International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.12 pp 996-1016, 2016

Assessment of Growth Performance, Hemato-Biochemical Parameters, Immunological and Histopathological Alterations Associated with New Bacterial Multistrain Probiotic (Gro-2-Max®) Supplementation on Broiler Chicken

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Abstract : Long time ago, attempts for enhancing the poultry health status, are concentrating on ways for modulating the indigenous intestinal flora by live microbial adjuncts, now called "probiotics". In the present study 126, one-day old commercial broiler chicks were used to evaluate the effect of supplementation with new bacterial multistrain probiotic (Gro-2-Max®) and were equally divided into 6 groups. Group 1 (control), were fed and drank without any treatment. Supplementation in ration was done at a rate of 500 g/ton starting from 1st day till the end of experiment (42 days) in group 2, from 15th to 42nd day in group 3, and from 1st to 10th and from 30th to 42nd day in group 4. Supplementation in drinking water was carried out at a rate of 1g/liter for 24 hours starting from 1st to 5th, 16th to 20th and from 30th to 35th day in group 5, and for 3 successive days/week till 42nd day in group 6. Evaluation included monitoring chicken performance (feed intake, body weight gain, feed conversion rate, immune index and cecal bacterial enumeration), clinicopathological alterations (hemogram and serum biochemistry), immune responses (humeral and cellular), and histopathological examinations (liver, kidney, spleen, bursa of Fabricious, thymus and ileum). Results concluded to, the positive effect of Gro-2-Max® on chicken performance especially groups 2 and 6, decreasing effect on lipogram especially total cholesterol, total triglycerides and low density lipoprotein cholesterol, nonspecific humeral and cellular immune responses, and improving effect on intestinal function through increasing the height of ileal villi.
Key words: Probiotics, Chicken performance, Clinical pathology, Immunology, Histopathology.

Introduction

Newly hatched chicks have little chance to be contacted with their mothers and consequently their normal microflora is slowly colonized to the intestine. During this early period, chicks are more susceptible for various stresses which poorly affect their growth performance, immunity and digestion because

of the incomplete development of their different physiological body functions[1]. Many researchers used probiotics as growth promoting agents[2]. Probiotics exhibit several ways of action including antagonistic action towards pathogenic bacteria, competition for locations to intestinal mucous membranes and competition for nutrients[3]. Probiotics, when fed, influence the intestinal morphology and function[4], promote feed conversion rate, progress immune system function and reduce chicken susceptibility to diseases[2]. Gro-2-Max® is a new bacterial multistrain probiotic used in poultry field. It contains naturally occurring four different species of beneficial bacteria (*Pedicoccus acidilactici*, *Pedicoccus pentosaceus*, *Acetabacter aceti* and *Bacillus amyloliquafaciens*) which are generally regarded as safe by American food and drug administration[5]. The present study aimed to evaluate supplementation of chicken with Gro-2-Max® in their ration and drinking water at different ages and to find the best way for its application in poultry field. This evaluation was done through studying its effect on chicken performance, hemato-biochemical parameters, immune responses, and histopathological alterations.

Materials and Methods

Chicks and Experimental Design

One hundred and twenty-six, one-day old commercial broiler chicks (Arbor Acres) were obtained and fed on basal ration from Cairo Poultry Corporate Egypt. All chicks were fed and watered *ad libitum* and were reared on a floor housing system at Animal Health Research Institute, Provincial Laboratory, Tanta. Chicks were divided equally into 6 groups each contains 21 chicks in 3 replicates. Group 1 was fed on a basal ration and drank water without any treatment and considered as control. Supplementation of Gro-2-Max® in ration was done at a rate of 500 g/ton starting from 1st day of age till the end of experiment (42 days) in group 2, from 15th to 42nd day in group 3, and from 1st to 10th and from 30th to 42nd day in group 4. Supplementation in drinking water was carried out at a rate of 1g/liter for 24 hours starting from 1st to 5th, 16th to 20th and from 30th to 35th day in group 5 and for 3 successive days/week till the end of experiment in group 6. All samples were collected at the 21st and 42nd day except those for chicken performance were collected weekly.

Vaccination

Chicks at hatchery were vaccinated via spray against Newcastle disease (ND) and by S/C injection against infectious bursal disease (IBD) and Avian influenza using Vitaberon L, Vaxxitic and Egyptian H9N1 vaccines, respectively. Booster doses against ND using MA5+Clone 30 vaccine at the 10th day and Aveinew vaccine at the 30th day via drinking water were administered.

Probiotic

Gro-2-Max® was manufactured by Bio-National American Institute and contains *Pedicoccus acidilactici* (3×10^5 cfu/g), *Pedicoccus pentosaceus* (4×10^3 cfu/g), *Acetabacter aceti* (2×10^5 cfu/g) and *Bacillus amyloliquafaciens* (4×10^4 cfu/g).

Chicken performance

Feed intake (FI), body weight gain (BWG), feed conversion rate (FCR) and mortality rate for each group was determined.

Immune index and Cecal Bacterial Enumeration

Three chickens from each group were weighed and scarified. Lymphoid organs including thymus, spleen and bursa of Fabricious were collected and weighed. Both organ weight and organ weight to body weight ratio, and immune index were calculated according to Lucio and Hitchner[6]. One gram of cecal digesta was used for enumerating *total coliform*, *E. coli* and *Lactobacillus spp.* using MacConkey, Eosin methylene blue (EMB) and Rogosa agars, respectively, according to Tuohy *et al.*[7].

Blood Samples for Clinicopathological and Immunological Examinations

Blood samples from 10 chickens of each group were collected (wing vein). Each blood sample was divided into two parts. First part was anticoagulated using EDTA for evaluating hemogram and cellular immunity. Second part was collected in clean plain centrifuge tube for serum separation to evaluate blood biochemistry and humeral immunity.

Clinicopathological Examinations

Hematological Examination

According to Campbell[8], the following hematological parameters were measured; red blood cells (RBCs) count, packed cell volume (PCV), hemoglobin (Hb) concentration, total leukocytic count (TLC), and differential leukocytic count (DLC).

Biochemical Examination

According to Warnick *et al.*[9], the following biochemical examinations were assayed; protein profile [total proteins, albumin (A), globulins (G) and A/G ratio], activity of hepatic enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)], lipogram [total cholesterol, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and total triglycerides] and concentrations of blood glucose, uric acid, creatinine, calcium and inorganic phosphorus. All the before mentioned parameters were assayed using reagent kits supplied by StanBio Laboratories incorporation, USA.

Immunological Examinations

Humeral Immunity

Measurement of antibody titers against ND using hemagglutination inhibition (HI) test and against IBD using enzyme-linked immunosorbent assay (ELISA) were carried out according to Snyder *et al.*[10].

Cellular Immunity

Phagocytic activity (PA), phagocytic index (PI) and lymphocyte transformation test (LTT) were assayed according to Nariuchi[11].

Histopathological Examination

Liver, kidney, spleen, bursa of Fabricious, thymus and ileum were collected and prepared for microscopic examination using hematoxylin and eosin stain (H &E).

Statistica Analysis

Results of the experiment were analyzed using ANOVA procedure using the mean ± SD by SPSS V.14. (2000).

Results

Chicken Performance

To accurately assess the chicken performance, weekly changes in patterns of FI, BWG and FCR during the whole experimental period (42 days) were recorded as shown in Table 1. At the beginning of the experiment, the initial average body weight of newly hatched broiler chicks in all groups was 46.4±4.2 g. By comparing the obtained results with those of the control group 1, fluctuated results were reported but the results took regular pattern at 21st and 42nd day as follow; at 21st day, FI showed significant increases in groups 2, 4, 5 and 6 with no significate change in group 3. BWG was significantly increased in groups 2, 3, 5 and 6 with no significate change in group 4. Significant decreases in FCR were recorded in groups 2, 3, 5 and 6 with no significate change in group 4. At 42nd day, FI showed significant decreases in all groups, BWG was significantly increased in groups 2, 3 and 6, significant decrease in group 4 with no significate change in group 5. FCR was significantly decreased in groups 2, 3 and 6, significant increases in group 4 and no significate change in group 5.

Table (1): Effect of probiotic (Gro-2-Max®) on FI, BWG, FCR and mortality on different experimental groups

Age/Day	Parameters	Group					
		1	2	3	4	5	6
7th	FI	24.00± 2.68^{b}	20.60± 2.29^{c}	24.00± 2.68^{b}	20.60± 2.29^{c}	27.00± 3.03^{a}	27.00± 2.96^{a}
	BWG	16.00± 1.66^{b}	14.00± 1.84^{c}	16.00± 3.82^{b}	14.00± 1.86^{c}	17.00± 1.44^{b}	19.00 ±1.55^{a}
	FCR	1.50± 0.17^{b}	1.40± 0.16^{c}	1.50± 0.17^{b}	1.40± 0.16^{c}	1.60± 0.18^{a}	1.40± 0.16^{c}
	Mortality	2.00	2.00	0	0	1.00	0
	%	9.52	9.52	0	0	4.76	0
14th	FI	51.00± 5.70^{b}	57.00± 6.34^{a}	51.00± 5.72^{b}	54.00± 6.05^{b}	59.00± 6.53^{a}	57.00± 6.32^{a}
	BWG	34.00± 5.85^{b}	34.60± 3.96^{b}	34.60± 4.52^{b}	35.60± 4.53^{b}	34.30± 7.69^{b}	49.00± 3.61^{a}
	FCR	1.50± 0.17^{c}	1.60± 0.18^{b}	1.50± 0.17^{c}	1.50± 0.17^{c}	1.70± 0.19^{a}	1.60± 0.18^{b}
	Mortality	1.00	0	0	0	0	0
	%	4.76	0	0	0	0	0
21st	FI	88.00± 9.80^{b}	96.00± 11.00^{a}	87.00± 10.00^{b}	99.00± 11.00^{a}	95.00± 11.00^{a}	90.00 ± 10.00^{a}
	BWG	47.00± 1.97^{c}	59.00± 4.68^{b}	68.00± 3.53^{a}	49.00± 6.46^{c}	53.00± 6.53^{b}	60.00± 3.32^{a}
	FCR	1.90± 0.21^{a}	1.60± 0.18^{b}	1.30± 0.16^{c}	2.00± 0.22^{a}	1.80± 0.19^{b}	1.50± 0.17^{b}
	Mortality	0	0	0	0	0	0
	%	0	0	0	0	0	0
28th	FI	146.00± 16.00^{c}	163.00± 18.00^{a}	155.00± 17.00^{b}	142.00± 15.00^{c}	137.00± 15.00^{d}	141.60± 13.00^{c}
	BWG	88.00± 13.07^{b}	93.00± 9.53^{a}	91.00± 11.79^{a}	74.00± 4.48^{b}	67.00± 9.73^{c}	83.00± 7.92^{b}
	FCR	1.60± 0.18^{b}	1.70± 0.20^{b}	1.70± 0.19^{b}	1.90± 0.21^{a}	2.00± 0.22^{a}	1.50± 0.17^{b}
	Mortality	0	0	0	0	0	0
	%	0	0	0	0	0	0
35th	FI	177.00± 16.00^{b}	180.00± 20.00^{a}	167.00± 18.00^{c}	167.00± 18.00^{c}	163.00± 18.00^{c}	166.00± 18.00^{c}
	BWG	91.50± 31.00^{a}	75.00± 26.00^{c}	75.00± 6.00^{c}	86.00± 21.00^{b}	93.00± 9.70^{a}	91.00± 7.90^{a}
	FCR	1.93± 0.19^{c}	2.40± 0.27^{a}	2.20± 0.25^{b}	1.90± 0.21^{c}	1.75± 0.20^{d}	1.80± 0.20^{d}
	Mortality	0	0	0	0	0	0
	%	0	0	0	0	0	0
42nd	FI	230.00± 26.00^{a}	217.00± 24.00^{c}	216.00± 24.00^{c}	221.00± 25.00^{b}	220.00± 24.00^{b}	221.00± 25.00^{b}
	BWG	81.00± 13^{c}	112.00± 14.00^{a}	85.00± 20.00^{b}	57.00± 20.00^{d}	81.00± 11.00^{c}	91.00± 23.00^{b}
	FCR	2.86± 0.30^{b}	1.90± 0.19^{d}	2.50± 0.28^{c}	3.90± 0.43^{a}	2.70± 0.30^{b}	2.43± 0.28^{c}
	Mortality	0	0	0	0	0	0
	%	0	0	0	0	0	0
Total means (from 1st - 42nd day)	FI	119.00± 13.40^{b}	122.00± 13.60^{a}	118.00± 13.14^{b}	117.00± 13.00^{b}	117.00± 13.00^{b}	114.00± 12.80^{c}
	BWG	60.00± 6.02^{b}	68.00± 9.55^{a}	60.40± 9.90^{b}	53.00± 10.76^{c}	58.50± 1.90^{c}	65.00± 11.50^{a}
	FCR	2.00± 0.20^{a}	1.80± 0.20^{b}	1.90± 0.21^{a}	2.2± 0.25^{a}	2.00± 0.22^{a}	1.80± 0.20^{b}
	Mortality	3.00	2.00	0	0	1.00	0
	%	14.29	9.52	0	0	4.76	0

Means with different superscripts (a,b,c,d) within a raw are significantly different.

Group (1): Control group was fed on a basal ration and drank water without any treatment.

Group (2): Supplemented with Gro-2-Max® in ration at a rate of rate of 500 g/ton starting from 1st to 42nd day.

Group (3): Supplemented with Gro-2-Max® in ration at a rate of rate of 500 g/ton starting from 15th to 42nd day.

Group (4): Supplemented with Gro-2-Max® in ration at a rate of rate of 500 g/ton starting from 1st to 10th and from 30th to 42nd day.

Group (5): Supplemented with Gro-2-Max® in drinking water at a rate of 1g/liter for 24 hours starting from 1st to 5th, 16th to 20th and from 30th to 35th day.

Group (6): Supplemented with Gro-2-Max® in drinking water at a rate of 1g/liter for 24 hours for 3 successive days/week till the end of experiment.

Mortality rate was lower in groups 2 and 5 than control group [9.52% and 4.76% via 14.29%], respectively and was absent in groups 3, 4 and 6.

Results of carcass yield in Table 2 clearly demonstrate the significant increased dressing values in groups 2 and 6, significant decreased values in groups 4 and 5, and insignificant changes in group 3. Insignificant changes of goblet weights and percentages of pancreas, fat, intestine and cecum in addition to the length of intestine and cecum of different experimental groups were recorded. Liver weight revealed significant increase in groups 2, 4, 5 and 6 while, heart weight revealed significant increase in groups 2 and 4 and significant decrease in group 6 in both weight and percentage. Groups 2, 3, 4 and 5 showed significant increase in gizzard weight while, group 6 showed significant decrease in weight and percentage, and group 2 showed significant decrease in percentage.

Immune Index

At 21st day all groups showed insignificant changes in weights and immune indices of thymus, spleen and bursa of Fabricious. At 42nd day significant increase in bursa weight of groups 2, 3, 4 and 5 and significant decrease in group 6 were recorded while, bursa immune index was insignificantly changed. Moreover, weights and immune indices of thymus and spleen at 42nd day were insignificantly changed in all groups (Table 3).

Cecal Bacterial Enumeration

At 21st day, *total coliform* count showed significant increase in groups 2 and 4, and significant decrease in groups 3, 5 and 6, *E. coli* count revealed significant decrease in groups 2, 3 and 6 and significant increase in groups 4 and 5 while, cecal *lactobacillus* count in all groups showed significant increase. At 42nd day, significant increases in *total coliform* and *lactobacillus* counts with significant decrease in *E. coil* count in groups 2, 5 and 6 were observed. Group 3 recorded insignificant change in *total coliform* and significant decrease in *E. coli* and *lactobacillus* counts. Significant increase in *total coliform* and *E. coli* counts with significant decrease in *lactobacillus* count was recorded in group 4 (Table 4).

Clinicopathological Results

Hematology

Insignificant increase in erythrogram parameters and significant lymphocytic leukocytosis were observed in all groups. These hematological changes were more pronounced in group 2 than other groups (Table 5).

Serum Biochemistry

Protein profile showed insignificant changes in albumin concentration, significant hyperproteinemia resulted from hyperglobulinemia and significant decrease in A/G ratio. Lipogram revealed insignificant change in HDL-c with significant decrease in total cholesterol, total triglycerides and LDL-c concentrations. Activities of AST, ALT and ALP, and concentrations of blood glucose, serum creatinine and uric acid showed insignificant changes. Insignificant changes in phosphorus concentration with significant hypercalcaemia were reported. These biochemical changes were more pronounced in group 2 than other groups (Tables 6 and 7).

Immunological Results

Humeral immunity at 21st day showed insignificant changes in antibody titers against ND and IBD vaccines except groups 4 and 6 showed significant increases against ND and IBD vaccines, respectively. At 42nd day insignificant changes were recorded except group 3 showed significant decrease against ND vaccine (Table 8).

Table (2): Effect of probiotic (Gro-2-Max®) on dressing value, weight and percentage of goblet in different experimental groups

Weight			Groups										ANOVA			
	1		2		3		4		5		6		F		P-Value	
Before slaughter	2.41±0.25		2.89±0.40		2.51±0.42		2.40±0.38		2.52±0.08		2.51±0.61		0.64		0.68	
After slaughter	1.70±0.17^{c}		2.14±0.39^{a}		1.76±0.32^{c}		1.61±0.25^{c}		1.70±0.05^{c}		1.81±0.01^{a}		1.21		0.04	
Dressing%	70.50±1.14^{c}		74.00±4.45^{a}		70.00±1.13^{c}		67.00±3.75^{d}		67.00±2.25^{d}		72.00±2.77^{b}		2.53		0.05	
Goblet	Weight	%	Weight	%	Weight	%	Weight	%	Weight	%	Weight	%	F	F	P-Valu	P-Value
Liver	55.00±8.00^{b}	3.23±0.25^{a}	78.00±11.53^{a}	3.64±0.95^{a}	57.00±12.02^{b}	3.24±0.01^{a}	79.00±18.77^{a}	4.12±0.16^{a}	70.00±4.73^{a}	4.12±0.16^{a}	61.00±15.87^{a}	3.37±0.07^{a}	1.54	2.44	0.25	0.10
Heart	12.00±3.21^{b}	0.70±0.11^{a}	15.00±2.89^{a}	0.70±0.14^{a}	13.70±3.79^{b}	0.78±0.09^{a}	14.00±1.00^{a}	0.87±0.13^{a}	12.00±2.08^{b}	0.70±0.11^{a}	7.70±1.15^{c}	0.42±0.12^{c}	3.26	3.50	0.04*	0.03*
Pancreas	6.00±1.73^{a}	0.35±0.06^{a}	7.00±2.00^{a}	0.33±0.12^{a}	5.60±70.58^{a}	0.32±0.05^{a}	5.70±2.25^{a}	0.35±0.10^{a}	5.70±1.15^{a}	0.33±0.05^{a}	6.00±1.15^{a}	0.33±0.06^{a}	0.30	0.05	0.91	1.00
Gizzard	35.67±5.51^{b}	2.10±0.10^{a}	40.30±5.69^{a}	1.88±0.35^{b}	40.00±5.29^{a}	2.27±0.12	45.70±7.51^{a}	2.86±0.25^{a}	42.00±5.29^{a}	2.47±0.31^{a}	28.70±8.08^{c}	1.58±0.25^{c}	2.60	6.51	0.08	0.001*
Fat	29.00±10.15^{a}	1.70±0.67^{a}	31.70±21.22^{a}	1.48±1.08^{a}	30.70±2.08^{a}	1.74±0.06^{a}	32.00±14.57^{a}	2.00±0.65^{a}	32.70±6.43^{a}	1.92±0.38^{a}	38.00±7.81^{a}	2.10±0.09^{a}	0.19	0.25	0.96	0.93
Intestine	135.00±23.29^{a}	7.94±0.53^{a}	178.00±27.15^{a}	8.31±2.18^{a}	135.67±14.57^{a}	7.71±1.59^{a}	157.67±36.56^{a}	9.79±1.96^{a}	148.00±29.54^{a}	8.71±1.40^{a}	140.67±38.94^{a}	7.77±0.08^{a}	0.94	0.51	0.49	0.76
Cecum	10.67±2.52^{a}	0.65±0.11^{a}	21.00±11.02^{a}	0.96±0.57^{a}	12.00 ±4.04^{a}	0.68±0.17^{a}	15.00±2.65^{a}	0.93±0.12^{a}	15.67±2.31^{a}	0.92±0.14^{a}	11.67±1.15^{a}	0.64±0.06^{a}	1.73	1.12	0.20	0.40
Intestine length	252.00±7.37^{a}	--	259.00±25.63^{a}	--	251.00±27.30^{a}	--	252.00±41.58^{a}	--	244.00±46.20^{a}	--	242.00±20.79^{a}	--	0.11	--	0.99	--
Cecum length	17.42±0.52^{a}	--	23.00±3.21^{a}	--	20.00±3.21^{a}	--	20.50±1.32^{a}	--	21.50±1.50^{a}	--	20.00±2.31^{a}	--	1.90	--	0.17	--

Means with different superscripts (a,b,c,d) within a raw are significantly different.

Table (3): Immune index of thymus, spleen and bursa of Fabricious at the 21st and 42nd day of age of all experimental groups

Items			Groups						ANOVA	
Age/ Day	Groups		1	2	3	4	5	6	F	P-Value
21st	Weight	Body weight	720±95.39	623±.33.15	731.67±25.66	713±75.88	720±112.69	601.67±85.78	0.99	0.46
		Thymus weight	0.88±0.26	1.33±0.47	1.17±0.37	1.02±0.25	0.87±0.11	0.92±0.14	0.66	0.66
		Spleen weight	0.78±0.23	1.00±0.53	0.78±0.19	0.66±0.18	0.75±0.13	0.80±0.22	0.49	0.78
		Bursa weight	1.18±0.44	0.83±0.23	1.20±0.40	0.97±0.45	1.38±0.25	1.47±0.04	2.07	0.14
	Immune Index	Thymus Index	1.20±0.21	1.87±0.58	1.59±0.44	1.45±0.49	1.24±0.28	1.54±0.22	1.17	0.38
		Spleen Index	1.07±0.22	1.53±0.49	1.08±0.28	0.91±0.15	1.07±0.30	1.38±0.59	1.19	0.37
		Bursa Index	1.63±0.58	1.35±0.22	1.65±0.58	1.36±0.66	1.93±0.12	2.48±0.37	2.46	0.09
42nd	Weight	Body weight	2413±252.91	2894±401.05	2514±15.55	2309±451.87	2522±78.51	2514±605.38	1.10	0.41
		Thymus weight	5.50±1.80	4.30±0.40	4.03±0.95	3.70±1.15	3.58±0.33	3.63±0.75	1.51	0.26
		Spleen weight	3.65±0.82	3.63±1.33	3.03±0.64	3.42±0.52	3.50±1.32	2.97±0.46	0.31	0.90
		Bursa weight	3.78 ± 0.63^{b}	4.62 ± 0.78^{a}	4.60 ± 0.88^{a}	4.67 ± 0.58^{a}	4.87 ± 0.81^{a}	2.97 ± 0.35^{c}	3.34	0.04*
	Immune Index	Thymus Index	2.35±0.94	1.49±0.07	1.63±0.47	1.58±0.21	1.40±1.38	1.33±0.59	1.36	0.30
		Spleen Index	1.51±0.26	1.28±0.55	1.20±0.21	1.53±0.44	1.38±0.48	1.19±0.11	0.46	0.80
		Bursa Index	1.59±0.37	1.59±0.09	1.83±0.16	2.05±0.31	1.93±0.36	1.23±0.36	2.95	0.06

Means with different superscripts (a,b,c,d) within a raw are significantly different.

Table (4): Effect of probiotic (Gro-2-Max®) on *total coliform*, *E. coli* and *lactobacillus* counts (log 10^{10}) in non-treated and treated groups at the 21st and 42nd day of age

Age/ Day	Intestine	Media	Groups						ANOVA	
			1	2	3	4	5	6	F	P
21st	Caecum	MacConkey	10.02±1.12^{c}	14.13±1.57^{b}	1.20±0.01^{d}	38.90±42.40^{a}	1.50±0.02^{d}	0.04±0.01^{d}	236.08	0.001
		EMB	74.17±8.25^{b}	3.51±0.39^{c}	5.01±0.56^{c}	107.20±11.90^{a}	170.40±19.00^{a}	3.01±0.30^{c}	151.97	0.001
		Ragusa	10.02±1.12^{c}	18.04±0.01^{b}	28.07±3.12^{a}	16.04±1.78^{b}	14.03±1.56^{b}	20.80±0.10^{a}	70.92	0.001
42nd	Caecum	MacConkey	0.10±0.02^{c}	1.60±0.02^{a}	0.10±0.01^{c}	1.24±0.14^{b}	1.403±0.16^{a}	1.34±0.10^{a}	134.63	0.001
		EMB	9.00±2.30^{b}	0.01±0.01^{d}	0.01±0.02^{d}	20.05±2.23^{a}	0.04±0.01^{d}	1.76±0.20^{c}	233.68	0.001
		Ragusa	6.01±0.67^{c}	12.13±1.35^{b}	0.90±0.10^{d}	4.53±0.50^{c}	14.03±1.56^{b}	18.20±2.00^{a}	84.70	0.001

Means with different superscripts (a,b,c,d) within a raw are significantly different.

Table (5) Hematological parameters of different experimental groups

Age/ Day	Parameter / Group	RBCs ($\times10^6$/µl)	PCV (%)	Hb (g/dl)	TLC ($\times10^3$/µl)	Heterophil count ($\times10^3$/µl)	Lymphocyte count ($\times10^3$/µl)	Monocyte count ($\times10^3$/µl)
21st	1	2.3±0.58^a	28.6±2.29^a	11.96±2.42^a	19.27±2.13^a	4.27±1.04^a	13.71±1.32^a	1.29±0.11^a
	2	2.5±0.33^a	31.4±2.58^a	13.20±1.03^a	22.23±3.58^b	4.42±1.48^a	16.64±1.25^b	1.17±0.13^a
	3	2.4±0.55^a	29.5±2.11^a	12.29±1.32^a	21.12±2.11^b	4.39±1.09^a	15.59±1.22^b	1.14±0.12^a
	4	2.3±0.34^a	28.8±2.23^a	11.97±2.11^a	19.34±3.49^{bc}	4.22±1.18^a	14.07±1.32^{bc}	1.06±0.11^a
	5	2.3±0.46^a	28.7±2.40^a	12.00±1.92^a	19.40±3.42^{bc}	4.26±1.22^a	13.98±1.26^{bc}	1.16±0.14^a
	6	2.4±0.33^a	30.0±2.19^a	12.93±2.06^a	21.05±2.23^b	4.30±1.30^a	15.65±1.34^b	1.10±0.10^a
42nd	1	2.8±0.65^a	31.4±2.01^a	13.59±1.80^a	20.64±2.15^a	5.31±0.80^a	14.03±1.21^a	1.30±0.10^a
	2	3.0±0.60^a	34.3±2.52^a	15.21±2.64^a	23.80±1.99^b	4.98±1.19^a	17.58±1.24^b	1.24±0.10^a
	3	2.7±0.58^a	32.1±2.09^a	13.62±1.62^a	21.00±2.01^{bc}	4.84±0.67^a	15.02±1.22^b	1.14±0.09^a
	4	2.8±0.61^a	31.6±1.90^a	13.88±2.79^a	21.20±2.32^{bc}	4.86±0.81^a	15.14±1.19^b	1.20±0.08^a
	5	2.9±0.59^a	32.3±1.92^a	14.01±2.60^a	21.09±1.89^{bc}	4.77±1.01^a	15.17±1.23^b	1.15±0.11^a
	6	2.9±0.63^a	33.4±2.48^a	14.54±1.79^a	22.99±1.90^b	4.92±1.21^a	16.84±1.27^b	1.23±0.09^a
F		**0.34**	**4.03**	**2.04**	**2.01**	**1.04**	**2.28**	**0.24**

Means with different superscripts (a,b,c,d) within a column are significantly different at probability P< 0.05.

Table (6) Levels of some serum biochemical parameters of different experimental groups

Age/ Day	Parameter / Group	Total proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G ratio	T. cholesterol (mg/dl)	T. triglycerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
21st	1	2.71±0.11^{a}	1.54±0.11^{a}	1.24±0.03^{a}	1.23±0.07^{a}	168.58±8.72^{a}	183.69±11.24^{a}	76.28±0.65^{a}	54.46±1.24^{a}
	2	3.27±0.20^{b}	1.56±0.12^{a}	1.73±0.08^{c}	0.92±0.05^{b}	135.48±6.13^{b}	134.08±3.09^{b}	71.17±1.08^{a}	36.39±1.27^{b}
	3	3.07±0.10^{bc}	1.50±0.10^{a}	1.53±0.05^{bc}	0.98±0.06^{b}	153.76±8.66^{c}	162.87±10.33^{c}	78.24±0.87^{a}	41.85±1.22^{b}
	4	2.92±0.13^{c}	1.47±0.09^{a}	1.55±0.03^{bc}	0.95±0.04^{b}	152.88±7.54^{c}	163.34±11.01^{c}	80.12±1.03^{a}	38.99±1.30^{b}
	5	2.97±0.15^{c}	1.43±0.12^{a}	1.52±0.07^{bc}	0.96±0.04^{b}	148.64±7.31^{bc}	156.92±9.93^{c}	76.32±0.74^{a}	39.84±1.28^{b}
	6	3.20±0.16^{b}	1.57±0.13^{a}	1.68±0.06^{c}	0.93±0.07^{b}	140.09±6.44^{bc}	138.26±4.88^{b}	73.91±0.98^{a}	37.43±1.24^{b}
42nd	1	2.88±0.05^{a}	1.58±0.04^{a}	1.35±0.02^{a}	1.21±0.02^{a}	177.76±8.58^{a}	170.10±7.23^{a}	78.02±1.25^{a}	64.62±2.44^{a}
	2	3.54±0.13^{b}	1.55±0.09^{a}	2.09±0.09^{b}	0.89±0.05^{b}	124.67±7.37^{b}	114.92±5.70^{b}	76.67±1.22^{a}	23.92±3.24^{b}
	3	3.27±0.05b^{c}	1.43±0.08^{a}	1.59±0.01^{bc}	0.97±0.03^{b}	146.73±8.45^{c}	134.61±7.21^{c}	78.89±1.12^{a}	39.82±2.56^{c}
	4	3.24±0.09b^{c}	1.52±0.07^{a}	1.61±0.06^{bc}	0.96±0.01^{b}	158.35±7.76^{c}	145.47±5.11^{c}	81.46±1.23^{a}	46.70±2.82^{d}
	5	3.21±0.12b^{c}	1.48±0.03^{a}	1.73±0.08^{c}	0.95±0.04^{b}	140.67±7.80^{cb}	132.19±6.99^{c}	79.34±0.99^{a}	33.79±3.14^{c}
	6	3.52±0.11^{b}	1.56±0.09^{a}	1.98±0.07^{b}	0.91±0.03^{b}	131.44±7.91cb	119.93±6.87^{b}	80.62±1.24^{a}	25.73±3.30^{b}
F		**0.19**	**0.16**	**0.12**	**0.14**	**13.62**	**16.19**	**5.96**	**6.55**

Means with different superscripts (a,b,c,d) within a column are significantly different at probability P< 0.05.

Table (7) Levels of some serum biochemical parameters of different experimental groups

Age/ Day	Parameter / Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Glucose (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
21st	1	161.78±7.31[a]	30.12±1.49[a]	144.81±6.54[a]	258.47±7.13[a]	0.31±0.02[a]	6.16±0.44[a]	8.43±0.43a	6.59±0.44[a]
	2	162.93±10.25[a]	32.14±1.62[a]	143.77±6.95[a]	261.13±10.15[a]	0.31±0.02[a]	6.27±0.47[a]	11.18±0.24[bc]	6.43±0.46[a]
	3	161.71±8.12[a]	30.22±1.54[a]	144.13±6.45[a]	254.34±8.96[a]	0.30±0.03[a]	6.18±0.39[a]	9.40±0.40[b]	6.40±0.43[a]
	4	161.98±9.98[a]	31.43±1.76[a]	143.82±6.42[a]	258.00±9.68[a]	0.30±0.04[a]	6.20±0.40[a]	11.00±0.34[bc]	6.49±0.44[a]
	5	162.69±7.25[a]	32.05±1.86[a]	144.45±6.39[a]	260.27±10.11[a]	0.31±0.03[a]	6.21±0.42[a]	10.19±0.29[c]	6.50±0.41[a]
	6	161.90±8.13[a]	31.82±1.67[a]	144.66±5.99[a]	259.93±10.09[a]	0.30±0.05[a]	6.24±0.43[a]	10.85±0.42[d]	6.52±0.46[a]
42nd	1	161.18±9.91[a]	31.12±2.09[a]	141.20±6.44[a]	260.10±11.68[a]	0.34±0.01[a]	6.15±0.41[a]	8.41±0.49[a]	6.26±0.43[a]
	2	165.73±8.08[a]	31.11±1.13[a]	139.88±6.46[a]	261.37±10.82[a]	0.31±0.02[a]	6.21±0.51[a]	11.99±0.45[b]	6.18±0.43[a]
	3	162.01±7.76[a]	30.99±1.28[a]	141.12±6.73[a]	260.89±10.96[a]	0.31±0.03[a]	6.11±0.44[a]	10.21±0.41[bc]	6.19±0.42[a]
	4	161.21±9.89[a]	31.21±2.41[a]	140.56±5.86[a]	261.32±10.83[a]	0.32±0.02[a]	6.09±0.49[a]	10.78±0.45[c]	6.20±0.31[a]
	5	163.76±8.43[a]	30.97±2.67[a]	139.98±5.95[a]	261.14±11.58[a]	0.34±0.01[a]	6.20±0.52[a]	10.21±0.43[bc]	6.23±0.39[a]
	6	163.65±8.23[a]	31.03±1.87[a]	141.10±6.01[a]	260.42±11.09[a]	0.33±0.04[a]	6.19±0.51[a]	10.99±0.39[d]	6.21±0.40[a]
F		**17.99**	**4.13**	**17.38**	**17.37**	**0.15**	**1.44**	**0.61**	**0.43**

Means with different superscripts (a,b,c,d) within a column are significantly different at probability P< 0.05.

Table (8): Effect of probiotic on antibody titers against ND, IB and IBD vaccines at the 21st and 42nd day of age of all experimental groups

Age/Day	Group	ND	IB	IBD
0	1	7	1101	9501
21st	**1**	2.6±1.15[b]	82±27.02[b]	2550±1216.90[b]
	2	2.6±0.58[b]	115±54.88[b]	3697±1001.21[b]
	3	2.7±0.58[b]	342±195.14[b]	3899±1203.30[b]
	4	4.3±1.00[a]	585 ±270.49[a]	2952±1512.96[b]
	5	2.3±0.58[b]	291±159.68[b]	2050±958.82[b]
	6	3.3±1.15[b]	247±176.77[b]	4581±2610.36[a]
ANOVA	**F**	1.44	3.45	1.14
	P-Value	0.28	0.04	0.39
42nd	**1**	6.0±2.65[a]	538±536.77[b]	9082±897.48[a]
	2	7.0±0.00[a]	571±126.58[b]	9713±1831.59[a]
	3	2.3±0.58[b]	640±106.35[b]	10129±888.79[a]
	4	5.3±4.62[a]	588±142.00[b]	10562±140.50[a]
	5	6.7±2.31[a]	3029±4110.41[a]	9727±2316.47[a]
	6	6.7±2.31[a]	3125±2726.35[a]	8287±1429.96[a]
ANOVA	**F**	1.38	1.21	0.65
	P-Value	0.30	0.36	0.67

Means with different superscripts (a,b,c,d) within a column are significantly different.

Table (9): Effects of probiotic on PA, PI and LTT at the 21st and 42nd day of age of all experimental groups

Age/Day	Test	Group					
		1	2	3	4	5	6
21st	PA	44.68± 2.33[a]	45.63± 4.37[a]	45.68± 2.01[a]	39.80± 4.89[a]	42.10± 3.87[a]	39.88± 3.98[a]
	PI	4.41± 0.50[ab]	4.28± 0.30[ab]	4.30± 0.08[ab]	3.55± 1.09[b]	4.97± 0.33[a]	4.31± 0.43[ab]
	LTT	26.95± 2.66[b]	33.33± 1.59[a]	31.35± 6.70[a]	26.40± 4.01[b]	30.35± 2.91[a]	26.63± 4.92[ab]
42nd	PA	46.17± 3.09[a]	46.17± 7.91[a]	48.83± 1.88[a]	45.63± 2.11[a]	48.23± 8.01[a]	45.20± 2.35[a]
	PI	3.44± 0.29[a]	3.12± 0.15[a]	3.21± 0.45[a]	3.73± 0.47[a]	3.65± 0.43[a]	3.48± 0.47[a]
	LTT	29.23± 1.92[a]	29.83± 7.60[a]	33.17± 1.38[a]	33.40± 5.82[a]	30.93± 6.39[a]	32.53± 5.98[a]

Means with different superscripts (a,b,c,d) within a raw are significantly different.

Cellular immunity at 21st and 42nd days cleared insignificant changes in PA and PI except at 21st day, group 5 showed significant increase in PI. Significant increase in LTT at 21st day in groups 2, 3 and 5, and insignificant changes in groups 4 and 6 were observed while, insignificant changes in all groups at 42nd day were recorded (Table 9).

Histopathological Findings

Liver

Examination at 21st day, showed normal hepatic parenchyma including hepatic cords, blood sinusoids and portal tract in groups 1 and 2 (Fig. 1, a). Different pathological alterations include mononuclear cells infiltration in portal tract of group 3 (Fig. 1, b), congested central vein and dilated blood sinusoids in group 4 (Fig. 1, c), and infiltrated necrotic hepatocytes with mononuclear inflammatory cells in group 5 (Fig. 1, d) were demonstrated. Apparently healthy hepatic parenchyma with slight leukocytic infiltration was noticed in group 6 (Fig. 1, e). At 42nd day, group 1 showed congested hepatoportal blood vessel (Fig. 1, f) while, other groups showed the same findings to those at 21st day.

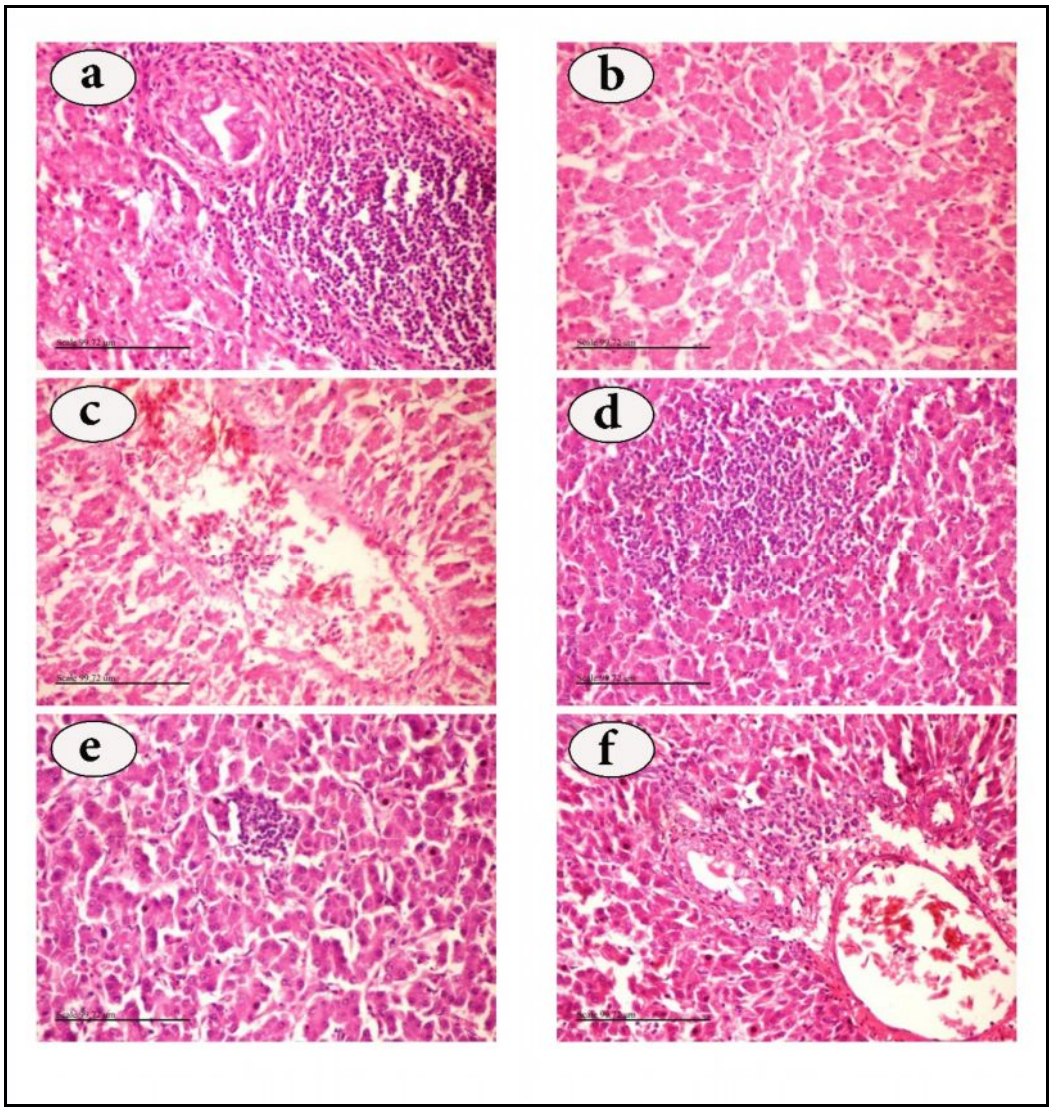

Fig. (1): Liver at 21st and 42nd day showing;
(a): Apparently normal portal area, hepatic cord and blood sinusoids in group 1 at 21st day and group 2 at 21st and 42nd day (H &E ×400).
(b): Mononuclear cells infiltration in portal area in group 3at 21st and 42nd day (H &E ×400).
(c): Congested central vein and dilated blood sinusoids in group 4 at 21st and 42nd day (H &E ×400).
(d): Infiltrated necrotic hepatocytes with mononuclear inflammatory cells in group 5 at 21st and 42nd day (H &E ×400).
(e): Apparently healthy hepatic parenchyma with slight leukocytic infiltration in group 6 at 21st and 42nd day (H &E ×400).
(f): Congestion of hepatoportal blood vessel with mononuclear cells infiltration in group 1 at 42nd day (H &E ×400).

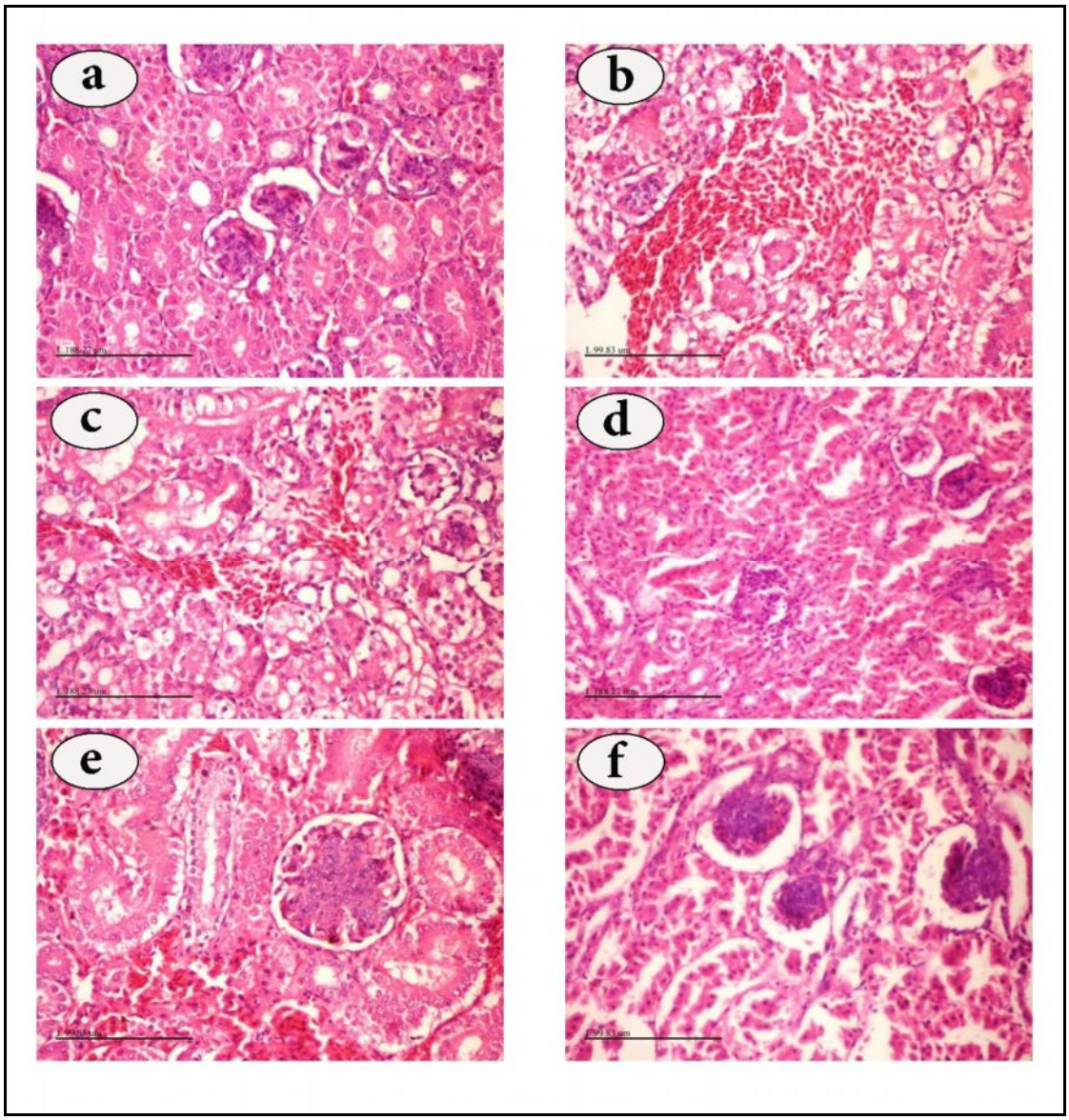

Fig. (2): Kidney at 21st and 42nd day showing;
(a): Apparently normal renal glomeruli and renal tubules in groups 1, 2 and 6 at 21st and 42nd day (H &E ×200).
(b): Edema and hemorrhages in the interstitial tissue in group 3 at 21st and 42nd day (H &E ×400).
(c): Vacuolated renal tubular epithelium and congested peritubular blood capillaries in group 4 at 21st day (H &E ×200).
(d): Infiltrated interstitial tissue with leukocytic cells in group 5 at 21st day (H &E ×200).
(e): Congested peritubular blood capillaries in group 4 at 42nd day (H &E ×400).
(f): Necrotic glomerular tuft in group 5 at 42nd day (H &E ×400).

Kidney

Examination at 21st day revealed apparently normal renal glomeruli and renal tubules in groups 1 and 2 (Fig. 2, a). Slight alterations were reported as interstitial edema and hemorrhages in group 3 (Fig. 2, b), vacuolated renal tubular epithelium and congested peritubular blood capillaries in group 4 (Fig. 2, c), interstitial leukocytic cells infiltration in group 5 (Fig. 2, d), and healthy renal tissue resemble to those observed in Fig. 2, a in group 6. At 42nd day, groups 1, 2 and 6 revealed normal microscopic findings (Fig. 2, a). Appearance of hemorrhages in the interstitial tissue still recorded in group 3 (Fig. 2, b). Congested peritubular blood capillaries in group 4 (Fig. 2, e) and necrotic glomerular tuft in group 5 (Fig. 2, f) were the main microscopic findings.

Ileum

Examination at 21st day revealed apparently normal mucosa and submucosa in group 1 (Fig. 3, a) while, groups 2 and 6 showed a lot of crypts which their lining was columnar epithelium giving increase in ileal villus height to crypts depth (Fig. 3, b). Microscopic findings of the rest groups including infiltrated mucosa with leukocytic cells in group 3 (Fig. 3, c), necrotic glands in group 4 (Fig. 3, d), and congested submucosal blood vessel in group 5 (Fig. 3, e) were recorded. At 42nd day, the same findings to those at 21st day were noticed in all groups.

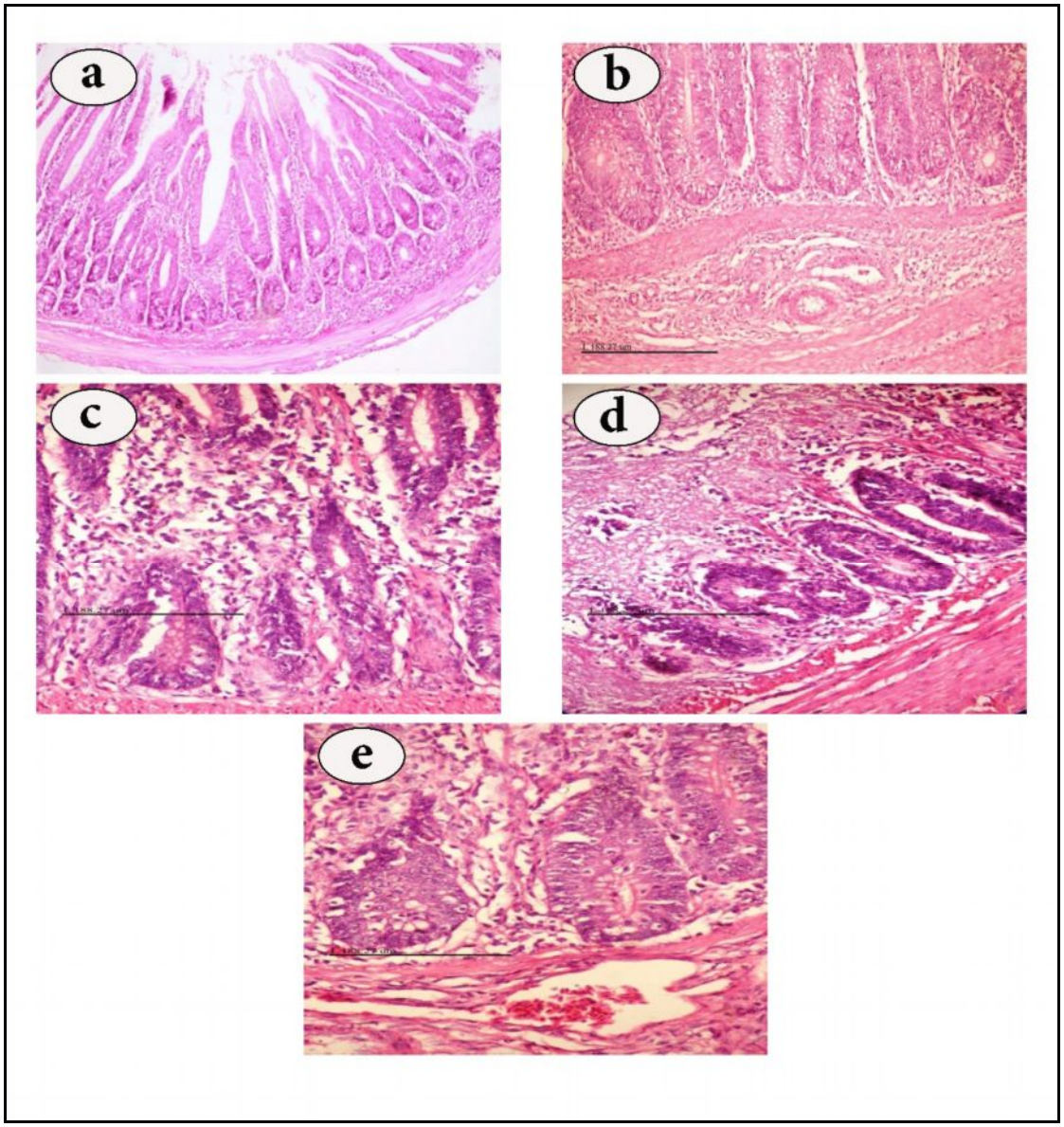

Fig. (3): Intestine (ileum) at 21st and 42nd day showing;
(a): Apparently normal mucosa and submucosa in group 1 (H &E ×100).
(b): Normal mucosa appeared with many crypts which lined with columnar epithelium and normal submucosa is in groups 2 and 6 (H &E ×200).
(c): Infiltrated mucosa with leukocytic cells in group 3 (H &E ×200).
(d): Necrotic glands in group 4 (H &E ×200).
(e): Congested submucosal blood vessels in group 5 (H &E ×400).

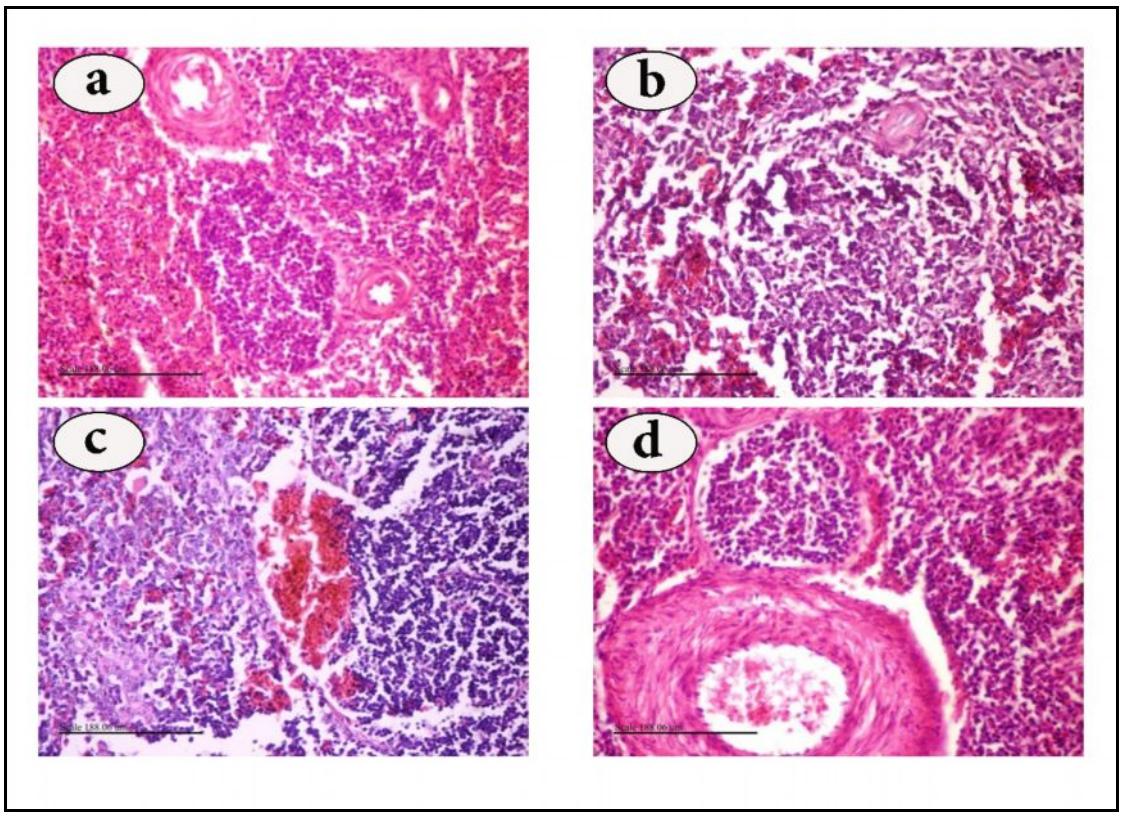

Fig. (4): Spleen at 21st and 42nd day showing;
(a): Apparently normal splenic follicles in groups 1, 2 and 6 (H &E ×400).
(b): Scanty splenic follicles in group 3 (H &E ×400).
(c): Congested splenic follicles in group 4 (H &E ×400).
(d): Atrophied follicles and thick walled blood vessels in group 5 (H &E ×400).

Spleen

Examination at 21st day, showed apparently normal white and red pulps in groups 1, 2 and 6 (Fig. 4, a). Main finding in group 3 was scanty splenic follicle (Fig. 4, b). Group 4, revealed the presence of congested splenic follicle (Fig. 4, c). Atrophied follicles and thicken wall blood vessel were detected in group 5 (Fig. 4, d). At 42nd day, same findings to those at 21st day were observed in all groups.

Bursa of Fabricious

Examination at 21st day, revealed apparently normal lymphoid follicles in groups 1 and 2 (Fig. 5, a). Atrophied follicles due to inbetween edematous pressure in group 3 (Fig. 5, b), thickening of interfollicular blood vessel wall in group 4 (Fig. 5, c), perifollicular fibrosis in group 5 (Fig. 5, d) and scanty follicles in group 6 (Fig. 5, e) were noticed. At 42nd day, the same findings were recorded in groups 1, 2 and 3. Microscopic findings in group 4 progressed into congestion of interfollicular blood vessel with interfollicular fibrosis (Fig. 5, f). Scanty follicles were observed in group 5 (Fig. 5, e). Group 6 showed healthy renal tissue as showed in Fig. 5, a.

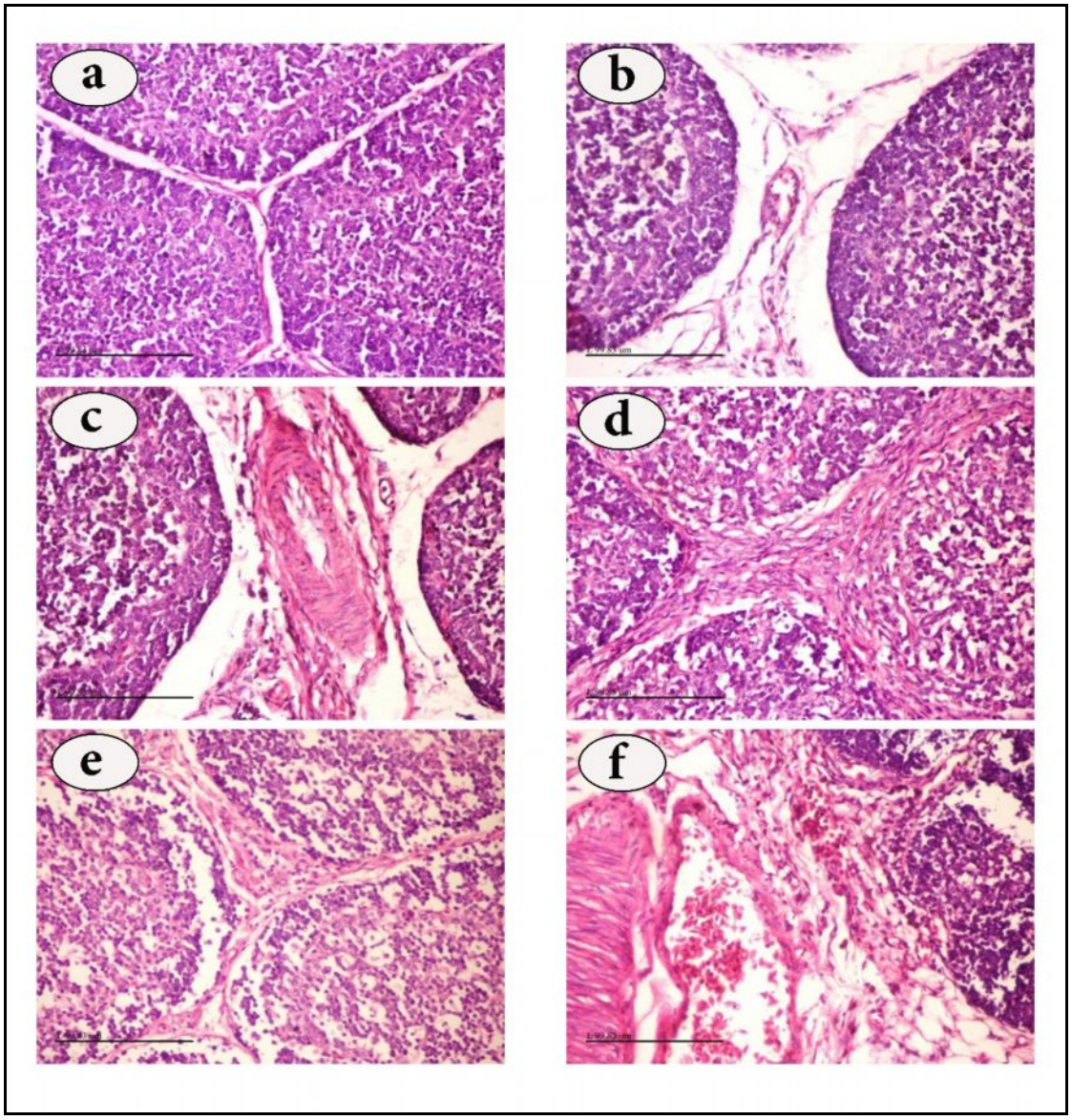

Fig. (5): Bursa at 21st and 42nd day showing;
(a): Apparently normal lymphoid follicles in groups 1 and 2 at 21st day and in group 6 at 42nd day (H &E ×400).
(b): Atrophied follicles due to edematous pressure in-between the follicles in group 3 at 21st and 42nd day (H &E ×400).
(c): Thickening of the interfollicular blood vessels wall in group 4 at 21st day (H &E ×400).
(d): Perifollicular fibrosis in group 5 at 21st day (H &E ×400).
(e): Scanty follicles in group 6 at 21st day and in group 5 at 42nd day (H &E ×400).
(f): Congestion of the interfollicular blood vessels with interfollicular fibrosis in group 4 at 42nd day (H &E ×400).

Thymus

Examination at 21st day, showed apparently normal thymic cortex and medulla in groups 1 and 2 (Fig. 6, a and b), respectively. Congestion inbetween the cortical lymphoid cells was the main recorded alteration in group 3 (Fig. 6, c). Scanty cortical lymphoid cells were seen in group 4 (Fig. 6, d). Scanty medullary lymphoid cells were observed in group 5 (Fig. 6, e). Congestion inbetween the medullary lymphoid cells were detected in group 6 (Fig. 6, f). At 42nd day, the same findings to those at 21st day were reported in all groups.

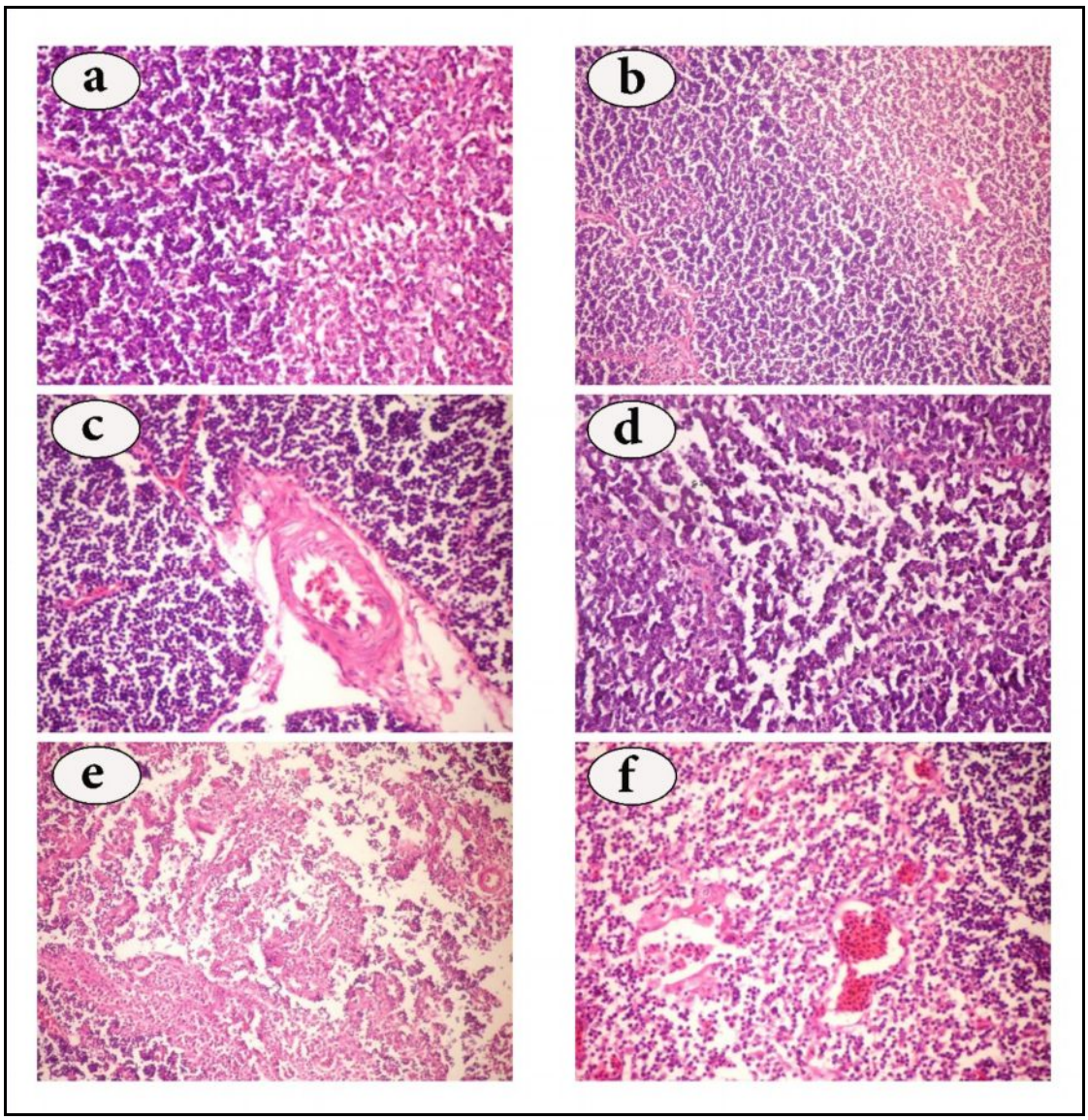

Fig. (6): Thymus at 21st and 42nd day showing;
(a), (b): Apparently normal thymic cortex and medulla in groups 1 and 2 (H &E ×400).
(c): Congestion in-between the cortical lymphoid cells in group 3 (H &E ×400).
(d): Scanty cortical lymphoid cells in group 4 (H &E ×400).
(e): Necrosed medullary lymphoid cells in group 5 (H &E ×400).
(f): Congestion in-between the medullary lymphoid cells in group 6 (H &E ×400).

Discussion

It could be seen from the present study that, administration of Gro-2-Max® had positive effect on broiler growth performance especially groups 2 and 6. This finding agrees with several reports that demonstrate probiotic supplemented to chickens remodel their performance[12] as a result of elaborating nutrient and energy utilization[13] or changing the intestinal bacterial structure which assist the growth of beneficial bacteria resulting in healthier intestinal system for superior nutrients absorption[14].

Increases in BWG and improvement in FCR associated with Gro-2-Max® may be resulted from the increases in villi height and the hyperplasia of their epithelial which observed during microscopic examination of ileal sections. These changes connected with increasing of intestinal absorptive surface area reverts the positive effect of Gro-2-Max® on digestive and absorptive functions[15].

Insignificant increase in erythrogram parameters could be attributed to production of more RBCs from bone marrow which evoked by erythropoietic factors released from activated hepatocytes by cause of probiotic used[16].

Significant lymphocytic leukocytosis may be resulted from the immuno-modulatory effect of probiotic[17]. The present lymphocytosis is suggestive of immunogenic stimulatory effect of the used probiotic as the lymphocytes represent the majority of leukocytes in the peripheral blood of normal chickens and play a principle role in chicken immunity[18].

Protein profile findings agree with Dimcho *et al*.[19] who found probiotic supplementation did not change albumin concentration of chicken. Significant decrease in lipogram including total cholesterol, total triglycerides and LDL-c concentrations may be related to the uses of probiotic which can digest cholesterol present in chicken gastro-intestinal tract for its own cellular metabolism thus downsizing the absorbed amount[20]. Another explanation is that, as Gro-2-Max® contains *Pedicoccus acidilactici* which is acidophilic, lowers the pH of the environment it occupies coupled with its high bile salt hydrolytic activity produce deconjugation of bile salts in intestine making it less soluble and less absorbable, thus preventing its action as cholesterol precursors result in reducing the amount of cholesterol present [21]. Similar lipogram results were reported by Arun *et al*.[22]. Hypercalcaemia recorded may be directly follow the uses of probiotic which increase calcium concentration[23] or may indirectly from the present hyperproteinemia as there is a linear relationship between total proteins and calcium concentration.

Results of our study concluded that, Gro-2-Max® has positive effect on chicken growth performance and it is recommended to supplement chicken with Gro-2-Max® in ration especially from the 1st day of age and in drinking water every 3 days per week till the end growing phases (42 days). Gro-2-Max® has reducing effect on lipogram especially total cholesterol, total triglycerides and LDL-c concentrations, and has improving effect on intestinal function through increases of ileal villi height. Nonspecific immune responses were recorded through studying its effect on humeral and cellular immunity.

Author's contribution:

AA and NS designed and executed the experiment. NS followed up the experiment. AA, NS and SA all did their work according to their specification. AA and NS analysed the data. AA wrote the manuscript. All authors interpreted the data, revised and approved the manuscript.

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