Effect of Prebiotic and Probiotic on Growth, Immuno-hematological responses and Biochemical Parameters of infected rabbits with *Pasteurella multocida*

Doaa. H. Abdelhady* and Moshira A. El-Abasy**

**Department of Clinical Pathology and **Department of Poultry Diseases, Faculty of Veterinary Medicine, Kafr Elsheikh University, 33516, Kafr Elsheikh, Egypt.*

ABSTRACT

This study was aimed to evaluate the effect of dietary supplementation of prebiotic (Bio-Mos®, mannoligosacchride), probiotic (Bio-Plus® 2B, Bacillus subtilis and Bacillus licheniformis) and their mixture on growth, biochemical parameters and immune-hematological responses of rabbits. Sixty four New Zealand White male rabbits were divided into 2 equal groups. The 1st group was uninfected and subdivided into 4 subgroups. The 1st subgroup fed basal diet (Control), the 2nd, 3rd and 4th subgroups fed on basal diet supplemented with 1 g Bio-Mos, 0.4 g Bio-Plus and 1g Bio-MOS + 0.4 g Bio-Plus / kg, respectively for eight weeks. The 2nd group was similar to the 1st group but experimentally infected with *Pasteurella multocida*. The results in 1st group showed significant increase (P<0.01) in body weight gain, phagocytic activity (PA), phagocytic index (PI) and total leukocytic counts (TLC) when compared with control group 1.1. In addition, there was significant decrease in serum total cholesterol, triglycerides and glucose when compared with control group 1.1. In 2nd group , the results showed significant increase (P<0.05) in body weight gain, (P<0.001) in phagocytic activity and phagocytic index, RBCS count, PCV, Hb concentration, and number of lymphocytes while TLC and number of heterophils showed significant decrease(P<0.001) when compared with control group 2.1 .Also there was significant decrease (P<0.05) in food conversion ratio (FCR), (P<0.01) in total cholesterol and creatinine and (P<0.001) in number of heterophils, triglycerides, glucose, alanine amino transferase (ALT), aspartate amino transferase (AST) and urea in all infected groups fed experimental diets compared with control group 2.1.Supplementing the diet with Bio-Mos, Bio-Plus or their mixture decreased the mortality and improved the adverse clinical signs and post mortem lesions in all experimentally infected groups compared with infected untreated control group. Our results indicate that rabbits received mixture of pre and probiotic groups 1.4 and 2.4 recorded the highest value of daily weight gain, PA, PI, TLC and lymphocytes number and recorded the lowest FCR followed by rabbits received probiotic. Dietary supplementation of prebiotic and probiotic and their mixture improves cell-mediated immune response, liver and kidney functions , decreased the mortality and improved the adverse clinical signs and post mortem lesions in in rabbits experimentally infected with *P. multocida*.

Keywords: Pasteurella multocida, phagocytic activity, prebiotic, probiotic

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1. INTRODUCTION:

Commercial rabbit production is an important industry for meat, fur and leather production. Disease has always been a critical issue in animal production, affecting not only animal health and wellbeing, but also the physical and economic condition of the producer. *Pasteurella multocida* is a bacterial pathogen affecting rabbits at different ages that causes rhinitis (snuffles), pneumonia, otitis media, septicemia, metritis, and death in domestic rabbits, which cause great economic losses due to high mortality among clinically affected rabbits, downgrading carcasses, abortion and infertility (Lebdah, 2009). For several decades, antibiotics and chemotherapeutic agents in prophylactic dosages have been

used in animal feed to improve animal welfare and to obtain economic benefits in terms of improved animal performance and reduced medication costs. The high incidence of drug-resistant bacteria possess a problem in clinical practice. Resistances in pathogenic bacteria in both human and livestock linked to the therapeutic and sub therapeutic use of antibiotics in livestock and pets (Flickinger and Fahey 2002). To prevent the emergence of drug resistance, new drugs have been developed and resulted in increased cost of rabbit products. Furthermore, drug- or antibiotic-residue in rabbit meat is potentially annoyance to consumer. Prebiotics are non-digestible, fermentable carbohydrates and fibers, such as inulin-type frucans and galacto-oligosaccharides, which exhibit health promoting properties to host through selective stimulation of growth and/or activities of a limited number of bacteria (i.e., probiotics) (Roberfroid *et al*.,2010). Probiotics have been introduced as an alternative to antibiotics. Probiotics come under the category of as Generally Recognized as Safe (GRAS) ingredients classified by Food and Drug Administration (FDA) (Bansal *et al.,* 2011). Probiotics are nonpathogenic bacteria that exert a beneficial influence on the health or physiology (or both) of the host, it neither has any residues in animal production nor exerts any antibiotic resistance by consumption (Rajput and Li, 2012). Various kinds of prebiotics and probiotics, as natural biological response modifiers, have the ability to enhance host defense mechanisms against infections and have been evaluated based on preventive and therapeutic effects on infectious diseases (El-Abasy, 2002). Therefore the purpose of this study was to evaluate the in vivo effects of dietary prebiotic, probiotic and their mixture on growth, immune response, liver and kidney functions in rabbits experimentally infected with *P. multocida.*

2. MATERIALS AND METHODS:

2.2. Rabbits:

Sixty four five-weeks-old New Zealand White male rabbits were obtained from Animal Production Research Center, Sakha, Kafr Elsheikh and were divided into 2 groups of 32 rabbits each with an average body weight of 604.2 ± 10.93 g.

2.3. Experimental diet:

Three types of experimental diets contained 1 g Bio-Mos, 0.4 g Bio-Plus and 1g Bio-MOS + 0.4 g Bio-Plus/kg diet, respectively were used in addition to the basal diet. Feed and water offered ad libitum. The composition of the basal experimental ration used for rabbit feed was described in Table (1).

2.4. P. multocida strains:

Field strains of *P. multocida* isolated from diseased rabbits were used for experimental infection using aerosol challenge. *P. multocida* isolates were passed through mice by intraperitoneal inoculation. Mice were euthanized after the first signs of disease, and the bacterium was re-isolated from the heart, liver, lung, and trachea and cultured on BHI agar at 37°C for 24 h before use. The bacterial mass was collected and diluted in glucose-enriched essential medium (MEM), achieving a final concentration of 10^7 CFU/mL (Suckow, *et al*., 1995) via counting and plating.

2.5. Blood samples:

Blood samples were collected from the lateral ear vein at the end of the study and divided into three parts. The first one was collected on EDTA for hematological parameters. The second one was collected on heparin for performing the phagocytic activity of heterophils. The third one was placed in plain centrifuge tubes, left to clot then centrifuged for serum separation. Serum samples were stored at -20°C until used for the biochemical parameters.

2.6. Experimental protocol:

Sixty four New Zealand White male rabbits were divided into 2 equal groups (thirty two each). The 1st group was uninfected and subdivided into 4 subgroups. The 1st subgroup

fed basal diet and kept as control**,** the 2nd, 3rd and 4th subgroups fed on basal diet supplemented with 1 g Bio-Mos, 0.4 g Bio-Plus and 1g Bio-MOS + 0.4 g Bio-Plus/kg, respectively. The 2nd group was similar to the 1st group but experimentally infected by *P. multocida*. Infected rabbits were kept under observation 3 weeks post infection, during which clinical signs and mortality were recorded. Dead and sacrificed rabbits were subjected to postmortem and bacteriological examinations for re-isolation of the inoculated organism. A scoring system for the lesions post *P. multocida* infection was adapted after Van Veen (2000). Goss lesions were scored as follow: sinus (Si): 0 = no abnormality, 1 = mucus discharge, 2 = purulent discharge; trachea (T): 0 = no abnormality, 1 = exudate in trachea, 2 = trachea filled with exudate; lungs (Lu): 0 = no abnormality, 1 = unilateral pneumonia, 2 = bilateral pneumonia and liver (L): 0 = no abnormality, 1 = congestion, 2 = severe congestion.

2.7. Evaluated parameters:

2.7.1. Growth Performance:

Rabbits were weighed at 5 weeks of age and then live body weights (LBW) (g) were recorded at 13 weeks of age. Average feed intake (FI) was recorded weekly. The average body weight gain (BWG) and feed conversion ratio (FCR) were calculated according to Brody (1968).

2.7.2. Immunological Parameters:

Candida albicans: A well identified strain was kindly supplied by the Dept. of Microbiology and Immunology, Fac. Vet. Med. Menofiya University. Candida albicans used for evaluating the phagocytic activity (PA) and phagocytic index (PI) according to Kawahara et al., (1991).

2.7.3. Hematological Parameters:

Packed cell volume (PCV), hemoglobin (Hb), red blood cell count (RBCs), total white blood cells (WBCs) and differential leukocytic count were evaluated according to Feldman et al., (2000).

2.7.4. Blood Biochemical Parameters:

Serum samples were analyzed for total proteins (TP), albumin (Alb) according to Henry et al., (1974), Globulins concentration (Glob) in serum was computed by subtracting albumin concentration from total Proteins, albumin to globulin ratio (A/G) was calculated according to Kaneko (1989). Serum enzymatic activities of alanine amino transferase (ALT) and aspartate amino transferase (AST) as described by Reitman and Frankel (1957), triglycerides (TG) and total cholesterol (TC) according to Richmond (1973) , glucose according to Trinder (1969), urea according to Henry et al., (1974) and creatinine according to Fabiny and Einghausen (1971) using spectrophotometer and commercial test kits of Randox (Antrim, UK) .

2.8.Statistical Analysis:

The data were presented as mean± standard error (SE) and were subjected to statistical analysis using one-way analysis of variance (ANOVA) according to Snedecor and Cochran (1980). Differences at $p < 0.05$ were considered significant**.**

3. RESULTS:

As shown in Table (2), rabbits in 1st group showed higher body weight gain and lower food conversion ratio ($P<0.01$) when compared with control group 1.1. Similarly, the 2nd group showed higher body weight gain and lower food conversion ratio ($P<0.05$) when compared with control group 2.1.Also rabbits in the 1st group showed significant increase ($P<0.01$) in phagocytic activity and phagocytic index in all treated groups when they compared with control group 1.1 .Similarly, rabbits in the 2nd group showed significant increase ($P<0.001$) in phagocytic activity and phagocytic index in all treated groups when

Table (1): Composition of the basic rabbit diet

Ingredients	%	Ingredients	%	Ingredients	%
Corn (yellow)	0.7	DL Methionin	0.27	Sodium chloride	0.3
Soy bean meal 44%	8.23	DL Lysine	0.13	Anti coccidia	0.1
Wheat bran	48.27	DI. Ca. ph.	1	Premix	0.3
Clover hay	31.7	Molass	4	Parly	4
Limestone	1				
Calculated analysis					
DE Kcal/kg	2510	C. fiber %	14.00	lysine	0.67
C. P.%	16.07	Calsium %	1.10	Methionin+cestein	0.61
C. Fat %	2.37	Total phosphorus %	0.80	Sodium	0.20

compared with control group 2.1 (Table 3). As shown in (Table 4), the 1st group revealed non-significant change in RBCs count, PCV, Hb concentration, MCV, MCH, MCHC and number of heterophils but revealed significant increase (P<0.01) in TLC and in number of lymphocytes(P<0.001) in all treated groups when compared with control group 1.1. In the 2nd group, control group 2.1 revealed marked reduction in RBCs count, PCV, Hb concentration, number of lymphocytes and MCHC, while MCV, MCH, TLC and number of heterophils were increased when compared with the infected treated groups .On the other hand, there was significant increase (P<0.001) in RBCs count, PCV, Hb concentration and number of lymphocytes but MCV (P<0.001), MCH (P<0.01), TLC (P<0.001) and number of heterophils (P<0.001) were significantly decreased in all infected treated groups when compared with control group 2.1. The 1st group in Table (5) showed no significant change in albumin, ALT, AST, urea and creatinine. But revealed significant increase (P<0.05) of total proteins and globulins concentration only in rabbit received mixture of prebiotic and probiotic group 1.4, while, (A/G & glucose) and (total cholesterol & triglycerides) were significantly decreased (P<0.05, and P<0.001) respectively in all treated rabbit groups when compared with control group 1.1. Rabbits in the 2nd group showed no change in albumin, globulins and A/G ratio but showed significant increase (P<0.05) of total proteins concentration only in infected rabbit group received mixture of prebiotic and probiotic group 2.4 , while TC, TG, glucose, ALT, AST, urea and creatinine were significantly decreased (P<0.001) in all infected-treated rabbit groups when compared with control group 2.1. The infected rabbits suffered from depression, coughing, sneezing, mucus and purulent nasal discharge, off food and decreased body weight. Mortality rate was 25%. The mortality and lesion score were described in Table (6). Supplementing the diet with Bio-Mos, Bio-Plus or their mixture decreased the mortality and improved the adverse clinical signs and post mortem lesions in all experimentally infected groups compared with infected untreated control group.

4. DISSCUSSION:

The goal of the present study was to clarify the effect of dietary supplementation with prebiotics, probiotics and their mixture on growth, immune-hematological responses as well as liver and kidney functions of normal and *P. multocida* infected rabbits. The results showed that, the daily body weight gain was significantly increased

Table (2): Effect of prebiotic and probiotic supplemented diet on growth of rabbits

Group No.	1st group (uninfected)				2nd group (infected with *P. multocida*)			
Subgroup	1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4
Initial BW (g)	589±34.3	594± 40.7	586±27.9	580±24.7	613±30	622±17	619±15.5	629±15.5
Final BW (g)	1530±3^{c}	1812±32^{b}	1720±86^{a} $_{b}$	1913±31^{a}	1350±32^{a}	1600±16^{b}	1680±15^{b}	1720±15^{b}
BWG (g)	941±21^{c}	1218±8^{ab}	1134±41^{b} $_{c}$	1333±2^{a}	737±6^{b}	978±2^{ab}	1061±3^{a}	1091±3^{a}
TFI (g)	2893±50^{a}	2606±89^{b}	2606±50^{b}	2606±23^{b}	2893±3^{a}	2893±50^{a}	2606±89^{b}	2606±89^{b}
FCR	3.07±0.1^{a}	2.13±0.2^{b}	2.29±0.1^{b}	1.95±0.1^{b}	4.2±0.02^{a}	2.96±0.3^{a} $_{b}$	2.46±0.1^{b}	2.44±0.1^{b}

a, b,….., Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table (3): Effect of prebiotic and probiotic supplemented diet on phagocytic activity (PA) and phagocytic index (PI) of rabbits

Group No.	1st group (uninfected)				2nd group (infected with *P. multocida*)			
Subgroup	1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4
PA	49±3.12^{c}	70±6.29^{b}	68±2.02^{b}	78±4.32^{a}	38±6.52^{b}	65±6.61^{a}	69±4.33^{a}	71±2.21^{a}
PI	1.9±0.07^{c}	3.04±0.1^{b}	2.64±0.2^{b}	3.5±0.07^{a}	2.04±0.29^{b}	2.6±0.13^{a}	2.9±0.3^{a}	3.3±0.13^{a}

a, b,….., Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table (4): Effect of prebiotic and probiotic supplemented diet on hematological parameters of rabbits

Group No.	1st group (uninfected)				2nd group (infected with *P. multocida*)			
Subgroup	1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4
RBCs (x10^{6} /µl)	5.44±0.07	5.84±0.05	5.56±0.16	5.94±0.07	3.22+5.2^{c}	4.48±0.11^{b}	5.2±0.05^{a}	5.64±0.07^{a}
Hb g/dl	9.0±0.06	9.02±0.05	9.04±0.05	9.2±0.02	6.9±0.31^{b}	8.12±0.06^{a}	8.42±0.04^{a}	8.5±0.02^{a}
PCV (%)	35±0.24	35±0.51	37±0.45	36±0.97	31±0.65^{b}	37±0.51^{a}	35±0.32^{a}	36±0.97^{a}
MCV (fl)	66.1±5.4	60.2±2	66.6±2.9	61.5±4.3	98±5.9^{a}	83±4.4^{b}	68±3.1^{c}	64±2.6^{c}
MCH (pg)	17±1.5	15.5±0.4	16.2±0.8	15.7±0.8	22±1.5^{a}	18±1.2^{ab}	16±1.05^{b}	15±0.8^{b}
MCHC (%)	26±0.15	26±0.4	24±0.2	26±0.7	22±0.5^{b}	22±0.5^{b}	24±0.7^{a}	23±0.4^{ab}
TLC (x10^{3}/ µl)	6.9±0.07^{b}	8.5±0.16^{a}	8.6 ±0.15^{a}	8.9±0.34^{a}	11.9±0.44^{b}	9.5±0.08^{a}	9.6±0.27^{a}	9.5±0.34^{a}
Lymphocytes 10^{3}/µl	3.45±0.37^{b}	4.0±0.37^{b}	4.1±0.37^{b}	5.3±0.20^{a}	3.1±0.18^{b}	6.6±0.37^{a}	6.4±0.58^{a}	6.3±0.2^{a}
Heterophils 10^{3}/ µl	3.45±0.49	3.57±0.37	3.28±0.32	2.87±0.58	8.8±0.25^{a}	2.9±0.37^{b}	3.2±0.37^{b}	3.2±0.58^{b}

a, b, c,….., Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table (5): Effect of prebiotic and probiotic supplemented diet on biochemical parameters of rabbits

Group No.	1st group (uninfected)				2nd group (infected with *P. multocida*)			
Subgroup	1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4
TP (g/dl)	5.3±0.13^{b}	5.76±0.16^{a}	5.44±0.14^{b}	6.02±0.08^{a}	5.30±8.7^{b}	5.66±0.11^{ab}	5.46±0.19^{ab}	5.84±0.13^{a}
Albumin (g/dl)	2.04±0.09	1.97±0.05	2±0.08	2.02±0.05	1.76±0.3	1.8±0.08	1.94±0.09	1.99±0.07
Globulin (g/dl)	3.28±0.18^{b}	3.79±0.13^{a}	3.44±0.13^{b}	4±0.09^{a}	3.54±0.7	3.86±0.13	3.52±0.25b	3.85±0.13
A/G	0.63±0.12^{b}	0.52±0.03^{a}	0.58±0.07^{b}	0.51±0.03^{a}	0.50±0.01	0.46±0.06	0.55±0.11	0.51±0.05
TC (mg/dl)	112±0.9^{a}	74±1.9^{bc}	78±2.7^{b}	72±1.2^{c}	172±1.2^{a}	135±3.5^{b}	137±3.7^{b}	130±2.8^{b}
TG (mg/dl)	161±5.3^{a}	135±1.8^{bc}	142±4.8^{b}	127±3.5^{a}	220±5.2^{a}	182±4.3^{b}	188±5.3^{b}	172±3.1^{b}
Glucose (mg/dl)	82±2.1^{a}	70±1.6^{b}	66±1.1^{b}	66±1.3^{b}	107±2.7^{a}	85±2.7^{b}	88±2.5^{b}	77±1.2^{c}
ALT (IU/L)	30±0.75	27±1.1	29±1.3	27±0.67	60±1.1^{a}	28±1.6^{b}	29±0.71^{b}	27±1.02^{b}
AST (IU/L)	34±1.6	32±1.6	34±1.8	31±1.02	91±4.2^{a}	64±2.1^{b}	63±1.95^{b}	52±2.3^{c}
Urea (mg/dl)	42±0.45	41±0.49	42±0.77	40±0.55	55±2.9^{a}	43±1.08^{b}	47±0.45^{b}	42±0.49^{b}
Creatinine (mg/dl)	1.1±0.55	1.04±1.08	1.06±0.51	1.0±1.1	2.2±1.8^{a}	1.3±0.07^{b}	1.6±0.11^{b}	1.2±2.5^{b}

a, b,c,….., Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table (6): Mortility and lesion score of experimentally infected rabbits with *P. multocida*

Group No.	2nd group (infected with *P. multocida*)															
Subgroup	2.1				2.2				2.3				2.4			
No. of rabbits	8				8				8				8			
Dose of *P. multocida*	10^{7} CFU/ml				10^{7} CFU/ml				10^{7} CFU/ml				10^{7} CFU/ml			
Lesion score after infection																
lesion	Week	Si	T	Lu	L	Si	T	Lu	L	Si	T	Lu	L	Si	T	Lu	L
1st week		2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
2nd week		2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
3rd week		2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Mortility No.		2				0				0				0			
Mortility %		25				0				0				0			

and food conversion ratio was significantly decreased in both uninfected and infected rabbit groups fed experimental diet compared with control groups. Similarly results were obtained by Kritas and Morrison (2005), Tellez et al., (2006), Mountzouris et al., (2010) and Bansal et al., (2011) as they reported beneficial effect of probiotic supplementation to broiler diet in terms of increased body weight and feed conversion through a natural physiological way and improving digestion by balancing

the resident gut microflora as they can improve the integrity of the intestinal mucosal barrier, digestive and immune functions of intestine. Improvement in digestion and absorption of intestine of nutrient transportation systems leads to immune resistance and productivity. Similarly Amat et al., (1996) and Ashayerizadeh et al., (2009) reported that prebiotics and probiotics are growth promoters that can be used as alternative non antibiotic feed additives because they improve growth indices of broiler chickens without side effects on the consumers. Similar findings on the positive effect of probiotics on growth performances have been well documented by Sieo et al., (2005), Apata (2008) and Yu et al., (2008).

Concerning the erythrogram, the 1st groups revealed non significant change in RBCs count, PCV , Hb concentration, MCV,MCH and MCHC as prebiotics and probiotics and their mixture could sustain the normal hematopoietic function of rabbits at the supplemented dose.This result is in agreement with the study of Dimcho et al. (2005) who found that the probiotic supplementation did not affect the blood constituents comprising, haemoglobin concentrations. Similarly, Ewuola et al. (2010) mentioned that weaned rabbits fed dietary prebiotics (Biotronic®) and probiotics (BioVET®-Yc) did not affect the erythrocytes and haemoglobin. However, unsupplemented infected control group 2.1 in the 2nd group revealed marked reduction in RBCs count, PCV, Hb concentration together with significant increase in MCV and decrease MCHC which reflects picture of anemia (hemolytic anemia) by *pasteurella* endotoxins . In rabbit groups fed experimental diets compared with un supplemented infected control group 2.1 ,this picture was much improved through increase in RBCs count, PCV, Hb concentration and return of the erythrocytic indices close to that of normal uninfected rabbits .Our results agree with Shoeib et al., (1997) who recorded that the improvement in RBCs count could be attributed to improved health status and physiological well-being of the rabbits fed diet supplemented with prebiotic and probiotic. Similarly, Yasuda et al., (2006) recorded that a diet supplemented by prebiotic inulin 4% increased iron bioavailability in iron deficient pigs. Piglets fed with a diet supplemented by prebiotic inulin 4% had a 15% higher Hb concentration after five weeks intervention compared with piglets fed with a basal diet.

Regarding the leukogram, the 1st group revealed significant increase in TLC and count of lymphocytes in rabbit groups fed experimental diets compared with control group1.1. However, un supplemented infected control group 2.1 revealed marked increase in TLC and number of heterophils with reduction in number of lymphocytes, which reflects stress picture of leukogram together with marked reduction in phagocytic activity and phagocytic index .The heterophilic leukocytosis might be viewed as the primary response to bacterial infection and presence of microorganisms in the respiratory tract. Similar findings were obtained by Ahamefule et al., (2006) who mentioned that high WBC count has been reported to be usually associated with microbial infection or the presence of foreign bodies or antigens in the circulatory system. This picture was improved by decrease in TLC and significant increase in number of lymphocytes together with marked decrease in number of heterophils in infected rabbit groups fed experimental diets compared with control group 2.1. The initial response of *P. multocida* was probably related to immunosuppression by endogenous corticosteroids triggered off by stress. Increased concentrations of glucocorticoids inhibit the immune response of animals by diminishing antibody production, diminishing lymphocyte blastogenesis, altering granulocyte and monocyte concentrations and functions and by inhibiting phagocytosis. Similar explanation was reported by Roth and Kaeberle (1982). The toxic effects of bacterial endotoxin give rise

to degeneration and degranulation of neutrophils and subsequently the chemotactic action of mononuclear cells and phagocytes [Markham and Wilkie, 1980]. Rabbits fed experimental diets compared with control group 2.1 revealed positive effect on the immune response through different ways; the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The direct effect might be related to stimulate the lymphatic tissue as reported by Kabir *et al*., (2004).Whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract. Shoeib *et al.* (1997) recorded that the bursa of probiotic-treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Similarly, Wintrobe (1983) reported an increase in the total leukocyte count on supplementation with a probiotic containing viable lactic acid bacteria. This was attributed to hyperplasia of white pulp in the spleen because of polymorphonuclear cell proliferation, increase in alkaline phosphatase activated B-lymphocytes in splenic red pulp. Additionally, Christensen *et al*., (2002) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Since probiotic- and prebiotic- induced health promoting effects are likely to be attributed to their ability to antagonize pathogenic bacteria and to modulate host immune responses (Yan and Polk 2011). Similarly, Glick (2000) found that the better microenvironment in intestines could cause pluripotent hemopoietic precursors to differentiate into clones of lymphocytes, which could be one of the factor for increase in total leukocyte count.

In this experiment it was found that, dietary supplementation with prebiotic, probiotics and their mixture has an immune-stimulating effect through the increased total leukocytic count and absolute number of lymphocytes as well as increased phagocytic activity and phagocytic index indicated a stronger innate immune response and higher resistance as Leucocytes play an important role in non-specific or innate immunity and their count can be considered as an indicator of relatively lower disease susceptibility. Similar findings were obtained by Falcao-e-Cunha *et al*., (2007) who reported that prebiotics may prevent the adhesion of pathogens to the mucosa and stimulate the immune responses in rabbits and Mateos *et al*., (2010) who reported that dietary supplementation with certain oligosaccharides stimulate the immune response of rabbits.

Concerning to serum biochemical parameters, unsupplemented infected control group 2.1 revealed markedly increased serum TC, TG, glucose, ALT, AST, urea and creatinine as well as marked reduction in total proteins and albumin, this could be duo to liver and kidney damage. The 1st group results revealed, non-significant change in the serum albumin in treated rabbits when compare with control group 1.1.

The results revealed significant reduction in glucose level in uninfected and infected rabbit groups fed experimental diet compared with control groups. Similar results were obtained by Everard *et al*., (2011) who found that, prebiotic treatments (0.3 g/mouse/day) exhibited anti-obesity, anti-diabetic, antioxidant, and anti-inflammatory effects in obese mice and altered intestinal microbial composition.

Serum cholesterol and triglycerides levels were significantly decreased by supplementing Bio-Mos, Bio-Plus or their mixture in rabbit diets. Similar findings were reported by Liong and Shah (2005), Sudha et al., (2009) and Ooi and Liong (2010). They hypothesized the effect of probiotic microorganism on lipid metabolism as: posing bile salt hydrolase activity and precipitation of cholesterol by some microorganisms such as Lactobacillus and Bifidobacterium, incorporation of cholesterol or binding to

bacteria and making of short-chain fatty acids by probiotic bacteria. Another explanation of the mechanism by which a probiotic can lower the serum cholesterol has been declared by Fukushima and Nakano (1995). The authors demonstrated that probiotic microorganisms inhibit hydroxymethyl-glutaryl-coenzyme A; an enzyme involved in the cholesterol synthesis pathway thereby decrease cholesterol synthesis. Similarly, reduction in serum cholesterol of broiler chickens fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan *et a*l., 1995 and 1996). In addition, it was speculated that Lactobacillus acidophillus reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis [Abdulrahim *et al*., (1996]. The activities of ALT and AST were measured as indicators of hepatocellular damage .The results of present study revealed non-significant change in ALT and AST activities in treated rabbits of 1st group when compare with control group 1.1. However greater liver enzymes (ALT and AST) reduction were detected associated with a greater improvement in liver enzymes by supplementing Bio-Mos, Bio-Plus or their mixture in rabbit diets of 2nd group when compared with un supplemented infected control group 2.1 . The decrease in ALT activity obtained in the present study agrees with the observations of Osman *et al*., (2007) who made studies on rats in which addition of L. plantarum and B. infantis to rat diets decreased ALT activity. Similarly, Praveen *et al*., (2009) found that probiotic ,prebiotic and symbiotic supplementation resulted in decreased bacterial translocation in the liver of mice challenged with *Salmonella typhimurium* and decreased levels of serum aminotranseferases, suggesting the protection role against *Salmonella* infection.

Urea and creatinine levels were significantly decreased by supplementing Bio-Mos, Bio-Plus or their mixture in rabbit diets of 2nd group when compared with unsupplemented infected (2.1control group). Similar results were reported by Cenesiza et al., (2008) and Alkhalf et al., (2010) in broiler chickens .

In conclusion, the present work sheds more light on the influence of dietary supplementation with prebiotic and probiotic and their mixture on rabbits either normal or experimentally infected with *P. multocida* and clarify their ability to correct the adverse alterations occur duo to infection with *P. multocida* as it improves their growth, cell-mediated immune response, hematological and serum biochemical parameters reflecting liver and kidney functions and decreased the mortality and improved the adverse clinical signs and post mortem lesions. Finally, it was found that, among the supplemented diets, the mixture of preparation had the superior overall effect followed by probiotic then prebiotic supplemented diets .

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