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Effect of Pedicoccus acidilactici on immunity, production and lipid profile in broilers

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Key words
Pedicoccus acidi lacti, S. enteritidis, broiler chicks, growth performance, blood biochemistry, hematology, NDV.

ABSTRACT:
The present study was conducted to investigate the effect of *Pedicoccus acidi lacti* supplementation to the ration of broiler chicks on growth performance, survival rate, immunity, hematological studied, serum biochemistry, response to NDV and its protective effect against artificial infection with *S. enteritidis* . One hundred and twenty day-old broiler chicks were allotted into four equal groups: group one fed on non- supplemented ration and not infected, group two fed on ration contain *Pedicoccus acidi lacti* 100 mg/kg ration and not infected, group three fed on non- supplemented ration and infected and group four fed on ration contain *Pedicoccus acidi lacti* 100 mg/kg ration and infected with *salmonella*. The results indicated that *Pedicoccus acidi lacti* supplementation improved the body weight and FCR in comparison to control group during the first two weeks (pre-infection), After infection the results indicated that the *Pedicoccus acidi lacti* supplementation protect chickens against bad effect of salmonella infection where both parameter (FCR and Body weights) still superior till 5th week. Also the addition of probiotics improved lipid profile and CBC. In the same time. It was evident that addition of *Pedicoccus acidi lacti* improved immune response to ND.

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1. INTRODUCTION

Probiotics have been defined as living microorganisms which upon ingestion inadequate numbers exert positive health effects (Fric, 2007). Probiotics are used an alternative to growth promoting antibiotics act by enhancing the three primary defense systems against pathogens: the intestinal microbiota, epithelial cell renewal and immune function (Patterson and Burkholder, 2003 and Biggs et al., 2007). A variety of microbial species have been used as probiotics, *Lactobacillus and Bifidobacterium* species have been used most extensively in humans, whereas species of *Bacillus, Enterococcus*, and *Saccharomyces yeast* have been the most common organisms used in livestock (Simon et al., 2001). It is often reported that a probiotic must be adherent and colonize within the gastrointestinal tract, it must replicate to high numbers, it must produce antimicrobial substances, and it must withstand the acidic environment of the gastrointestinal tract (Ziemer and Gibson, 1998 & Mombelli and Gismondo, 2000). *Pedicoccus acidilactici* is a species of gram positive cocci homo-fermentative that can grow in a wide range of pH, temperature and osmotic pressure and exert antagonism against other microorganisms, including enteric pathogens, primarily through the production of lactic acid and secretion of bacteriocins known as pediocins (Klaenhammer, 1993). *Pedicoccus acidilactici* has not been stated in any literature to have toxic effects. Health benefits include improvement of the normal microflora, prevention of infectious diseases (Ooi and Liong, 2010), reduction of serum cholesterol (Lee and Salminen, 1995), stabilization of gut mucosal barrier (Farkas, 1977), immune adjuvant properties (Duffy, 2000). *Pedicoccus acidilactici* is also known to prevent colonization of the small intestine by pathogens like *Shigella, Salmonella, Clostridium* difficile and Escherichia coli among small animals (Jamila, et al., 2011). The effects of Probiotics may be classified in three modes of action: Probiotics might be able to modulate the host's defenses including the innate as well as the acquired immune system (Fooks, et al., 1999 and Nermes, et al., 2011). Probiotics can also have a direct effect on other microorganisms, commensal and/or pathogenic ones. This principle is in many cases of importance for the prevention and therapy of infections and restoration of the microbial equilibrium in the gut; (Jin et al., 2000 and Ibrahim et al., 2010).

Infection by *S. typhimurium* is an important cause of morbidity and mortality in poultry, and infection with *Salmonella enteritidis* is a major cause of food-borne illness (Cohen, et al, 1994). *Salmonella enterica* serovars continue to be among the most important food borne pathogens worldwide due to the considerable human rates of illness reported the wide host species that are colonized by members of this remarkable pathogen genus, which serve as vectors and reservoirs for spreading these agents to animal and human populations. Furthermore, the public concern for the appearance of resistant strains to many antibiotics, particularly among zoonotic pathogens such as common *Salmonella* isolates, is also challenging the poultry industry to find alternative means of control (Boyle et al., 2007). Using the *Salmonella* challenge model, (Higgins et al., 2010), have observed an increase in *S.enteritidis* incidence in cecal tonsils over the initial 12 h post-treatment which indicates that the *SE* is continuing to cause infection despite theoretically coming into contact with the probiotic organisms within approximately 2 h (neonatal chick gastrointestinal transit time). Because *Salmonella* is an intracellular pathogen, we suggest that increased apoptosis may be a mechanism by which some probiotic reduces *Salmonella* infection (Higgins et al., 2011). Experimental evaluations have confirmed improved body weight gain as well as Salmonella sp. Or Clostridium perfringens reduction in commercial turkey and broiler operations fed on probiotic when compared with medicated or control non medicated diets respectively, (Tellez, et al., 2012).

Serum protein of avian blood ranges between 3 and 6 gm/dl. Albumin is considered the large fraction of the total protein, so a reading less than 3gm/dl indicates hypo-albuminemia. Chronic renal or hepatic diseases, malnutrition and malabsorption cause hypo-protenemia (Embert, 1986). The changes in liver metabolism caused by endotoxins treatment or live bacterial challenge have been observed in both mammals and birds (Curtis et al., 1980). Liver is affected greatly due to infection or sepsis which in turn affects its function, (Kokosharov et al., 1997), who reported degenerative changes in the liver to which attribute the decreased protein synthesis. Also, (Kokosharov 2007), reported significant decrease in either serum total protein or albumin due to *S. gallinarium* infection. Also he added that, the acute *S. gallinarium* infection caused reduction in albumin whereas globulin fractions increased. Serum albumin levels in *S.gallinarium* infection were lower in susceptible birds five days post-inoculation as compared to healthy birds, also, there was an increase in the activity of aspartate-aminotransferase (AST) in birds five days post-inoculation as compared to the mean value in birds of the same group (Freitas et al., 2007). These changes are correlated with the liver lesion at five-days post-inoculation, (multifocal necrosis), higher AST levels, and lower albumin levels. This may be interpreted as inability of the liver to synthesize protein due to the lesion intensity, macroscopically evidenced by hepatomegaly and measurable loss of protein in the affected kidney. Therefore, the damage in the glomerular filtration barrier may result from the presence of plasma proteins in the urine (Relford and Lees 1996).

Aim of the work: Due to many products are introduced to the Egyptian markets as an immunostimulant, antibacterial and growth enhancer, so it necessitates assessing the effect of these products. *Pedicoccus acidilactici* is one of these products, so this study was performed to evaluate its effect on bacterial infection, blood chemistry, lipids profile, performance and immunity in broilers.

2. MATERIALS AND METHODS

Experimental design:

Birds used:

A total of 125 two day–old chicks assumed to be free of *salmonella* by (15 fecal swabs and organs culture from 5 sacrificed chicks). Broiler chicks were allotted into 4 groups (25 bird /group) by ranking method and treated as shown in (table 1). Where *Pediococcus acidilactici* were added to gp2&gp4 at a rate of 100mg/kg ration, other gps fed plain ration, till 35day. Broiler chicks were housed in experiment room with initial temperature set at 32°C then gradually decreased to 23°C. These chicks were exposed to a photoperiod of 24 h of light. All gps were vaccinated against ND and IBD at 7 & 12 day respectively. Feed and water were provided ad libitum. The birds were fed formulated ration that meet the nutritional requirements according to the NRC (1994) as shown in Table 2. Feed samples were analyzed for moisture, crude protein (CP), ether extracts (EE), crude fiber (CF) according to AOAC, (1990) (table 2).

Table (1): Experimental design outline.

Group No.	Diet type	Pedicoccus supplementation*	S.enteritidis infection*
1	Basal diet	-	-
2	"	100mg/kg ration	-
3	"	-	+ve
4	"	100mg/kg ration	+ve

* *Pedicoccus* is a Probiotic (Bactocell) a commercial product each 1gm contains 1x109 CFU. EGAVET, Giza, Egypt.

Table (2) Physical and chemical composition of the basal experimental diets.

Ingredient composition	Experimental diets	
	Starter	Grower and finisher
Yellow corn (8.5%)	53.00	59.50
Soybean meal (44%)	29.26	29.00
Corn gluten meal (62%)	9.00	3.427
Sun flower oil	4.50	4.50
Dicalcium phosphate	1.80	1.30
Limestone	1.30	1.30
Lysine	0.08	0.29
DL-Methionine	0.10	0.044
Common salt	0.40	0.40
Choline chloride 50%	0.26	0.20
Trace minerals and vit. premix	0.30	0.30
Values between parentheses are determined crude protein content (N×6.25).		
Analysed chemical composition percentage	Experimental diets	
	Starter	Grower and finisher
Dry matter %	89.60	89.69
Moisture	10.40	10.31
CP %	21.87	19.16
EE	5.64	5.99
Ash	6.81	6.89
CF	3.02	3.43
NFE	52.26	54.22
ME(Kcal/Kg)	3147.1281	3176.7282

Metabolisable energy (ME) estimation was done acc. to the equation of Lodhi et al. (1976).

The used premix (multimix broiler) without choline composed of vitamin A 12000000 ıU, vitamin D3 2200000 ıU , vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, Niacin 30000 mg, Biotin 50 mg, Folic acid 1000 mg, pantothenic acid 10000 mg, Iron 30000 mg, Manganese 60000 mg, Copper 4000 mg, Zinc 50000 mg, Iodine 1000 mg, Cobalt 100 mg, Selenium 100 mg, Calcium carbonate(CaCo3) carrier to 3000 g.

Bacterial infection:

Experimental infection via oral route with 1 ml containing $3x10^8$ of *S. enteritidis* at 15 day –old. S.enteritidis strain used in artificial infection was supplied by A.M. Hegazy (Animal Health Res. Instit., Kafr El-Sheikh Regional lab).

Measurements:

Growth performance parameters:

Body weights (BW), feed conversion ratio (FCR) and relative growth rate were estimated acc. To Vohra and Roudybush (1971), Ensminger (1980), Brody (1968) respectively. Body weight gain was calculated by the difference between two successive weeks or periods weights, feed intake (FI) and mortality rate were recorded weekly.

Immun response:

HI titre against ND was preformed as described by (king and Seal 1998). The ND virus and positive control serum was kindly provided by A.Y.Tahoon, Animal Health Research Institute

(Kafr El-Sheikh Branch).H.I against ND was measured.

Blood pictures and biochemical changes:

Blood was collected weekly post-infection from five randomly selected birds from each group through the brachial vein. 1 ml of this blood was mixed immediately in Ependorff tubes with EDTA (Anticoagulant) and used for hematological analysis. The rest of blood centrifugate at 3,000 x g for 15 minutes for serum separation. Where erythrocyte and leukocyte count (Dacie and Lewis 1984), hemoglobin content (Vankampen, 1961) and packed cell volume (PCV), (Britton 1963). While biochemical parameters were assayed calorimetrically by using of commercial diagnostic kits of total protein (Weichselbaum, 1946), albumin (Doumas, 1971), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Retiman and Francle, 1957). Creatinine (Husdan and Rapaport, 1968), uric acid (Arliss and Entvistle, 1981), total cholesterol (Allain et al., 1974), serum LDL (Wieland and Seidel, 1982), serum HDL (Lopez-Virella et al., 1977), serum triglyceride (Fossati and Prencipe, 1982).

S. enteritidis colonization:

Fecal shedding, organ colonization were recorded.Confirmation of the results of colonization and shedding was done by *S. enteritidis* antiserum prepared in rabbit as described by (Seleim, 1999).

Symptoms and P/M lesions:

All gps kept under observation for symptoms, P.M and mortality along the experimental period .

Statistical analysis:

Using the General Linear Model for analysis of variance (SPSS.16, 1997). Duncan's multiple range test (Duncan, 1955) was used for test the significance ($p<0.05$) of differences among means.

Table (3): Effect of *pediococcus acidi lactici* supplementation without or with *SE* infection on growth performance of broiler chicks.

Age/week	Supplementation	Without infection	With SE infection
2nd day	Control	56 ± 1.32ax	56 ± 1.32ax
	pediococcus acidi lactici	53 ± 0.87ax	53 ± 0.87ax
1	Control	135.2 ± 0.44ax	131 ± 0.58ax
	pediococcus acidi lactici	157.3 ± 0.67bx	142.1 ± 0.59bx
2	Control	285.1 ± 0.47ax	280.3 ± 0.67ax
	pediococcus acidi lactici	338.2 ± 0.60bx	332.2 ± 0.62bx
3	Control	558.3 ± 0.33ax	518.2 ± 0.39by
	pediococcus acidi lactici	621.1 ± 0.58bx	562.3 ± 0.65ay
4	Control	970.2 ± 0.6 ax	865.0 ± 0.58by
	pediococcus acidi lactici	981.0 ± 0.57ax	945 .0± 0.58ay
5	Control	1439.1 ± 0.64ax	1211.1 ± 0.59by
	pediococcus acidi lactici	1405.0 ± 0.59ax	1397.3 ± 0.64ax
RGR	Control	185.02	182.32
	pediococcus acidi lactici	185.46	185.38

Relative growth rate (RGR): was calculated acc. to the equation described by Brody (1968). Values are expressed as mean ± standard errors. Means between different groups in the same column (a-c) and same raw (x-y) with different letters significantly differ at ($p\leq0.05$).*= time of infection.

3. RESULTS AND DISCUSSION

Growth performance:

Effect of Pediococcus acidi lactici supplementation without or with SE infection on broiler chicken body weight development are presented in table 3. Statistical analysis of the obtained data revealed that no significant difference between different groups at the start of the experiment, while Pediococcus acidi lactici supplementation significantly ($P\leq0.05$) improved broiler chick body weight at 1st, 2nd and 3rd weeks of chicks age by about 16.3%, 18.6% and 11.2% respectively when compared with control group. However Pediococcus supplementation non significantly increased body weight at 4th weeks of age and reduced ($P\geq0.05$) final body weight when compared with broiler chick group fed on the control diet. Moreover, it was observed that SE infection reduced body weight of broiler chick fed on control or Pediococcus supplementation at 3rd, 4th and 5th week of broiler chickens age by about (7.2%, 10.8% and 15.8%) and (9.5%, 3.7% and 0.5%) respectively when compared with broiler chicks fed on the same diet

without infection. Pedicoccus supplementation slightly improved relative growth rate of broiler chicks and had protection against the bad effect of SE infection.

Regarding feed conversion ratio (table, 4) it was observed that pediococcus acidi lactici significantly improved (P≤0.05) FCR of broiler chicken at 1st, 2nd and 3rd week of age by about 6.9%, 5.8% and 8.9% respectively when compared with control one. On the other hand pediococcus acidi lactic supplementation had no significant effect on FCR during 3 -5 week of broiler age, moreover pediococcus supplementation non significantly (P≥0.05) improved FCR throughout the whole experimental period by about 4.8% when compared with control group. Broiler chicken infection by SE and fed on control or Pediococcus acidi lactici supplementation deteriorate FCR throughout the whole experimental period by about 7.8% and 3.2% respectively when compared with boiler chick group fed on the same diet without SE infection. The present data are supported by those obtained by Stella et al., 2005; Tollba et al., 2007 and Satheesh et al., 2012.

The obtained data proofed that Pediococcus acidi lactici supplementation more effective as growth stimulant during the first three weeks of broiler age while acting as a protective effect to counter act the bad result of SE infection during the finisher period. The present data are in harmony with those obtained by (Stella et al.,2005 and Tollba et al., 2007 and Djezzar, et al., 2013).

Table (4): Effect of *pediococcus acidi lactici* supplementation without or with *SE* infection on feed conversion ratio (FCR).

period / week	Supplementation	Without infection	With SE infection
0 - 1	Control	1.44 ± 0.006ax	1.46 ± 0.005ax
	pediococcus acidi lactici	1.34 ± 0.012bx	1.36 ± 0.023bx
1 – 2	Control	1.54 ± 0.003ax	1.53 ± 0.008ax
	pediococcus acidi lactici	1.45 ± 0.006by	1.56 ± 0.009ax
2 – 3	Control	1.68 ± 0.008ay	1.82 ± 0.007ax
	pediococcus acidi lactici	1.53 ± 0.020by	1.62 ± 0.012bx
3 – 4	Control	1.75 ± 0.007ay	2.04 ± 0.017ax
	pediococcus acidi lactici	1.68 ± 0.017ax	1.73 ± 0.005bx
4 – 5	Control	1.91 ± 0.008ay	2.11 ± 0.015ax
	pediococcus acidi lactici	1.89 ± 0.006ax	1.90 ± 0.011bx
0 – 5 (average FCR)	Control	1.66±0.08ay	1.79±0.13ax
	pediococcus acidi lactici	1.58±0.1ax	1.63±0.09ax

Feed conversion ratio (FCR) was estimated acc. to the equation by Ensminger (1980). Values are expressed as mean ± standard errors. Means between different groups in the same column (a-c) and same raw (x-y) with different letters significantly differ at (p≤0.05).

Immune response:

Immune response against ND vaccination as evaluated by HI titre revealed differences in log of the base 10 or the numerical values of GM. (Table, 5). Although the geometric mean titers is higher in non infected groups 70.2 in control negative and 76.99 in *pedicoccus* control vs.33.51 in *pedicoccus-salmonella* group and 26.59 in *salmonella* control we are not going to discuss it as it depend on many factor . Yet the coefficient of variation (CV) is a critical point as it measures the homogeneity within the flock. The CV was affected and may be attributed to the *salmonella* infection (11.13) while *pedicoccus* addition decreased the effect of *salmonella* CV (6.5%) and slightly improve CV (3.17%) than the control negative (3.53%). the curative effect of probiotic has been reported previously, and one mechanism may be the non-specific stimulation of immunity. The increase of local IgA levels resulting from ingestion of the probiotic may contribute to enhancement of the mucosal resistance against GIT infections (Fukushima et al., 1998 and Fooks et al., 1999).

Table (5) HI titer for ND expressed numerically* and as a log of base 10[a]

Parameters		Supplementation	Without infection	With SE infection
1st wpi	GM	Control	*32 [a]1.51	*12.1 [a]1.08
		pediococcus acidi lactic	*25.3 [a]1.38	*16 [a]1.2
2nd wpi	GM	Control	147 2.17	32 1.51
		pediococcus acidi lactic	168.9 2.23	64 1.81
3rd wpi	GM	Control	73.5 1.87	48.5 1.69
		pediococcus acidi lactic	111.4 2.05	36.8 1.57
Overall GM GSD CV		Control	70.2 1.85 2.48 3.53%	26.59 1.42 2.96 11.13%
		pediococcus acidi lactic	76.99 1.89 2.44 3.17%	33.51 1.53 2.179 6.5%

GM: geometric mean. GSD: geometric standard deviation. CV: coefficient of variation

Blood pictures:

Changes that happened in the blood picture and biochemical values are a mirror of the changes occurred in the tissues and organs as a result of bacterial infection. Values of Hb, PCV, RBCS and WBCS (table, 6) varied in significance among groups and within group relative to the period post challenge. Regarding the overall average of these values follow the same pattern as they were higher in salmonella treated groups vs. non salmonella treated gps., increase of Hb, PCV, and RBCS (being related to each other) may be attributed to hemo- concentration resulted from diarrhea . These findings disagree with those reported by, (Assoku et al., 1970) and may be due to that they used *S. gallinariumm* in the infection and this serotype is documented as host –specific and is more pathogenic than *S. enteritidis*. The WBCS did not differ between *pedicoccus* treated gps and negative control gp in the 1st 2nd weeks. This disagrees with the findings of (Chafai et al., 2007).

Liver and renal function:

Serum protein of avian blood ranged between 3 and 6 gm/dl. Albumin is considered the large fraction of the total protein, so a reading less than 3gm/dl indicates hypo- albuminemia. Chronic renal or hepatic diseases, malnutrition and malabsorption cause hypoprotenemia (Embert, 1986). The changes in liver metabolism caused by endotoxins treatment or live bacterial challenge have been observed in both mammals and birds (Curtis et al., 1980).

Blood serum lipid profile:

It seems that infection with *salmonella* decreases cholesterol level (table, 7) as in the present work it level was 125mg/dl vs.133 in control gp. Also *Pedicoccus* decreases the cholesterol 119 in *pedicoccus* treated gp vs. 133 in control gp. (Mohan et al., 1996; Jin et al., 1998; Chafai, et al., 2007 and Alkhalf et al., 2010). Yet the highest drop 111mg/dl was seen in *Pedicoccus-salmonella* treated gp. The decrease in cholesterol level could be due to the coprecipitation of cholesterol with deconjugated bile salts (Klaver and Van der Meer, 1993), but there is no study to show this ability and also its mechanism in *pedicoccus acidilactici.* There are many significant variation between the values of triglyceride (TG), HDL and LDL among groups (table, 8) but concerning the overall average it seems that *salmonella and pedicoccus* cause slight increase in TG and LDL while HDL is slightly decreases.

Liver is affected greatly due to infection or sepsis which in turn affects its function, (Kokosharov et al., 1997), who reported degenerative changes in the liver to which the decrease was attributed. Also, (Kokosharov 2007), reported significant decrease in either serum total protein or albumin due to *S. gallinarium* infection, also he added that, the acute *S. gallinarium* infection caused reduction in albumin whereas globulin fractions increased.

Table (6): Effect of *pediococcus acidi lactici* supplementation without or with *SE* infection on blood picture (CBC.)

Parameters	Supplementation	1 week		2 week		3 week		Overall average	
		Without infection	With SE infection	Without infection	With SE infection	Without infection	With SE infection	Without infection	With SE infection
Hb (g/dl)	Control	8.69 ± 0.12cx	8.63 ± 0.32cx	8.21 ± 0.61ax	8.18 ± 0.54ax	8.5 ± 0.12bcx	8.7 ± 0.17abx	8.46 ± 0.14a,b	8.5 ± 0.16a,b
	pediococcus acidi lactici	7.84 ± 0.32 acx	9.44 ± 0.32bcx	7.72 ± 0.42ax	8.08 ± 0.36ay	7.69 ± 0.45cx	8.31 ± 0.24bcy	7.75 ± 0.05a	8.61 ± 0.42b
PCV %	Control	32.33 ± 0.33ax	36.67 ± 1.76axz	30.33 ± 2.60ax	29.67 ± 2.33ayz	31 ± 1.53ax	32.67 ± 0.88az	31.21 ± 0.59a	33.04 ± 2.03a
	pediococcus acidi lactici	31.33 ± 2.33ax	33.67 ± 2.33axy	26.67 ± 0.33ax	26.33 ± 0.88ayz	30 ± 1.15az	31 ± 1.53ay	29.29 ± 1.38a	30.36 ± 2.14a
RBCs $x10^6/mm^3$	Control	2.7 ± 0.06ax	2.91 ± 0.12ax	2.78 ± 0.19ax	3.07 ± 0.09ax	2.7 ± 0.1ax	2.8 ± 0.16ax	2.7 ± 0.05a,b	2.93 ± 0.08a
	pediococcus acidi lactici	2.47 ± 0.29ax	3.1 ± 0.15axy	2.43 ± 0.15ax	2.42 ± 0.22ayz	2.46 ± 0.16ax	2.7 ± 0.02ay	2.45 ± 0.02b	2.74 ± 0.2a,b
WBCs $x10^3/mm^3$	Control	28.5 ± 0.87ax	29 ± 0.32ax	21.33 ± 0.44ax	23.83 ± 0.37ax	27.44 ± 0.54bcx	31.64 ± 1.14abx	25.81 ± 2.26a	28.16 ± 2.29a
	pediococcus acidi lactici	28.16 ± 0.30ax	31 ± 0.15ax	21.83 ± 0.12ay	25.83 ± 0.22ax	27.38 ± 1.13bcx	29.16 ± 0.59bx	25.66 ± 1.9a	28.73 ± 1.53a

Values are expressed as mean ± standard errors. Means between groups in the same period (a-c) and between periods in the same group (x-y) with different letters significantly differ at ($p \leq 0.05$).

Screening of some blood biochemical parameters (table, 8) showing hypoproteinemia was evident due to *S. enteritidis* infection, as there is a significant decrease in the average level of total protein (3.43g /dl) if compared with the control group (4.179 g/dl), this effect may be attributed to the pathological effect of *salmonella* on liver as judged by isolation of the organism from the liver tissue (Freitas et al., 2007), in the same time pedicoccus did not improve liver total protein(4.04g/dl),but protect acute drop 3.43 in salmonella infected gp vs.3.85 in *pedicoccus-salmonella* treated gp. Serum albumin levels in *S. gallinarium* infection were lower in susceptible birds five days post-inoculation as compared to healthy birds, also, there was an increase in the activity of aspartate-aminotransferase (AST) in birds five days post-inoculation as compared to the mean value in birds of the same group (Freitas et al., 2007) these changes are correlated with the liver lesion at five-days post-inoculation, (multifocal necrosis), higher AST levels, and lower albumin levels. This may be interpreted as inability of the liver to synthesize protein due to the lesion intensity, macroscopically evidenced by hepatomegaly and measurable loss of protein in the affected kidney. Therefore, the damage in the glomerular filtration barrier may result in the presence of plasma proteins in the urine; in addition, inflammation of the renal parenchyma or epithelial damage of the tubules may cause loss of protein to the urine, Relford and Lees (1996).

Albumin, is synthesized by the liver and has a half life about 2 weeks, so decrease in albumin level may be due to decrease production by the liver or albumin loss either from the kidney (nephropathy) or loss from intestine (enteropathy). In the present work, the albumin followed the same pattern of total protein, due to close relationship between them (Table, 8).

Alanine amino transferase (ALT) formally termed serum gultamic pyruvic transferase (SGPT), present mostly inside hepatocyte so it is specific for the liver of human and other animal but not in birds (Lohr, 1975). Mean while in this work, salmonella infection increase ALT 11 u/l vs. 8 u/l in control negative one (Table, 8). While *pedicoccus* had no affect on ALT. On the other hand aspertate transferase (AST) formally termed serum gultamic oxaloacetic transferase (SGOT), present in liver cell , intestine and muscles, in acute infection it proceed ALT, a significant increase in the levels of aspartate aminotransferase was detected 82u/l in salmonella infected group vs.66u/l in control one (Itoh et al,. 1996). Although serum AST is not liver specific in birds, increased activity has been associated with hepatocellular damage in chicken and turkeys (Rivtez et al., 1977 and Pearson et al., 1979).

Table (7): Effect of *pediococcus acidi lactici* supplementation without or with *S* infection on lipid profile.

Parameters	Supplementation	1 week		2 week		3 week		Overall average	
		Without infection	With SE infection	Without infection	With SE infection	Without infection	With SE infection	Without infection	With SE infection
Cho mg/dl	Control	137.97 ± 4.82ax	103 ± 10.51by	132.33 ± 4.67bx	151.67 ± 4.33ax	130.4 ± 4.35ax	120.33 ± 7.51ay	133.57 ± 2.27a	125 ± 14.24a
	pediococcus acidi lactici	108.5 ± 8.67bxz	86.3 ± 7.26bz	118 ± 6.66 bcz	114 ± 4.93cy	133.73 ± 3.77ayz	135 ± 4.16ax	119.95 ± 7.25a	8.61 ± 0.42b
TG mg/dl	Control	47.6 ± 5.48ax	60.3 ± 13.83ax	45 ± 5.77 ax	64 ± 5.19ax	33.33 ± 3.33cx	48 ± 4acx	41.98 ± 4.39a	57.43 ± 4.84a
	pediococcus acidi lactici	57.13 ± 5.51ax	53.93 ± 3.17az	51.67 ± 3.33ax	57.67 ± 8.19axz	30 ± 5.77bcy	32.67 ± 6.06cyz	46.27 ± 8.28a	48.09 ± 7.79a
HDL mg/dl	Control	83.8 ± 10.45acxz	66.63 ± 0.47cxz	61.9 ± 5.4az	62.57 ± 6.62az	53.63 ± 4.17cbyz	49.43 ± 5.42byz	66.44 ± 9a	59.54 ± 5.19a
	pediococcus acidi lactici	59.03 ± 7.65bcx	71.9 ± 6.29cxz	46.43 ± 5.96ax	52 ± 4.32ayz	66.63 ± 3.41 acx	66.07 ± 4.08acz	57.36 ± 5.89a	63.32 ± 5.91a
LDL mg/dl	Control	34.51 ± 5.57by	23.11 ± 7.77by	61.97 ± 1.98bx	76.3 ± 5.80abx	64.77 ± 6.06 ax	64.63 ± 5.25ax	53.75 ± 9.65a	54.68 ± 16.14a
	pediococcus acidi lactici	34.71 ± 5.64by	3 1.16 ± 1.56by	65.9 ± 5.80bx	57.13 ± 5.75cbx	71.1 ± 2.11ax	71.13 ± 4.19ax	57.24 ± 11.36a	53.14 ± 11.71a

Values are expressed as mean ± standard errors. Means between groups in the same period (a-c) and between periods in the same group (x-y) with different letters significantly differ at ($p \leq 0.05$).

Transient impairment of kidney function that has been noted during acute phase-infection (First, 1996). Uric acid is the primary catabolic product of protein, the avian kidney excrete uric acid primarily by tubular excretion, the normal serum uric acid of the most bird is 2-15 mg/dl (Embert,1986). Hyper uricemia in birds is associated with starvation, gout, tissue destruction and renal disease (Osbaldiston, 1968 and Rivtez, et al., 1977). Under the circumstances of the present work we can state that all groups revealed hyperuricemia, according to the findings of (Embert, 1986), but salmonella infection significantly increased uric acid level to 13.06 mg /dl vs. 9.55 mg/dl in control negative group (Table, 8). Also, it is evident that *Pedicoccus* decrease uric acid level either in infected or control groups from 10.9 to 8.47 mg/dl, respectively.

Furthermore creatinine is a product of protein metabolism, so its serum level increase indicates a defective excretion from the kidney. Creatinine is not a major non protein nitrogenous component of avian blood, the normal serum creatinine of the most birds' ranges from 0.5- 1.5 mg/dl (Rivtez et al., 1977).

In the present work, *salmonella* infection, significantly increase serum creatinine level 0.82mg/dl in salmonella infected group, vs. 0.58 mg/dl in control negative group. In the same time the addition of *pedicoccus* in control gp showed no effect but in *pedicoccus-salmonella* group slightly relief the *salmonella* effect on creatinine level 0.67 mg/dl vs. 0.58 in control negative group and 0.82 mg/dl in *salmonella* infected group.

S. enteritidis colonization:

S. enteritidis differed in their colonization of different organs where it shows the rates of 60, 40, 33, and 33% for each of intestine, liver, spleen and gall bladder respectively (table, 9). Similar observations were reported by (Barrow, 1991 and Gorham et al., 1991). Several experiments have demonstrated that prevention of *Salmonella* colonization in chickens can be achieved by many treatments, probiotics is one of them (Johannsen et al., 2004). This was true as in the present work *S. enteritidis* was capable to colonize different organs with different rates (Table, 9) intestine, 60 vs. 30% , liver, 40 vs.13% , spleen, 33 vs.20% and gallbladder, 33vs.13% in each *salmonella* infected control group and *pedicoccus* vs. *salmonella* group respectively, and this could be supported by the findings of (Tollba et al,. 2007 and Jamila, et al., 2011) who reported that probiotics, prebiotic or both, suppressed the counts of pathogenic intestinal bacteria and decreased colonization of *salmonella*.

Table (8): effect of *pediococcus acidi lactici* supplementation without or with *SE* infection on serum proteins, liver and renal function.

Parameters	Supplementation	1 week		2 week		3 week		Overall average	
		Without infection	With SE infection	Without infection	With SE infection	Without infection	With SE infection	Without infection	With SE infection
TP g/dl	Control	3.75 ± 0.17ayz	3.25 ± 0.27ax	4.18 ± 0.19az	3.5 ± 0.49ax	4.59 ± 0.23axz	3.53 ± 0.26ax	4.17 ± 0.24a	3.43 ± 0.08b
	pediococcus acidi lactici	3.75 ± 0.25ayz	3.75 ± 0.13ax	4.24 ± 0.38az	3.87 ± 0.27ay	4.12 ± 0.13axz	3.93 ± 0.28ay	4.04 ± 0.15a	3.85 ± 0.05a,b
AL g/dl	Control	2.85 ± 0.08ax	1.93 ± 0.11bx	2.81 ± 0.07ax	1.79 ± 0.02bx	2.70 ± 0.09ax	1.87 ± 0.06bx	2.79 ± 0.04a	1.86 ± 0.04b
	pediococcus acidi lactici	2.47 ± 0.23cx	2.66 ± 0.04acx	2.63 ± 0.02ax	2.91 ± 0.02ay	2.73 ± 0.04ax	2.02 ± 0.06by	2.61 ± 0.07a	2.53 ± 0.261a
ALT u/l	Control	7.33 ± 1.3ax	9.33 ± 1.33bx	9.33 ± 1.60ay	13.67 ± 1.86by	7.67 ± 1.33ax	10.33 ± 1.33bx	8.11 ± 2.15a	11.11 ± 3.01a
	pediococcus acidi lactici	7.33 ± 1.33ax	7 ± 0ay	10 ± 0ay	8.67 ± 1.20ay	7.33 ± 1.33ax	12.33 ± 1.33bx	8.22 ± 0.89a	9.33 ± 1.57a
AST u/l	Control	55.33 ± 7.33ay	70 ± 4bz	69.33 ± 7.33ax	82 ± 5.77by	74.67 ± 6.28ax	96.67 ± 6.96cx	66.44 ± 5.77a	82.99 ± 7.8a
	pediococcus acidi lactici	54 ± 4ay	57 ± 2ax	73.33 ± 6.36ax	63.67 ± 2.67ay	79 ± 5.77abx	83 ± 3.14bz	68.78 ± 7.57a	67.89 ± 7.8a
Uric Acid mg/dl	Control	7.79 ± 0.89ay	12.32 ± 0.94by	9.87 ± 0.58ax	10.17 ± 1.57bx	11 ± 0.26bz	16.69 ± 0.98dz	9.55 ± 0.94a,b	13.06 ± 1.92a
	pediococcus acidi lactici	7.04 ± 0.65ay	8.72 ± 0.42ax	9.97 ± 1.44ax	11 ± 0.47bx	8.4 ± 0.1ayx	12.99 ± 1.88cx	8.47 ± 0.85b	10.9 ± 1.2a,b
Creatinine mg/dl	Control	0.23 ± 0.03az	0.27 ± 0.01ay	0.64 ± 0.08bcy	0.6 ± 0.09cdy	0.87 ± 0.07bx	1.6 ± 0.23ax	0.58 ± 0.19a	0.82 ± 0.39a
	pediococcus acidi lactici	0.26 ± 0.01az	0.23 ± 0.03ax	0.42 ± 0.04ady	0.68 ± 0.04cy	0.93 ± 0.07bx	1.09 ± 0.06bz	0.54 ± 0.2a	0.67 ± 0.25a

Values are expressed as mean ± standard errors. Means between groups in the same period (a-c) and between periods in the same group (x-y) with different letters significantly differ at ($p \leq 0.05$).

Table (9): Colonization of *S. enteritidis* and rate of shedding as judged by intestinal colonization.

	liver			G.bladder			spleen			intestine			Total(T)		
	+	T	%	+	T	%	+	T	%	+	T	%	+	T	%
S. E + P	2	15	13	2	15	13	3	15	20	5	15	33	12	60	20
S.E	6	15	40	5	15	33	5	15	33	9	15	60	25	60	42
total	8	30	27	7	30	23	8	30	27	14	30	47	37	120	31

*S.E+P = *S.enteritidis + Pedicoccus* *S.E = S.enteritidis*

Symptoms and P/M lesions:

Experimental infection revealed suggestive clinical and gross pathological lesions, in the form of depression which appeared after 48h pi which was associated with whitish diarrhea unabsorbed yolk sac, distended gall bladder, enlarged congested liver, distended cecum and sometimes cecal core (Gast and Benson, 1995) and the appearance of intestinal ulcer characteristics for ND specifically in the duodenum were obvious in gp.3. All the previously mentioned symptoms and p.m changes were less prominent in gp.4 in comparison with gp.3 this may be attributed to the effect of *pedicoccus* sp. Mortality: No significant variation in mortality (one chick died 4hr after salmonella inoculation in gp.3) as it was 3&2 chicks for each of gp.3 & gp.4 respectively (table 10). Deaths were restricted to 1st 5days pi, the mortality rate was lower than that recorded by other workers and this may be attributed that older birds were considerably less susceptible to the lethal effects of *Salmonella paratyphoid* and may experiences intestinal colonization and even systemic dissemination without significant morbidity or mortality, or the fact that the paratyphoid bacteria are not host specific and produced mortality only in young chicks (Gordon, 1977).

Table (10): effect of *pediococcus acidi lactici* supplementation without or with *SE* infection on mortality rate pi.

Parameters	Supplementation	Without infection	With SE infection
Total no.	Control	25	25
	pediococcus acidi lactici	25	25
Dead no.	Control	0	3
	pediococcus acidi lactici	0	2
Survival %	Control	100%	88%
	pediococcus acidi lactici	100%	92%
Mortality %	Control	0%	12%
	pediococcus acidi lactici	0%	8%

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