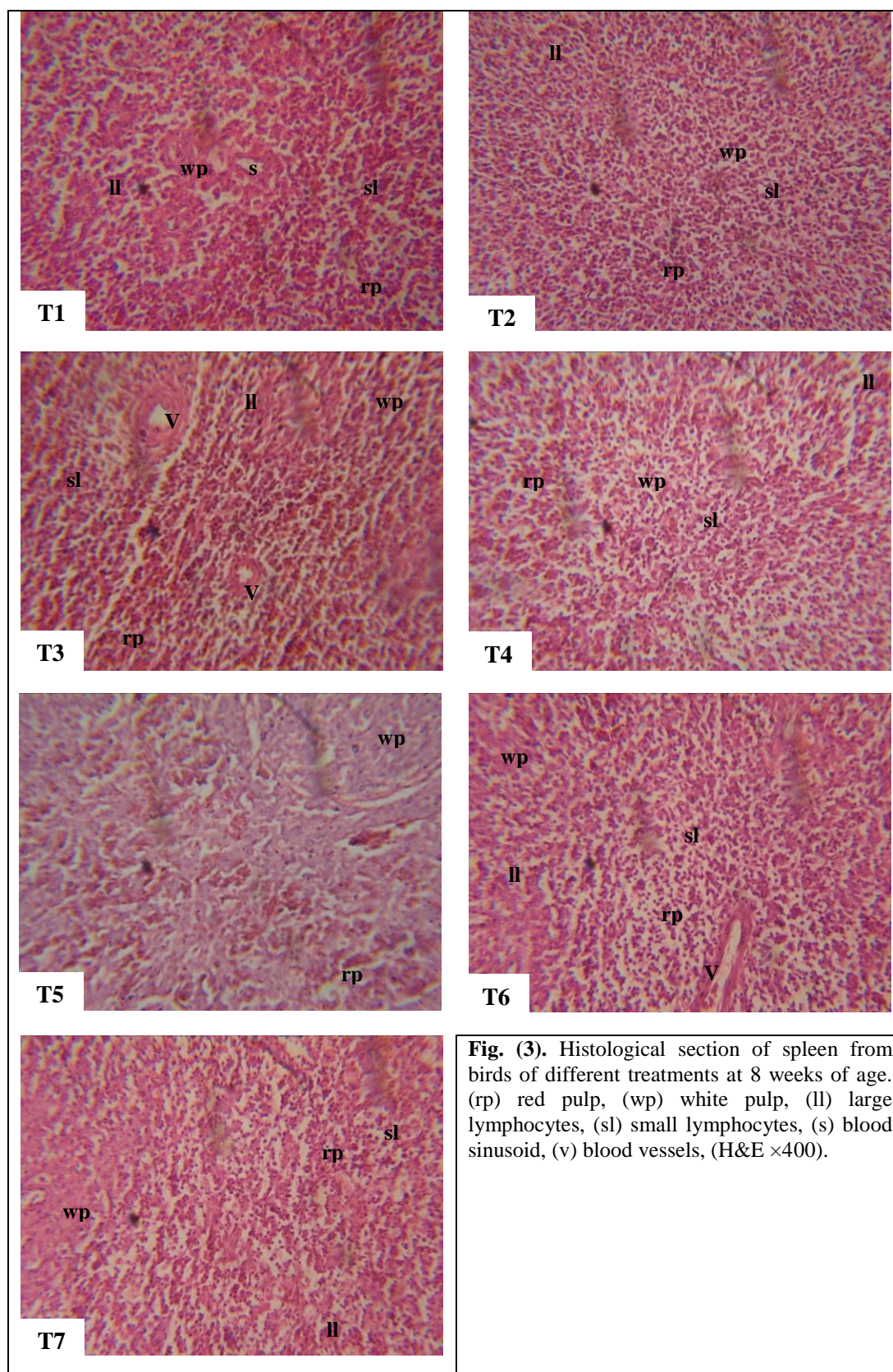
T1: Basal diet, T2: Basal diet + Ascorbic acid 500 mg/ Kg, T3: Basal diet + Ascorbic acid 1000 mg/ Kg, T4: Basal diet + L-Carnitine 75 mg/ Kg, T5: Basal diet + L-Carnitine 150 mg/ Kg, T6: Basal diet + Probox 0.5 g/ Kg, T7: Basal diet + Probox 1.0 g/ Kg.



**Fig. (2).** Histological section of liver from birds of different treatments at 8 weeks of age. (RP) red pulp, (WP) white pulp, (LL) large lymphocytes, (SL) small lymphocytes, (S) sinusoid, (V) blood, (H&E ×400).

T1: Basal diet, T2: Basal diet + Ascorbic acid 500 mg/ Kg, T3: Basal diet + Ascorbic acid 1000 mg/ Kg, T4: Basal diet + L-Carnitine 75 mg/ Kg, T5: Basal diet + L-Carnitine 150 mg/ Kg, T6: Basal diet + Probiotics 0.5 g/

Kg, T7: Basal diet + Probax 1.0 g/ Kg.



**Fig. (3).** Histological section of spleen from birds of different treatments at 8 weeks of age. (rp) red pulp, (wp) white pulp, (ll) large lymphocytes, (sl) small lymphocytes, (s) blood sinusoid, (v) blood vessels, (H&E ×400).

T1: Basal diet, T2: Basal diet + Ascorbic acid 500 mg/ Kg, T3: Basal diet + Ascorbic acid 1000 mg/ Kg, T4: Basal diet + L-Carnitine 75 mg/ Kg, T5: Basal diet + L-Carnitine 150 mg/ Kg, T6: Basal diet + Probax 0.5 g/ Kg, T7: Basal diet + Probax 1.0 g/ Kg.

## تأثير استخدام لـكارنيتين، حمض الأسكوربيك والبروبيوتيك على الأداء الإنتاجي، الحمل الميكروبي والتركيبة النسيجية في دجاج التسمين المستنبت محليا

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<sup>2</sup> المركز الإقليمي للأغذية والأعلاف - مركز البحوث الزراعية - الجيزة - مصر.

في تجربة أجريت لدراسة تأثير استخدام لـكارنيتين، حمض الأسكوربيك والبروبيوتيك على الأداء الإنتاجي، وخصائص الذبيحة وبعض الخواص البكتيرية والنسجية في سلالة الدجاج اللحم المستنبتة محليا. تم توزيع عدد 252 ككتوت غير مجنس عمر أسبوعين تم رعايتهم حتى عمر 8 أسابيع من حيث تم توزيعهم على 7 معاملات، في 6 مكررات كل منها أحتوى على 6 طيور. كانت المجموعات التجريبية على النحو التالي:

T1: مجموعة كونترول تم تغذيتها على العليقة القاعدية.

T2: العليقة القاعدية مضاف إليها 500 ملجم حمض اسكوربيك / كجم عليقة.

T3: العليقة القاعدية مضاف إليها 1000 ملجم حمض اسكوربيك / كجم عليقة.

T4: العليقة القاعدية مضاف إليها 75 ملجم لـكارنيتين / كجم عليقة.

T5: العليقة القاعدية مضاف إليها 150 ملجم لـكارنيتين / كجم عليقة.

T6: العليقة القاعدية مضاف إليها 0.5 جرام بروبيوتيك / كجم عليقة.

T7: العليقة القاعدية مضاف إليها 1.0 جرام بروبيوتيك / كجم عليقة.

النتائج المتحصل عليها يمكن تلخيصها على النحو التالي:

1. عدم وجود فروق معنوية خلال فترة الأسبوعين الأولى من فترة الدراسة بين جميع المعاملات في وزن الجسم الحى، والوزن المكتسب اليومي، واستهلاك العلف المستهلك اليومي ومعامل التحويل الغذائي.
  2. الطيور المغذاة عليقة T2 اكتسبت مزيد من الوزن بشكل معنوي عند مقارنتها مع المجموعات أخرى على طول فترة الدراسة. بينما معامل التحويل الغذائي للطيور التي غذيت على علائق T4، T5، T6 أو T7 سجل قيم أفضل بشكل معنوي بالمقارنة مع جميع المعاملات الأخرى.
  3. إزدادت النسبة المئوية للذبيحة مع جميع المعاملات التجريبية بشكل معنوي مقارنة مع مجموعة الكونترول. بينما الطيور المغذاة علائق T3 أو T5 سجلت أقل معدل من دهن البطن بشكل معنوي بالمقارنة مع جميع المعاملات.
  4. أظهرت الدراسات الميكروبية أن البكتيريا الهوائية إنخفضت كنتيجة للمعاملات الغذائية المختلفة في منطقة الأعرور والأمعاء الدقيقة كمقارنة مع مجموعة المقارنة. كما وقد ارتفع العد البكتيرى للبكتيريا اللاهوائية (النافعة)، حيث ارتفعت في منطقتى الأعرور والأمعاء الدقيقة عند مقارنتها بمجموعة الكونترول. وكذلك فإن تغذية الطيور على بروبيوتيك أو فيتامين C تسبب في القضاء تماما علي كامبيلوباكتر في لحم الدجاج التسمين. وكانت معاملة البروبيوتيك هي الأكثر فاعلية في خفض العدد الكلي للبكتيريا في لحم الذبيحة.
  5. أظهرت نتائج الدراسة الهستولوجية لعضلات الصدر أن إضافة حمض الاسكوربيك وإلـكارنيتين والبروبيوتك في العليقة أدى إلى زيادة عدد وحجم الألياف العضلية لعضلة الصدر وبالتالي زيادة حجم الحزم العضلية والتي بدورها تزيد من حجم ووزن العضلة والذي يترتب عليه زيادة في الوزن ومعدلات النمو للطيور في نهاية فترة التسمين وقد لوحظ تفوق لمستويات البروبيوتك مقارنة بباقي المعاملات.
  6. التركيب الهستولوجي للكبد كان طبيعياً في كل المعاملات ولم تظهر أى إختلافات جوهرية أو وجود علامات مرضية ناتجة عن المعاملات.
  7. بالفحص الهستولوجي للطحال لم تكن هناك أى علامات أو تركيبات غير طبيعية لجميع المعاملات فيما عدا المعاملات T2 و T3 و T5 والتي أبدت زيادة في أعداد كلاً من الخلايا الليمفاوية الكبيرة والصغيرة فيما أبدت المعاملات T5 و T7 زيادة في أعداد الخلايا الليمفاوية الكبيرة مقارنة بأعداد الخلايا الليمفاوية الصغيرة.
- من واقع البيانات المتحصل عليها، يقترح أن لـكارنيتين، فيتامين C أو البروبيوتيك، يمكن أن يتم استخدامهم بشكل آمن كإضافات غذائية لتحسين الأداء الإنتاجي لطيور التسمين دون أي تأثير سلبي على أعضاء الجسم أو المجتمع البكتيري في القناة الهضمية للطيور.