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EFFECT OF MANNAN OLIGOSACCHARIDES (MOS) ON GROWTH, PHYSIOLOGICAL AND IMMUNE PERFORMANCE OF BROILER CHICKENS

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Abstract

Mannan oligosaccharides (MOS) are obtained from the outer cell wall of Saccharomyces cerevisiae. These (MOS) are among widely searched class to be used as feed additives in broiler chicken diets. Mannan oligosaccharides (MOS) can be used in poultry production to modulate immunity and improve growth performance. Supplementation of Mannan oligosaccharides (MOS) are judiciously valuable in getting better growth performance, boosting immunity and attaining better physiological conditions during rearing of broiler chickens due to its capability of attaining equilibrium between beneficial and harmful microflora in the digestive tract, which is essential for regular working of gastro-intestinal tract. This study was intended with the objectives of finding out the influence of MOS supplementation on growth parameters, boost in immune status and physiological performance parameters to be tested in broilers. Significantly improved (P<0.05) growth performance (average daily gain, body weight, and feed conversion ratio) was achieved by the supplementation of mannan oligosaccharides. Significantly (P<0.05) boost in parameters of immunity (Relative lymphoid organ weights, hemagglutination inhibition (HI), sheep red blood cell (SRBC) antibody reaction against Newcastle disease (ND), (IgG) resistant mercaptoethanol-2 and (IgM) sensitive mercaptoethanol-2) in mannan oligosaccharides supplemented groups of broiler chickens was achieved for Newcastle disease virus (NDV) titres. There was non-significant (P>0.05) difference among bursa, thymus and spleen weight. Effects of supplementing different concentration (0, 0.5, 1.0 and 1.5 grams/kg of feed for 42 days) of MOS on different haematological (erythrocyte sedimentation rate (ESR), haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), platelets) and serum biochemical parameters (ALT, AST, protein, globulin, albumin, cholesterol urea and creatinine) showed a non-significant difference (P>0.05) in all treatment groups.

Keywords: *Hemagglutination inhibition (HI), IgG, IgM, Mannan oligosaccharides, Newcastle disease, SRBC*

INTRODUCTION

Extensive and conventional poultry farming in most of the areas of Pakistan is liable for sudden disease outbreaks and poor growth of broiler chickens. Therefore use of antibiotics is seen to be a part and parcel during rearing not only to control infections but

also as feed additives to get better live weights and feed efficiencies. These practices of using antibiotic growth promoters are creating resistance against microorganisms along with a potential source of antibiotics residues in chicken meat. Due to these reasons European Union and many advanced countries completely imposed a ban to use antibiotics as growth promoters (1). The current scenario is compelling the poultry nutritionists to seek the unconventional ways to improve the performance of broiler chickens. Among these substitutes feed additives other than antibiotics, probiotics, prebiotics and immune modulators might be a good option to get better weight gain, improved feed efficiency, superior physiology of body and boost in immune responses. Now a days mannan oligosaccharides (MOS), derivatives of yeast cell wall, are the most extensive exploration class of oligosaccharides due to their remarkable ability in decreasing pathogenic microflora of the gut (2, 3) enhancing the strength of intestinal mucosa which help in creating a balance between useful and harmful intestinal microorganisms (4, 5). With the increases in number of useful microbes the eradication of the harmful microbes from the nutrition and also from the wall of gut is inexpensive. Mannan oligosaccharides triggers macrophages of gut associated lymphoid tissues that are capable of producing humoral, cellular and cutaneous immunity (6, 7)). The study was intended to identify the effect of MOS supplementation on growth performance, boost in immunity and physiology of broiler chickens.

METHODOLOGY OF THE STUDY

One hundred and eighty (180) day old broiler chicks (45 chicks per group with 3 replicates of 15 chicks each) were divided in 4 treatment groups. Basal feed was given to control group whereas other 3 groups were supplemented with 0.5, 1 and 1.5 grams of mannan oligosaccharides (MOS) per kilogram of diets from day 1-42. The chicks were offered daily restricted but calculated feed with *ad-libitum* provision of drinking water. Pre-starter (day 1-11), starter (12-24 days) and finisher feed (25-42 days) were offered. Chicks were vaccinated for Newcastle disease virus (7th and 21st day) and Infectious Bursal disease virus (14th and 28thday). Data of growth performance parameters (carcass weight, live weight gain, daily feed consumption) were collected from day 1st to 42nd on weekly basis. Dressing percentages and relative visceral organ weight % (RVOW) were recorded at the age of week 3 and 6 by slaughtering chickens (6, 8). For Relative lymphoid organ weights, at day 21st and 42nd of experiment, five birds/treatment groups were slaughtered and relative weights of lymphoid organs were recorded (7, 9). For haematological study, blood samples were collected in collection tubes containing anticoagulants (1-2 mg/ml) Ethylene diamine tetra acetic acid (EDTA). At 42nd day of trial, six (n=6) blood samples (2 from each replicate) were randomly taken from wing veins of birds. Blood samples were examined within 2 hours of collection. To find physiological state of birds, different blood tests (erythrocyte sedimentation rate (ESR), white blood cells (WBC), erythrocytes/red blood cell (RBC), haemoglobin (Hb) and platelets) were conducted in all treatment groups (10). Serum biochemical analysis was performed from each experimental group at 42nd day of trial. Approximately 3-4 ml of blood was taken from wing veins of 6 birds. Blood samples were kept in tubes in inclined position for 3 hours, and these tubes were

centrifuged at 1200 revolutions per minutes (rpm) for 10-12 minutes. Separated serum was preserved at -20 °C for biochemical analysis (ALT, AST, total protein, globulin, albumin, cholesterol, urea and creatinine) with commercial kits (9, 11).

DATA ANALYSIS

Collected data were summarized (MS excel) and subjected to statistical analysis (SPSS 20). Significant differences (P<0.05) were calculated by Duncan's Multiple Range (DMR) test.

RESULTS

MANNAN OLIGOSACCHARIDES (MOS) AND GROWTH PERFORMANCE

As far as the outcomes of supplementations of MOS on growth performance in different treatment groups was concerned, improvements in daily weight gain (DWG), feed consumption, average daily gain, carcass percentage, and feed conversion ratio were identified in all MOS treated groups. Growth performance parameters in broiler chicken of all treatment groups during different phases (starter 1-3, finisher 4-6 and overall growth1-6 weeks) are presented in Table I.

Table I. Effect of mannan oligosaccharides (MOS) on daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR) and average daily gain (WG) on finisher phase of age (1-3, 4-6 and 1-6 weeks)

	Groups	Weight gain (grams)	Feed intake (grams)	FCR	ADG (grams)
Week 1-3	CONTROL	680.65±1.64^{c}	871.33±2.63	1.28±0.01^{c}	32.41±0.08^{c}
	MOS0.5	696.73±0.99^{b}	875.45±3.12	1.26±0.01^{b}	33.18±0.05^{b}
	MOS1	724.14±2.09^{a}	874.7±1.5	1.21±0^{a}	34.48±0.10^{a}
	MOS1.5	727.06±3.48^{a}	876.68±4.34	1.21±0^{a}	34.62±0.17^{a}
Week 4-6	CONTROL	1799.57±6.18^{b}	2950.5±7.73	1.63±0.01^{b}	85.69±0.29^{b}
	MOS0.5	1837.53±9.69^{a}	2936.50±4.13	1.60±0.01^{a}	87.50±0.46^{a}
	MOS1	1860.34±5.59^{a}	2934.25±7.85	1.58±0.01^{a}	88.59±0.27^{a}
	MOS1.5	1861.91±9.05^{a}	2940.50±6.12	1.58±0.01^{a}	88.66±0.43^{a}
Week 1-6	CONTROL	1799.57±6.18^{b}	2950.5±7.73	1.63±0.01^{b}	85.69±0.29^{b}
	MOS0.5	1837.53±9.69^{a}	2936.50±4.13	1.60±0.01^{a}	87.50±0.46^{a}
	MOS1	1860.34±5.59^{a}	2934.25±7.85	1.58±0.01^{a}	88.59±0.27^{a}
	MOS1.5	1861.91±9.05^{a}	2940.50±6.12	1.58±0.01^{a}	88.66±0.43^{a}

^{abc} Values in a column followed by various means (±SE) within a column presenting different superscripts are significantly different (P<0.05)

In all three phases, significant differences (P<0.05) in live body weight, feed conversion ratio and daily weight gain (DWG) with non-significant effect (P>0.05)) on visceral organ and carcass percentage (Table II) was found in treatment groups.

EFFECTS OF MANNAN OLIGOSACCHARIDES (MOS) ON IMMUNE PARAMETERS

As far as the effects of supplementing mannan oligosaccharides on immune parameters (Relative lymphoid organ weights, Hemagglutination inhibition (HI), total antibody reaction to sheep red blood cell (SRBC), Immunoglobulin M (IgM) Mercaptoethanol-2 sensitive and Immunoglobulin G (IgG) Mercaptoethanol-2 resistant responses) in different treatment groups for various stages of rearing were concerned, improvement in immune status of all MOS treated groups was evident.

Table II. Effect of mannan oligosaccharides (MOS) on relative visceral organ weight (%) and carcass percentage at 3 and 6 weeks

	Groups	Carcass	Liver	Heart	Gizzard	Proventiculous	Intestine
week 3	CONTROL	52.62±1.20	2.85±0.23	0.64±0.08	3.15±0.34	0.57±0.03	16.56±0.49
	MOS0.5	54.15±1.29	2.56±0.08	0.59±0.06	3.29±0.31	0.51±0.04	15.99±0.73
	MOS1	54.86±1.08	2.40±0.15	0.57±0.04	3.58±0.09	0.5±80.03	15.91±0.56
	MOS1.5	54.55±0.30	2.33±0.13	0.66±0.03	3.59±0.25	0.47±0.03	15.82±0.63
week 6	CONTROL	62.1±0.36	2.6±0.07	0.51±0.04	2.72±0.16	0.4±0.02	7.24±0.34
	MOS0.5	61.12±0.82	2.42±0.07	0.46±0.03	2.65±0.12	0.48±0.04	7.31±0.29
	MOS1	61.45±0.76	2.38±0.04	0.43±0.02	2.33±0.13	0.41±0.03	7.30±0.42
	MOS1.5	63.38±1.09	2.48±0.12	0.45±0.01	2.50±0.09	0.44±0.03	7.62±0.36

(P>0.05) Non-significant (NS), standard error of the mean (±SEM)

The results of the effect of mannan oligosaccharides (MOS) on relative lymphoid organ weight at 3 and 6 weeks of age in broiler chicken of all treatment groups are presented in Table III. No significant effect (P>0.05) in relative lymphoid organ (bursa, thymus and spleen) weights was obtained. At 42nd day, highest Hemagglutination (HI) titers (6.67±0.42) were found in MOS1.5 treatment group whereas least titers (5.17±0.17) were in control group (Table IV). MOS1.5 had significantly higher (P<0.05) SRBC titers against ND (7.50±0.22) at the age of 42 days followed by 7.33±0.21, 7.17±0.17 and 5.33±0.33 in MOS1, MOS0.5 and control group respectively (Table V). Same trend was obtained for IgM sensitive mercepto-ethanol 2 (ME) and IgG resistant mercepto-ethanol 2 (ME) antibody titers (Table V).

Table III. Effect of mannan oligosaccharides (MOS) on relative lymphoid organ weight (%) at 3 and 6 weeks

	Groups	Bursa	Thymus	Spleen
week 3	CONTROL	0.13±0.03	0.33±0.03	0.18±0.03
	MOS0.5	0.13±0.02	0.31±0.05	0.19±0.02
	MOS1	0.14±0.01	0.28±0.06	0.20±0.02
	MOS1.5	0.17±0.02	0.37±0.04	0.21±0.02
week 6	CONTROL	0.23±0.02	0.27±0.05	0.08±0.02
	MOS0.5	0.25±0.04	0.32±0.04	0.11±0.01
	MOS1	0.26±0.04	0.34±0.04	0.09±0.02
	MOS1.5	0.32±0.05	0.33±0.01	0.12±0.02

MANNAN OLIGOSACHARIDES (MOS) AND BLOOD PHYSIOLOGY

Results presented in Table VI and VII revealed the influence of various concentrations of mannan oligosaccharides (MOS) on haematology and serum biochemistry. Outcomes of the study indicated a non-significant difference (P>0.05) in erythrocyte sedimentation rate (ESR), haemoglobin (Hb), WBC, RBC, platelets, alanine aminotransferase (ALT), aspartate aminotransferase (AST), protein, globulin, albumin, cholesterol, urea and creatinine in all treatment groups.

Table IV. Effect of mannan oligosaccharides (Reciprocal of log_2 GMT ±SE) on hemagglutination inhibition (HI) titer against Newcastle disease virus of broiler chicken (0-6 weeks)

Groups	Week0	Week1	Week2	Week3	Week4	Week5	Week6
CONTROL	5.33±0.42	4.17±0.31^{b}	4.50±0.22^{b}	5.00±0.26^{b}	5.00±0.26^{b}	4.67±0.21^{b}	5.17±0.17^{b}
MOS0.5	5.17±0.31	5.67±0.42^{a}	5.50±0.43^{a}	6.17±0.31^{a}	5.83±0.31^{ab}	5.17±0.31^{b}	5.33±0.33^{b}
MOS1	5.83±0.31	5.83±0.31^{a}	5.67±0.33^{a}	6.33±0.42^{a}	6.17±0.31^{a}	6.50±0.43^{a}	6.33±0.21^{a}
MOS1.5	6.00±0.26	6.01±0.26^{a}	5.83±0.31^{a}	6.50±0.43^{a}	6.33±0.33^{a}	6.67±0.21^{a}	6.67±0.42^{a}

^{abc} Values in a column followed by various means (±SE) within a column presenting different superscripts are significantly different (P<0.05)

Table V. Effect of mannan oligosaccharides (Reciprocal of log_2 GMT ±SE) on SRBC, IgM and IgG titers against Newcastle Disease virus of broiler chicken (week 3-6)

	Groups	Week3	Week4	Week5	Week6
SRBC	CONTROL	5.50±0.34^{b}	5.50±0.22^{b}	5.00±0.26^{b}	5.33±0.33^{b}
	MOS0.5	7.33±0.21^{a}	6.67±0.21^{a}	7.17±0.31^{a}	7.17±0.17^{a}
	MOS1	7.33±0.21^{a}	7.00±0.26^{a}	7.50±0.22^{a}	7.33±0.21^{a}
	MOS1.5	6.83±0.31^{a}	7.17±0.17^{a}	7.17±0.17^{a}	7.50±0.22^{a}
IgM	CONTROL	2.17±0.17	2.33±0.21	2.50±0.22	3.00±0.26^{b}
	MOS0.5	3.17±0.31	2.50±0.22	2.83±0.17	4.67±0.21^{a}
	MOS1	2.50±0.22	2.50±0.22	2.83±0.17	4.67±0.33^{a}
	MOS1.5	2.67±0.49	2.67±0.33	3.17±0.31	4.50±0.22^{a}
IgG	CONTROL	3.33±0.33^{b}	3.17±0.31^{b}	2.50±0.22^{b}	2.03±0.21
	MOS0.5	4.17±0.17^{a}	4.17±0.17^{a}	4.33±0.21^{a}	2.50±0.22^{a}
	MOS1	4.83±0.17^{a}	4.50±0.43^{a}	4.67±0.21^{a}	2.67±0.21^{a}
	MOS1.5	4.17±0.31^{a}	4.50±0.22^{a}	4.00±0.26^{a}	3.00±0.26^{a}

^{abc} Values in a column followed by various means (±SE) within a column presenting different superscripts are significantly different (P<0.05).

Table VI. Effect of mannan oligosaccharides (MOS) on blood haematology at week 6

Groups	ESR (mm/hr)	WBC (10^3 /mL)	RBC (10^6 /mL)	PLATELETS (10^3 /mL)	HAEMOGLOBIN g/dL
CONTROL	3.00±0.26	21.72±0.73	2.78±0.15	56.42±6.97	10.50±0.86
MOS0.5	3.17±0.31	21.85±1.80	3.28±0.28	56.42±6.19	10.78±0.33
MOS1	3.00±0.37	22.78±1.48	2.97±0.19	61.77±6.03	10.38±0.67
MOS1.5	3.17±0.31	20.43±1.28	2.88±0.38	64.33±2.39	9.93±0.69

(P>0.05) Non-significant (NS), standard error of the mean (±SEM)

Table VII. Effect of mannan oligosaccharides (MOS) on serum biochemistry at week 6

Groups	ALT (u/L)	AST (u/L)	Protein g/dL	Globulin g/dL	Albumin g/dL	Cholesterol mg/dL	Urea mg/dL	Creatinine mg/dL
CONTROL	29.08±3.78	230.70±21.20	4.02±0.16	1.42±0.09	1.32±0.06	54.50±9.24	3.00±0.45	4.33±0.49
MOS0.5	24.87±7.90	282.23±19.92	4.07±0.15	1.42±0.07	1.53±0.07	183.67±13.48	3.00±0.52	4.83±0.17
MOS1	29.58±4.34	242.53±27.22	3.98±0.48	1.37±0.09	1.45±0.13	180.50±21.54	3.67±0.84	4.00±0.26
MOS1.5	15.22±1.57	252.80±28.02	3.52±0.25	1.38±0.06	1.45±0.14	183.33±23.25	3.83±0.70	4.17±0.31

(P>0.05) Non-significant (NS), standard error of the mean (±SEM)

DISCUSSION

MANNAN OLIGOSACCHARIDES (MOS) AND GROWTH PERFORMANCE

As far as the impact of supplementations of MOS on growth in different treatment groups were concerned, improvement in daily weight gain (DWG), feed consumption,

average daily gain (ADG), carcass percentage, and feed conversion ratio was seen in all MOS treated groups. The results were in agreement with the findings of (12) who described that prebiotics have ability to accelerate the growth. The usefulness of prebiotics may be built on two kinds of functions, growth of beneficial microbe and suppression of harmful microbes especially bacteria (13). When animals are offered prebiotics as fermentable substrates as feed additives, usual objectives of these practices are to escalate weight gain and feed conversion ratio (8). Mookiah et al., 2014 also reported better efficacy of broiler chicken diets supplemented with prebiotics regarding growth performance parameters as it was seen in this experiment (14). Equilibrium between beneficial and harmful microflora in the digestive tract is required for regular working of gastro-intestinal tract (15) and MOS has ability to maintain this stability by dropping the burden of harmful bacteria and improving the growth of useful bacteria through its brush-like structure. This distinctive structure enables MOS efficient within the bird's gut by attaching it with receptors prevailing on digestive wall and membranes of microorganism (6, 16). In this trial significant ($P<0.05$) improvement in body weight, feed conversion ratio (FCR) and average daily gain with the supplementation of MOS was found. Numerous reports, studies, research articles and meta/holo analysis (8, 17, 18) proved MOS as a good prebiotic with greater height of villi (19) and effecting intestinal microbiota (3) showing equivalent or higher effect of mannan oligosaccharides (MOS) on performance to those antibiotic growth promoters (20) that can be supplemented as protein source in broiler production to get economic benefits without any negative impact on bird's growth (21), increasing the number of beneficial microbes (lactobacilli, bifido bacteria) in intestine which results in reduction of harmful microbes creating immunomodulatory and physiological effects and ultimately enhance growth, boost immunity and better feed conversion ratio (22). In other experiments (23) equivalent results were also reported. Non-significant differences ($P>0.05$) in carcass, liver, heart, gizzard proventiculous and relative intestine length among non-supplemented control group and supplemented MOS groups were observed. The results were in agreement with carcass parameters (24) in broilers raised in a hygienic atmosphere ($P>0.05$).

MANNAN OLIGOSACHARIDES (MOS) AND IMMUNITY

Prebiotics (MOS), probiotics and immune modulators are helpful for immunomodulation (6, 7) to intensify the resistance potential of chickens against antibody reactions to Newcastle disease virus (NDV), infectious bronchitis (IB) and infectious bursal disease (IBD) by enhancing humoral, cellular and mucosal immunities and antibiotic suppression (25), proving them a tool to improve immune status successfully in intense poultry production areas (26, 27).

The results were in line with the outcomes of this trial with positive effect on lymphoid organ weight (9). Mannan oligosaccharides have been considered to have an influence on the immune cells with the help of its mannan molecule. Better immune capabilities in the intestine (28, 29), and improved jejunal gene expression (4). Better immune titers for hemagglutination inhibition (HI), SRBC, IgM and IgG were achieved (7)

in this trial. There were non-significant differences (P>0.05) in relative bursa, thymus and spleen weight. But weights of bursa, thymus and spleen with numerically greater values in MOS1.5, MOS1 and MOS0.5 treated groups as compared to control. In this trial, highest HI titers against ND were achieved in MOS1.5 and MOS1 with non-significant difference (P>0.05) between them. Similar findings were reported by Sheikh et al., 2020 (7), however, Raza et al., 2017 (23) and Zhang et al., 2012 (30) found positive effect of yeast cell wall (YCW) on the humoral immune response by means of antibody titers production against Newcastle disease (ND) vaccination at day 7 and 14 after vaccination. MOS might be influential in motivating the humoral immune reactions against infectious Bursal Disease (IBD) and Newcastle Disease viruses (31). Supplementation of yeast cell wall derived mannan oligosaccharides (MOS) in broiler diet showed better antibody response against Newcastle Disease virus by means of hemagglutination inhibition (HI) test (32, 33) in the 3rd, 5th and 6th weeks of age as were apparent in this experiment, supporting the findings of this experiment. Supplementation of manna oligosaccharides at different concentrations showed significant effect (P<0.05) on the SRBC, mercepto-ethanol 2 (ME) sensitive IgM antibody and mercepto-ethanol 2 (ME) resistant IgG antibody titers at week 3, 4, 5 and 6. Similarly, Bozakova et al., 2018 also found an increased level of IgM in layers by the supplementation of immune modulators in feed (34), whereas, Akhtar et al., 2015 observed significant positive effect of MOS on total immunoglobulins (Ig) content as were found in trial (9).

MANNAN OLIGOSACHARIDES (MOS) AND PHYSIOLOGICAL PARAMETERS

Feed additive are able to increase lymphocyte count in blood (P<0.05) along the reduced levels of uric acid and aspartate aminotransferase (ALT) in blood plasma upon the supplementation of feed additive were observed (35). The effects of different levels of mannan oligosaccharides on erythrocyte sedimentation rate (ESR), haemoglobin (Hb), WBC, RBC, and platelets in the trial indicated a non-significant difference (P>0.05) in ESR, TLC, RBC, platelets count and haemoglobin concentration of treatment groups. Non-significant difference (P>0.05) among serum biochemical parameters (ALT, AST, protein, globulin, albumin, cholesterol urea and creatinine) of treatment groups were indicated in the results. Akhtar et al., 2015 (9) supported the findings of this trial but the results were not in line with explanations of Jameel 2014 (36), who indicated a significant increase in total protein, albumin, and globulin, while glucose, ALT, AST and ALP were decreasing these differences might be due to inclusion of different pre/probiotics, experimental plan executed, management differences during rearing and environment conditions.

CONCLUSION

There are several mechanisms due to which MOS are capable of producing a positive influence on the growth of broilers. Various levels of supplementation of MOS are proficient in increasing physiological and immunomodulatory effects ultimately enhance growth; boost immunity and better feed conversion ratio.

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Statement of conflict of interest

The authors state no conflict of interest.

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