Indian Journal of Animal Sciences **86** (4): 460–467, April 2016/Article
https://doi.org/10.56093/ijans.v86i4.57790

Evaluation of mannan-oligosaccharides (MOS) in broiler chicken during hot humid summer using zoo technical, molecular and physio-biochemical tools

J J ROKADE[1], S K BHANJA[2], A S SHINDE[3], SAJAD[4], MUKESH KAPGATE[5] and A B MANDAL[6]

ICAR- Central Avian Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 6 April 2015; Accepted: 11 September 2015

ABSTRACT

Climate resilient poultry production is a major challenge especially for hot regions like India. Accordingly, the efficacy of mannan-oligosaccharides as anti-heat stressor was studied. Broiler chicks were reared on a standard diet up to 14th day of age. Thereafter, the chicks were randomly distributed into three dietary treatment groups viz. T1 (Control group: Standard diet, T2 (Standard diet with MOS @ 0.3%) and T3 (Standard diet with MOS @ 0.5%) each with 40 birds divided in 5 replicates of 8 birds each upto 42 days of age. Experiment was carried out during hot-humid (August-September, 26.0±0.12° to 34.25±0.37°C, Rh%: 76.95±0.90 to 86.15±0.61) summer. Growth performance, immunity (4th week), physiological (4th and 6th week), biochemical (4th and 6th wk) and molecular parameters (4th and 6th wk) were recorded. Feed intake, live weight gain and FCR improved significantly (P<0.001) on MOS supplementation. The relative yield of immune organs at 4th and 6th week of age, humoral (P<0.001) as well as cellular (P<0.01) immunity also improved significantly. The percentage of haemoglobin, protein, aspartate transaminase and alanine transaminase increased significantly (P<0.001) due to MOS supplementation at 4th as well as 6th week of age. While H:L ratio, serum corticosterone and serum cholesterol decreased significantly (P<0.001) in MOS supplemented groups. Supplementation of MOS at both the levels (0.3 and 0.5%) caused significant down regulation of relative expression of HSP70 in jejunum tissues during 28th or 42nd day of age. From the results of the present study, it can be concluded that MOS supplementation @ 0.3% or 0.5% in diet of heat stressed broilers improved performance as well as welfare.

Key words: Broiler, Corticosterone, Hot-humid conditions, HSP-70, Immunity, MOS

The poultry industry, especially meat production is growing at much faster rate in Asia and South America since the past decade. The potential for growth is obvious due to ever increasing demand of meat for human consumption, but increasing environmental temperature and humidity posing challenges to growing poultry industry. There has been significant impact on efficiency, production, morbidity and mortality with the rise in global temperature (Ahmad Mujahid 2011). The patho-physiology of heat stress involves inappetance, poor feed conversion, production of free radicals, immune insufficiency, several derangements in synthesizing antioxidant enzymes, imbalance of electrolyte balance and change in haematological profile (Mandal 2010). Heat stress over a period of time also enhances Heat Shock Protein 70 (HSP70) expression in different organs like jejunum, liver and brain of broiler chicken (Edens *et al.* 2001). It has been suggested that HSP70 might be involved in cellular protection in adverse situations and a relationship between the development between this protein and thermo tolerance has been established (Givisiez *et al.* 2001). Also during heat stress, serum cholesterol level goes up, which increases serum corticosterone production and H: L ratio.

It is difficult to follow the suitable management system in low-cost open houses especially in developing countries; therefore, dietary approach seems to be most user-friendly to curb heat stress. To alleviate marginal nutrient deficiencies that are considered the primary cause of economic losses associated with heat stress antioxidant, feed additive, vitamins and minerals are commonly added as a part of a nutritional manipulation tool (Sahin and Kucuk 2003). Mannan-oligosaccharides (MOS) is a feed supplement which helps to restore intestinal microbial ecology, inhibit the growth of pathogenic microorganisms and provide digestive enzymes (Martin *et al.* 2008). It also increases synthesis of lactic acid leading to lowering of the intestinal pH, adhesion or colonization to the intestinal mucosa and prevention of ammonium synthesis. MOS helps to improve the intestinal absorption of trace minerals and also its antioxidant activities helps in stimulating immunity. However, to the best of our knowledge, meagre literature

Present address: [1]Scientist (jaydeepvet@gmail.com), [4]PhD Scholar (sajadhassan5191@gmail.com), [6]Principal Scientist and Head (abmcari@rediffmail.com), Department of Avian Nutrition and Feed Technology, [2]Principal Scientist (subratcari @gmail.com), [3]PhD Scholar (anil.ivri@gmail.com), Poultry Housing and Management, [5]PG Scholar (dr.mgkapgate08 @gmail.com), Animal Genetics and Breeding.

is available regarding the study of potential effects of MOS supplementation at molecular level in broilers reared under hot and humid conditions. Keeping all above information in view, the present study is proposed to evaluate MOS supplementation in broiler during hot humid summer using zoo technical, molecular and physio-biochemical tools.

MATERIALS AND METHODS

Experimental birds, management and diet: The fertile eggs were received from flock of synthetic broiler dam line and were set at experimental hatchery. The day old chicks obtained from these eggs were wing banded and weighed individually. Broiler chicks (120) were reared on a standard diet up to 14th day of age and then chicks were randomly distributed into three dietary treatments viz. T1 (Control group: Standard diet as per BIS), T2 (Standard diet with MOS @ 0.3%) and T3 (Standard diet with MOS @ 0.5%) each with 40 birds in 5 replicates (8 per replicate) and reared up to 42 days of age. Out of 5 replicates, one replicate was solely kept to study the blood biochemical and HSP70 expressions in the jejunum at the end of 4th wk period. Experiment was carried out during hot humid (August-September, 26.0±0.12°C to 34.25±0.37°C, Rh%: 76.95±0.90 to 86.15±0.61) summer. All birds were reared under uniform and standard management conditions. Broiler starter (21.44% CP and 2839 kcal ME/kg) and broiler finisher (19.75% CP and 2891 kcal ME/kg) mash were prepared. The experimental diets were isocaloric and isonitrogenous but with variable MOS level. The representative samples of practical feed ingredients and test diets used in the study were analyzed (AOAC 1990).

Table 1. Ingredients and nutrient composition of basal diet used during starting and finishing phase

Ingredients (kg/100kg)	Starting (%)	Finishing (%)
Yellow maize	54.03	58.88
DORB	1	1
Soybean meal	36	31.5
RSM	3	3
Fish meal	3	3
Limestone powder	1	1
Dicalcium phosphate	1.3	1
Common salt	0.2	0.2
DL-Methionine	0.1	0.05
Constant*	0.415	0.415
Total	100	100
Nutrient composition (As fed basis)		
ME, kcal/kg***	2838.57	2891.25
Crude Protein,%**	21.25	19.75
Lysine,%***	1.20	1.10
Methionine,%***	0.50	0.43
Calcium,%**	1.06	0.99
Total Phosphorus,%**	0.75	0.70
Available Phosphorus,%***	0.45	0.40
Ether extract,%**	2.80	2.02
Crude fiber,%**	4.58	4.60
Total ash,%**	5.20	5.30

*Constant includes trace mineral premix 0.1, vitamin premixes 0. 15, toxin binder 0.05 and coccidiostat 0.05%. Trace mineral premix supplied: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn,30; Cu, 4 mg/kg diet. The vitamin premixes supplied: vitamin A, 8250 IU; vitamin D_3, 1200 ICU; vitamin K, 1 mg; vitamin E, 40 IU; vitamin B_1, 2 mg; vitamin B_2, 4 mg; vitamin B_{12}, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline chloride, 500 mg/kg diet. ** Analyzed values as fed basis. ***Calculated values as fed basis.

Growth parameters: Data regarding growth performance such as body weight gain, feed intake, feed conversion ratio in the control and experimental group were recorded every week from 0–42nd days of age.

Immune responses: Lymphoid organ weights (bursa of Fabricius, spleen and thymus) were recorded on 28th and 42nd day from 6 birds (three male and three female) in each treatment and was expressed as per cent (relative yield) of live weight. Cell mediated immunity (CMI) measured as foot web index in response to mitogen PHA-P (Corrier and Deloach 1990) and humoral immune response measured as serum haemagglutination (HA) titre to sheep red blood corpuscles (Siegel and Gross 1980) on 28th day post-hatch. Eight birds (equal sex) from each treatment were used for CMI and humoral immunity.

Haematological parameter: Blood samples (1ml) were collected from the wing vein using 24 gauge needle in K_3-EDTA tubes on 28th and 42nd day of age from 6 birds (3 male and 3 female). Haemoglobin concentration (g/dl) in the whole blood was estimated by cyanomethemoglobin method. Blood smears prepared from fresh blood smear were stained by Geimsa stain (1:9 Dilution for 45 min) to calculate Heterophil to lymphocyte (H:L) ratio.

Biochemical parameter: On 28th and 42nd day of age, blood samples were collected from 6 birds (equal sex) of each treatment and allowed to clot at room temperature. Serum was separated and subjected to different blood biochemical tests like Aspartate amino transferase (AST), Alanine amino transferase (ALT), total protein and total cholesterol using standard KIT.

Serum corticosterone: Corticosterone EIA Kit (ELISA test) was used for the estimation of serum corticosterone. The intensity of the colour, determined spectrophotometrically at 412nm, is proportional to the amount of corticosterone tracer bound to the well, which is inversely proportional to the amount of free corticosterone present in the well during incubation; or

$$\text{Absorbance} \propto [\text{Bound Corticosterone Tracer}] \propto 1/[\text{Corticosterone}]$$

Expression of heat shock protein (HSP70) gene: Tissue collection for gene expression studies: At 4th and 6th wk of age, about 2 cm of the proximal portion of the jejunum was collected from four birds per treatment. 50 mg of homogenized tissue was used for the total RNA isolation.

RNA isolation and reverse transcription: Total cellular RNA from jejunum of each treatment group was isolated using RNAgents® Total RNA Isolation System, purity and quantity were assessed by measuring the optical density of each sample at 260 versus 280 nm in a nanodrop.. Each DNase treated total RNA sample (2 µg) was reverse

transcribed using the RevertAid First Strand cDNA Synthesis Kit according to the manufacturer's instructions. The resultant cDNA was stored at –20°C for further use.

Standardization of primers for PCR: The primers for this study were designed using DNASTAR Lasergene software (1997). The specificity of primers was checked by the NCBI blast program. The qPCR assays were evaluated by the generation of a standard curve. Calibration curves for each gene were done on an iQ5 cycler with five 10–fold serial dilutions (in triplicates) and were calculated by the Bio-Rad Optical System Software (2010) with the analysis mode "PCR base line substracted". Efficiency (E) of qPCR reactions was calculated according to the equation:

$E = 10^{(-1/slope)}$ Expression of HSP 70 was studied in real time PCR. The amplifications of genes were carried out using an iQ5 cycler in 25 µl volume containing 1X QuantiTect® SYBR® Green PCR Master Mix (SYBR® Green 1 dye, HotStartTaq DNA polymerase, and dNTPs in optimized buffer components; a 0.2 µM concentration of each gene-specific primer, and 1 µl of cDNA template. For each gene of interest, negative and positive controls were also included. A melting curve was performed for each sample after completion of amplification and analyzed in comparison to negative and positive controls to determine the specificity of PCR reaction.

The relative expression ratio (ER) of a target gene is computed, based on its real-time PCR efficiencies (E) or a static efficiency of 2, and the cycle threshold (Ct) difference (Δ) of mean control versus each unknown sample (ΔCt control – treatment) as described below (Pfaffl 2001) using GAPDH as the reference housekeeping gene (Table 6):

$$ER = \frac{(E\ target)^{\Delta Ct\ target\ (control - treatment)}}{(E\ ref)^{\Delta Ct\ ref\ (control - treatment)}}$$

Statistical analysis: Data emanated from different treatments were analysed for statistical significance using completely randomized design (CRD) by following standard methods (Snedecor and Cochran, 1989**).** All data were statistically analysed using SPSS software package version 16.0. Variables having unequal observations were analysed following least square design method and the Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Growth parameters: Chicks fed with MOS either 0.3% or 0.5% had significantly (P<0.01) higher body weight gain (BWG) at 2–6 wk and also at overall 0–6 wk growth phase (Table 2). FI was significantly (P<0.05) higher in MOS fed birds during 0–6 wk than control (un-supplemented) birds. Significant (P<0.001) improvement in FCR was observed in MOS fed birds than the un-supplemented birds in all phases. The present findings got support from earlier work of Sohail *et al.* (2013), who concluded that feeding diet supplemented with MOS not only improved overall growth performance but also improved immunity in heat stressed birds. Similar to present report Sohail *et al.* (2012) reported that in hot humid environment supplementation of the MOS helps in reduction of stress and improved FI, FCR and BWG of the birds. The feed conversion efficiency of the birds fed different strains of probiotics and prebiotic was found to be better as described by Apata (2008**).** The significant improvement in the body weight during the finisher phase in probiotic + prebiotic supplemented birds may be attributed to a better microbial environment in the gut and higher enzymatic activity which in turn have enhanced digestion, absorption and utilization of feed (Apata 2008). The fact that MOS improves gut integrity and function via a prebiotic control of potentially pathogenic bacteria (Baurhoo *et al.* 2007), it is reasonable to assume that reduced stress in the gastrointestinal tract might be translated into decreased stress at the whole organism level.

Table 2. Effect of supplemental mannan-oligosaccharide on production performance in different growth phases during hot humid summer

Parameter	Phase (d)	Control	MOS 0.3%	MOS 0.5%	SEm	Prob
Live wt gain (g)	14–21	171.6^{a}	247.1^{b}	247.6^{b}	11.83	P<0.001
	21–42	1097.7	1124.9	1135.0	8.59	NS
	14–42	1269.3^{a}	1372.0^{b}	1382.6^{b}	17.49	P<0.01
	0–42	1475.7^{a}	1578.7^{b}	1589.6^{b}	17.17	P<0.01
Feed intake (g)	14–21	287.6^{a}	402.8^{b}	404.2^{b}	18.45	P<0.01
	21–42	2211.1	2209.9	2198.2	15.59	NS
	14–42	2498.7	2612.7	2602.4	22.64	NS
	0–42	2837.1^{a}	2950.0^{b}	2939.9^{b}	21.65	P<0.05
Feed Conversion ratio	14–21	1.68^{b}	1.63^{a}	1.63^{a}	0.007	P<0.001
	21–42	2.01^{c}	1.96^{b}	1.94^{a}	0.010	P<0.001
	14–42	1.97^{c}	1.90^{b}	1.88^{a}	0.011	P<0.001
	0–42	1.92^{c}	1.87^{b}	1.85^{a}	0.010	P<0.001

Values bearing different superscript differed significantly (P<0.05); NS, Non significant.

Immune competence: Supplementation of MOS caused significant improvement in relative weights of spleen, thymus and bursa at 28th day post-hatch during hot humid climate (Table 3). Also on 42nd day of age, the relative weight of bursa and spleen improved significantly as compared to control due to supplemental of MOS in the diet. MOS supplementation either at 0.3% or 0.5% diet improved humoral and cell mediated immune response than un-supplemented (control) birds during hot-humid climate. This findings were in line with Mashaly *et al.* (2004) who observed that heat stress not only adversely affected production performance but also inhibited immune function which could be overcome by antioxidant or prebiotic like MOS. Accumulating evidence also suggests that probiotics/ prebiotic play an important role in stimulating the immune system during stressful conditions like higher environmental temperature (Jin *et al.* 1997). Khalaji *et al.* (2011) also reported that dietary MOS supplementation (0.5–1.5 g/kg) beneficially affected the cellular as well as humoral immunity during heat stress. MOS supplementation stimulated the immunity of chickens in two ways (a) flora from prebiotic migrated throughout the gut wall and

Table 3. Effect of supplemental mannan-oligosaccharides immune response during hot humid summer

Parameter	Sex	Control	MOS 0.3%	MOS 0.5%	Mean	SEm	Prob.
CMI	Male	0.27	0.37	0.41	0.35^{N}	0.020	NS^{S}
PHA-P foot	Female	0.25	0.34	0.38	0.32^{M}	0.016	NS^{I}
Web Index (mm)	Mean	0.26^{a}	0.35^{b}	0.39^{c}	0.33	0.013	$P<0.001^{T}$
Humoral	Male	7.25	9.00	9.50	8.58	0.398	NS^{S}
HA Titer Against	Female	7.00	8.50	9.25	8.25	0.411	NS^{I}
SRBC (Log_2)	Mean	7.13^{a}	8.75^{b}	9.38^{b}	8.42	0.282	$P<0.01^{T}$
28d Lymphoid wt. (% of live wt.)							
Thymus	Male	0.37	0.43	0.44	0.41	0.019	NS^{S}
	Female	0.32	0.41	0.44	0.39	0.023	NS^{I}
	Mean	0.34^{a}	0.42^{ab}	0.44^{b}	0.40	0.015	$P<0.05^{T}$
Bursa	Male	0.18	0.21	0.26	0.22	0.015	NS^{S}
	Female	0.16	0.20	0.24	0.20	0.016	NS^{I}
	Mean	0.17^{a}	0.21^{b}	0.25^{b}	0.21	0.011	$P<0.01^{T}$
Spleen	Male	0.14	0.19	0.20	0.18	0.011	NS^{S}
	Female	0.16	0.16	0.21	0.18	0.012	NS^{I}
	Mean	0.15^{a}	0.18^{ab}	0.21^{b}	0.18	0.008	$P<0.05^{T}$
42d Lymphoid wt. (% of live wt.)							
Thymus	Male	0.28	0.30	0.26	0.28	0.008	NS^{S}
	Female	0.27	0.30	0.29	0.29	0.010	NS^{I}
	Mean	0.27	0.30	0.28	0.28	0.006	NS^{T}
Bursa	Male	0.16	0.19	0.31	0.22	0.024	NS^{S}
	Female	0.16	0.17	0.30	0.21	0.024	NS^{I}
	Mean	0.16^{a}	0.18^{b}	0.31^{c}	0.22	0.016	$P<0.001^{T}$
Spleen	Male	0.17	0.23	0.21	0.21	0.011	NS^{S}
	Female	0.16	0.22	0.19	0.19	0.013	NS^{I}
	Mean	0.17^{a}	0.22^{b}	0.20^{ab}	0.20	0.008	$P<0.05^{T}$

Values bearing different superscript differed significantly (P<0.05); NS, Non significant.

multiply to a limited extent or (b) antigen released by the dead organisms were absorbed and thus stimulates the immune system (Havenaar and Spanhaak 1994). MOS supplementation reduced serum cortisol, an immunosuppressive, inhibiting the production and actions of antibodies, lymphocyte function and leukocyte population. This factor also helps birds to stimulate the immunity during heat stress.

Haematological parameter: As seen in Table 4 and 5, haemoglobin was significantly increased in broiler fed either 0.3% or 0.5% MOS at 28th as well as 42nd day of age. H:L ratio was significantly (P<0.001) lower in MOS fed birds as compared to unsupplemented birds at 28th as well as 42nd day of age. In present study, significantly (P<0.001) lower H:L ratio in female as compared to male was observed at 42nd day of age due to MOS supplementation. Similar to present study Roul *et al.* (2008) observed that MOS supplementation of pro and prebiotic in combination improved body weight, FCR, haemoglobin and lowered the H:L ratio and cost of production. Also Cetin *et al.* 2005, proved that probiotic supplementation caused statistically significant increase in the erythrocyte count, haemoglobin concentration and haematocrit values of turkeys. Adding probiotic/prebiotic diet could inhibit any stress which causes an increases in H/L ratio because the stress could cause an increases in the stimulation of adrenal gland to produce some hormones such as estrone which has a direct effect to analyze a lymphatic cell which causes an increase in H/L ratio (Seifi *et al.* 2013). The present results were in concurrence with the observation of Hanamanta *et al.* (2009) who reported that 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 as a result of polymer pholymphocytic cell proliferation, increase in alkaline. MOS supplementation helps in reducing stress by improved gut functioning by normalizing micro flora balance and enhancing mineral, vitamin and amino acid absorption.

Biochemical parameter: As shown in Table 4 and 5, the serum total protein was significantly (P<0.001) higher in MOS supplemented birds as compared to control birds at 28th as well as 42nd day of age during hot humid summer. Male had significantly (P<0.01) higher protein concentration than female at 28th as well as 42nd day of age. In accordance with present report, Mashaly *et al.* (2004) observed that exposure to high temperature decreased plasma protein concentration and plasma calcium concentration this serum protein level was increased by supplementation of antioxidant or probiotics. Also Haldar *et al.* (2011) concluded that yeast (*Saccharomyces*

Table 4. Effect of supplemental mannan-oligosaccharide on haematological and biochemical parameter at 28th day of age during hot humid summer

Parameter	Sex	Control	MOS 0.3%	MOS 0.5%	Mean	SEm	Prob.
42nd day							
Haemoglobin (g%)	Male	13.10	14.20	15.03	14.11	0.336	NS^S
	Female	12.53	14.03	14.70	13.76	0.351	NS^I
	Mean	12.82^a	14.12^b	14.87^c	13.93	0.240	$P<0.001^T$
H:L ratio	Male	0.44	0.33	0.31	0.36^N	0.019	$P<0.001^S$
	Female	0.42	0.31	0.30	0.35^M	0.019	NS^I
	Mean	0.43^c	0.32^b	0.31^a	0.35	0.013	$P<0.001^T$
Total Protein (g/dl)	Male	6.32	7.46	7.95	7.25^N	0.210	$P<0.01^S$
	Female	6.11	7.38	7.80	7.10^M	0.217	NS^I
	Mean	6.22^a	7.42^b	7.88^c	7.17	0.149	$P<0.001^T$
Total cholesterol	Male	217.46^Z	163.83^Y	146.33^X	175.87^M	9.151	$P<0.001^S$
(mg/dl)	Female	240.41^Z	168.50^Y	161.79^X	190.23^N	10.776	$P<0.001^I$
	Mean	228.93^c	166.16^b	154.06^a	183.05	7.073	$P<0.001^T$
AST (I.U./l)	Male	99.75	120.44	130.17	116.79^N	3.873	$P<0.05^S$
	Female	98.54	119.18	125.54	114.42^M	3.525	NS^I
	Mean	99.15^a	119.81^b	127.85^c	115.60	2.573	$P<0.001^T$
ALT (I.U./l)	Male	0.67^X	0.86^Y	1.10^Z	0.88^M	0.053	$P<0.001^S$
	Female	0.59^X	1.18^Y	1.43^Z	1.07^N	0.106	$P<0.001^I$
	Mean	0.63^a	1.02^b	1.26^c	0.97	0.061	$P<0.001^T$

^{abc}(Treatment),^{MN}(Sex), ^{XYZ}(Interaction) values bearing different superscript differed significantly (P<0.05); NS, Non significant.

cerevisiae) and yeast protein concentrated supplement positively serum protein concentration in heat stressed birds. MOS supplementation significantly (P<0.001) reduced the serum cholesterol concentration at 28th and 42nd day of age as compared to un-supplemented (control) birds during hot-humid summer. The total cholesterol concentration was significantly lower in male birds as compared to female birds at 28th as well as 42nd day of age in MOS supplemented birds. Interaction effect of sex with treatment did show significance in broiler birds only at 42nd day of age due to MOS supplementation. In accordance with our study Maziar *et al.* 2007 reported that prebiotic/probiotic supplementation significantly decreased the serum cholesterol in heat stressed birds. Also Abdul-rahim *et al.* (1996) observed that lower serum cholesterol concentration was observed in the MOS supplemented groups compared with the heat stress group. Sohail *et al*. (2011) reported that supplementation of probiotic into laying hen diets has been shown to reduce not only serum cholesterol but also egg yolk cholesterol concentration. It is possible that some of the organisms present in the probiotics preparation could assimilate the cholesterol present in the gastrointestinal tract for their own cellular metabolism, thus reducing the amount absorbed. Serum cholesterol is precursor of serum corticosterone, thus it can be said that MOS has stress amelioration effect thus reduction of corticosterone level or alternatively by reducing serum cholesterol value as there is probability of destruction of cholesterol due to production of favourable organisms or reduced absorption of cholesterol.

On 28th and 42nd day of age, aspartate amino transferase (AST) levels increased significantly (P<0.001) with higher concentration in MOS fed birds as compared to control (un-supplemented) birds. At 28th day, female had significantly (P<0.001) higher concentration but at 42nd day of age, males had significantly (P<0.05) higher concentration compared to female. Interaction of sex with treatment had significant (P<0.001) effect on AST concentration at 28th day but not at 42nd day of age. On 28th and 42nd day alanine aminotransferase (ALT) had significant (P<0.001) effect with higher concentration in MOS fed birds as compared to control (un-supplemented) birds. At 28th and 42nd day, post hatch female had significantly (P<0.001) higher concentration than males. Interaction of sex with treatment had significant (P<0.001) effect on ALT concentration at 28th as well as 42nd day post hatch during hot humid summer. Similar to present study, was Hassaan *et al.* (2008) reported that significant increase in AST as well as ALT serum values due to MOS supplementation in heat stressed birds. Sohail *et al*. (2011) concluded that single or combined supplementation of mannan-oligosaccharides and probiotic resulted into significant increase in AST as well as ALT serum values. This will help in reducing deleterious effect due to heat stress and might promote protein synthesis. It can be assumed that the significant increase in AST and ALT level in MOS supplemented groups was due to lower cortisol level.

Serum Corticosterone: As seen in Figure 1., serum corticosterone in MOS supplemented group shows significant (P<0.001) reduction at 42nd day of age during hot humid environment. Elevation of serum corticosterone

Table 5. Effect of supplemental mannan-oligosaccharide on haematological and biochemical parameter at 42th day of age during hot humid summer

Parameter	Sex	Control	MOS 0.3%	MOS 0.5%	Mean	SEm	Prob.
28 day							
Haemoglobin (g%)	Male	12.59	13.97	14.17	13.57	0.269	NS^S
	Female	12.23	13.60	14.09	13.31	0.356	NS^I
	Mean	12.41^a	13.78^b	14.13^b	13.44	0.219	$P<0.01^T$
H:L ratio	Male	0.40	0.32	0.31	0.34	0.014	NS^S
	Female	0.39	0.32	0.30	0.34	0.015	NS^I
	Mean	0.40^b	0.32^a	0.31^a	0.34	0.010	$P<0.001^T$
Total Protein (g/dl)	Male	5.98	7.19	7.30	6.82^N	0.184	$P<0.01^S$
	Female	5.95	6.94	7.10	6.66^M	0.154	NS^I
	Mean	5.96^a	7.07^b	7.20^c	6.74	0.119	$P<0.001^T$
Total cholesterol	Male	163.46	128.62	127.84	139.97^M	5.051	$P<0.001^S$
(mg/dl)	Female	167.67	134.15	132.08	144.63^N	4.927	NS^I
	Mean	165.56^b	131.38^a	129.96^a	142.30	3.484	$P<0.001^T$
AST (I.U./I)	Male	68.10^X	95.60^Y	100.98^Z	88.23^M	4.391	$P<0.001^S$
	Female	78.17^X	99.49^Y	102.58^Y	93.41^N	3.354	$P<0.05^I$
	Mean	73.13^a	97.54^b	101.77^c	90.82	2.756	$P<0.001^T$
ALT (I.U./I)	Male	1.57^X	4.81^Y	5.33^Z	3.90^M	0.502	$P<0.001^S$
	Female	3.53^X	4.99^Y	5.20^Z	4.57^N	0.226	$P<0.001^I$
	Mean	2.55^a	4.90^b	5.26^c	4.24	0.278	$P<0.001^T$

^{abc}(Treatment),^{MN}(Sex), ^{XYZ}(Interaction) values bearing different superscript differed significantly (P<0.05); NS, Non significant.

Table 6. Oligonucleotide sequence of HSP-70 gene primers

SrNo.	Gene	Primer	Annealing temperature	Length(bp)	Accession number
1	HSP-70	F-GGCACCATCACTGGGCTT R-TCCAAGCCATAGGCAATAGCA	56°C	74	HM587997
2	GAPDH	F-CCGTCCTCTCTGGCAAAGTCC R-AGCCCCAGCCTTCTCCATG	57.5°C	266	NM_204305

level is considered as an indicator of heat stress. The hypothalamus pituitary axis (HPA) axis is a complex neuroendocrine pathway that controls reactions to stress. The rise in the serum corticosterone concentrations is considered as an indication of over activation of the HPA axis which is deleterious for birds. In the present study, we observed that MOS supplementation helped to normalize the serum corticosterone. As these supplements are believed to influence the gut health and microbiome, it can be postulated that a healthy and balanced microbial community may have helped normalize adrenal gland activity (Sohail *et al.* 2012). In accordance with the present study, Ghareeb *et al.* (2008) found that dietary supplementation of MOS could reduce corticosterone level in stressed birds. It is postulated that serum cholesterol which is considered as precursor of corticosterone is decreased due to MOS supplementation which may lead to decreasing corticosterone value in MOS supplemented birds. The reduction in serum corticosterone along with reduced H:L ratio in MOS supplemented group is clear indicator that

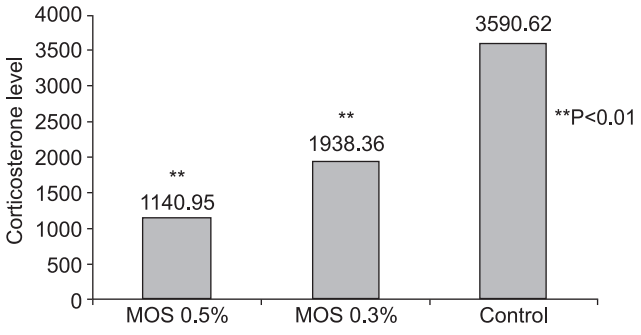

Fig. 1. Effect of supplemental MOS on serum corticosterone level at 42th day of age during hot.

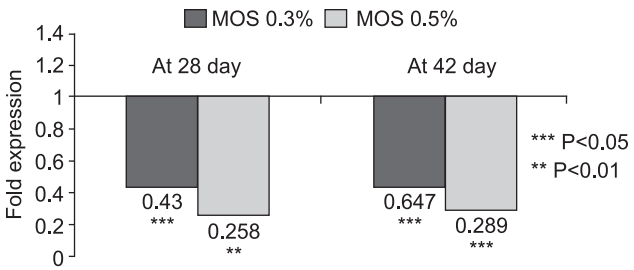

Fig. 2. Effect of MOS supplementation of HSP70 expression during hot humid summer on 28th and 48th day.

stress in MOS supplemented group were reduced.

Expression of heat shock protein (HSP70) gene: In present study, at 28th as well as 42nd day post-hatch expression of HSP70 of jejunum tissue was significantly (P<0.001) down regulated in MOS fed birds either at 0.3% or 0.5% diet comparison to control (un-supplemented) birds (Figure 2 and 3). At the cellular level, elevated temperature increases the synthesis of heat shock proteins (HSP), also known as stress proteins. Increased HSP protect cells against the additional stress, via protecting the cells against harmful insults and making the cells resistant to apoptosis. In acccordence with present study, Lowman *et al.* (2014) reported that lower HSP mRNA expression in Actigen (a second generation MOS) fed birds indicated that the supplement can modify the HSP response while allowing continued good performance during heat-exposure. Xiao *et al.* (2012) and Fang Gan *et al.* (2013) also observed similar result that yeast supplementation during heat stress significantly reduce, HSP70 gene expression. *Saccharomyces cerevisiae* is believed to interact with carbohydrate and mannose receptors on the intestinal epithelial cell. Zachary (2012) observed that there was significant influence of probiotic (*Saccharomyces cerevisiae*) on the induction or suppression of various HSPs. There were significant decreases in HSP90 (P=0.0038), HSP90 (P=0.0040) and HSP70 (P=0.0211) expression due to probiotic (*Saccharomyces cerevisiae*) in the livers. An explanation for this phenomenon could be that probiotic affects many pathways where multiple heat-related changes could occur (Xiao *et al.* 2012). This change might be related to secondary effects in the liver which allows for decreased HSP production in the livers. Also probiotic increases numbers of gut microbes, which potentially release a bioactive substance that could prevent oxidative damage and ultimately lowers expression of HSP (Sohail *et al.* 2011).

In conclusion from all above parameters, it is clear that supplementation of MOS either at 0.3% or 0.5% diet improved body weight and feed conversion, immune response, haematological and biochemical profile and decreases serum corticosterone level during hot-humid summer than in un-supplemented diet. Also MOS at any level down regulated expression of HSP70 in jejunum at 28th as well 42nd day. In conclusion, it's clear that MOS had beneficial functions in heat stressed birds, and MOS @ 0.3% can suitably be used for heat stressed broilers for improved performance and welfare.

REFERENCES

Abdul-rahim S M, Haddadin M S Y, Hashlamoun E A R and Robison R K. 1996. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol contents of plasma and egg yolk. *British Poultry Science* **37**: 341–46.

Ahmad Mujahid. 2011. Nutritional strategies to maintain efficiency and production of chickens under high environmental temperature. *Journal of Poultry Science* **48**: 145–54.

Apata D F. 2008. Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. *Journal of Science of Food and Agriculture* **88**: 1253–58.

Association of Official Analytical Chemists. 1990. *Official Methods for Analysis of the Association of Official Analytical Chemists*, 15th Edn. Association of Official Analytical Chemists, Suite 400, 2200 Wilson Boulevard, Arlington Virginia 22201, USA.

Baurhoo B, Phillip L and C A Ruiz-Feria. 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poultry Science* **86**: 1070–78.

Cetin N, Guclu B K and Cetin E. 2005. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in Turkeys. *Journal of Veterinary Medicine Series A* **52** (6): 263–67.

Corrier D E and Deloach J R. 1990. Evaluation of cell mediated, cutaneous basophil hypersensitivity in young chickens by an inter digital skin test. *Poultry Science* **69** (3): 403–08.

Duncan D B. 1955. Multiple ranges and multiple F test. *Biometrics* **11**: 1–42.

Edens F W, Givisiez P E N, Mahmoud K Z and Sefton A E. 2001. Influence of Escherichia coli-challenged broiler chickens. *Proceedings for the 50th Western Poultry Disease Conference.* University of California, Davis California.

Fang Gan, Fei Ren, Xingxiang Chen, Chenhui Lv, Cuiling Pan, Gengping Ye, Jun Shi, Xiuli Shi,Hong Zhou, Shituleni Andreas Shituleni and Kehe Huang. 2013. Effects of Selenium-Enriched Probiotics on Heat Shock Protein mRNA Levels in Piglet under Heat Stress Conditions. *Journal of Agriculture and Food Chemistry* **61** (10): 2385–91.

Ghareeb K, Awad W A, Nitsch S, Abdel-Raheem S and Bohm J. 2008. Effects of transportation on stress and fear responses of growing broilers supplemented with prebiotic or probiotic. *International Journal of Poultry Science* **7**: 678–85.

Givisiez P E N, Ferro J A, Ferro M I T, Kronka S N, Decuypere E and Macari E. 2001. Hepatic concentration of heat shock protein 70 kd HSP 70 in broilers subjected to different thermal treatments. *British Poultry Science* **40**: 292–96.

Haldar S, Ghosh T K, Toshiwati and Bedford M R. 2011. Effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with Salmonella enteritidis. *Animal Feed Science Technology* **168**: 61–71.

Hanamanta N, Narayana S M, Veena T, Narayana H D and Jayakumar K. 2009. Effect of prebiotic, probiotic and g-probiotic spl® on certain haematological parameters in broiler chickens. *Veterinary World* **2** (9): 344–46.

Hassaan S F, Elsalmoney M and Fathi M M. 2008. *Egypt Poultry Science* **18**: 251–63.

Havenaar R and Spanhaak S 1994. Probiotics from an immunological point of view. *Current Opinion in Biotechnology* **5**: 320–25.

Jin Z, Ho Y W, Abdullah N and Jalaludin S. 1997. Probiotics in poultry: Modes of action. *World's Poultry Science Journal* **53**: 351–68.

Khalaji S, Zaghar M and Nezafati S. 2011. The effects of mannan-oligosaccharides on cecal microbial populations, blood parameters, immune response and performance of broiler chicks under controlled condition. *African Journal of Biochemistry Research* **5**: 160–64.

Lowman Z S, Edens F W, Ashwell C M and Nolin S J. 2014. Actigen Influence on the gene expression of heat ® shock

proteins in ross 708 broiler chickens. *International Journal of Poultry Science* **13** (2): 114–23.

Mandal A B. 2010. Dietary modulation to curb impact of changing climate on livestock and poultry. *International conference on physiological capacity building in livestock under changing climate scenario* **1**: 127–36.

Martin F P J, Wang Y, Sprenger N, Yap I K S, Lundstedt T, Lek P, Rezzi S, Ramadan Z, Van-Bladeren P, Fay L B, Kochhar S, Lindon J C, Holmes E and Nicholson J K. 2008. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Molecular Systems Biology* **4**: 157.

Mashaly M M, Hendricks G L, Kalama M A, Gehad A E, Abbas A O and Patterson P H. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science* **83**: 889–94.

Maziar Mohiti Asli, Seyed Abdollah Hosseini, Houshang Lotfollahian and Farid Shariatmadari. 2007. Effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. *International Journal of Poultry Science* **6** (12): 895–900.

Pfaffl M W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**: 2002–07.

Roul G, Panda N, Mishra P K, Mishra R, Behera P C and Panigrahi B. 2013. Effect of pro- and pre-biotic supplementation on growth and immune status of broilers during summer. *Animal Nutrition and Feed Technology* **13**: 261–70.

Sahin K and Kucuk O. 2003. Heat stress and dietary vitamin supplementation of poultry diets. *Nutrition Abstracts and Reviews. Series B: Livestock Feeds and Feeding* **73**: 41R–50R.

Seifi S, Shirzad M R and Habibi H. 2013. Effects of probiotic yoghurt and prebiotic utilization on performance and some haematological parameters in broiler chickens. *Acta Scientiae Veterinariae* **41**: 1103.

Siegel P B and Gross W B. 1980. Production and persistency of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poultry Science* **59**: 1–5.

Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 8th edition Iowa State University Press, Ames, Iowa, Reprinted in 1994. East-West Press Pvt. Ltd., New Delhi.

Sohail M U, Rahman Z U, Ijaz A, Yousaf M S, Ashraf K, Yaqub T, Zaneb H, Anwar H and Rehman H. 2011. Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. *Poultry Science* **90** (11): 2573–77.

Sohail M U, Hume M E, Byrd J A, Nisbet D J, Ijaz A, Sohail A, Shabbi M Z and Rehman H. 2012. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat. *Poultry Science* **91** (9): 2235–40.

Sohail M U, Ijaz A, Younus M, Shabbir M Z, Kamran Z, Ahmad S, Anwar H, Yousaf M S, Ashraf K, Shahzad A H and Rehman H. 2013. Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. *Journal of Applied Poultry Research* **22** (3): 485–91.

Xiao R, Power R F, Mallonee D, Routt K, Spangler L, Pescatore A J, Cantor A H, Ao T, Pierce J L and Dawson K A 2012. Effects of yeast cell wall-derived mannan-oligosaccharides on jejunal gene expression in young broiler chickens. *Poultry Science* **91**: 1660–69.

Zachary L. 2012. 'Antigen influence on the gene expression of heat shock proteins in ross 708 broiler chickens.' M.Sc. thesis submitted to the graduate faculty of North Carolina State University.