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Effect of mannan-oligosaccharides (MOS) supplementation on performance, immunity and HSP70 gene expression in broiler chicken during hot-dry summer

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ABSTRACT

An experiment was conducted on broiler chicks (n=120), reared in cages on a standard diet up to 14th day of age and thereafter up to 42nd day (14-42d) on test diets with or without MOS. The 14th day old chicks were randomly distributed into three dietary treatment groups *viz.*, T_1 (control diet), T_2 (control diet with MOS @ 0.3%) and T_3 (control diet with MOS @ 0.5%). Each dietary treatment comprised of four replicates of eight birds each. Experiment was carried out during hot-dry summer. It was found that production indices improved (P<0.001) significantly during all phases. Cellular and humoral immunity at 28th day of age improved significantly (P<0.05) due to MOS supplementation. The percentage of hemoglobin, protein, AST and ALT increased significantly (P<0.001) in MOS supplemented group at 28th as well as 42nd day of age. While H:L ratio, serum corticosterone and cholesterol levels decreased significantly (P<0.001) in MOS supplemented groups. Supplementation of MOS at the both levels caused significant (P<0.001) down regulation of HSP70 expression in jejunum tissues during 28th and 42nd day. Based on this study it was concluded that supplementation of 0.3% MOS in broiler diets significantly improved their performance and welfare during heat stressed conditions.

Key words: Broilers, Corticosterone, Heat stress, HSP70, MOS, Production performance.

INTRODUCTION

Climate model projections summarized in the Intergovernmental Panel on Climate Change report indicated that the global surface temperature will probably rise by 1.1 to 6.4°C during the twenty-first century (IPCC, 2007). High environmental temperature affects poultry birds severely, it leads to poor production, production of free radicals, immune insufficiency, imbalance in electrolyte balance and expression of heat shock proteins (Mandal, 2010). Managing the hypothalamic-pituitary-adrenal (HPA) axis activity to avoid overproduction of corticosterone is important because immunity and infection response are preferentially affected by over activation of the HPA axis (Siegel, 1960). Also the cells, in culture or *in vivo*, respond to heat stress by decreasing significantly the synthesis of almost all cellular proteins except a selected group of highly conserved proteins, the heat shock protein 70 (HSP70) (Leandro *et al.* 2004).

In developing countries dietary approach seems to be more user-friendly strategy to curb heat stress. Prebiotics and probiotic as functional foods influence intestinal micro architecture, microbial profiles and broiler performance during heat stress (Sohail *et al.* 2013) Prebiotics are non-digestible carbohydrates. Mannan oligosaccharides (MOS) enhances the growth of beneficial organisms in the gut, and is thought to function as competitive attachment sites for pathogenic bacteria (Gibson and Roberfroid, 1995) MOS supplementation helps to improve bird's performance, gut health, pathogenic micro flora inhibition, nutrient digestion and absorption, also immunity through its antioxidant activities under heat stress.

However, literature and understanding of the effects of prebiotic supplementations under heat stress conditions in broilers is lacking, especially in terms of the immunity, gene expression and corticosteron changes. Therefore, the present study was aimed at investigating the potential role of MOS on selected biological health markers in broilers exposed to heat stress.

MATERIALS AND METHODS

All the experiments were carried out as per the code of practice approved by the Institute of Animal Ethics Committee at Central Avian Research Institute, Izatnagar, UP-24122, India.

Experimental birds, management and diet: The present study was conducted during hot-dry (April-May: 30.0±0.7-37.0±1.4°C, Rh%:58.05±1.3-70.11±0.82)summer. Temperature Humidity Index (THI) during study was in the range of 80 to 92. Straight run broiler chicks (n = 120) of CARIBRO VISHAL were procured, wing banded and raised

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in cages on a standard diet up to 14 days of age. Thereafter, the chicks were randomly distributed into three dietary treatment *viz.*, T_1 (Control diet), T_2 (Control with MOS @ 0.3%) and T_3 (Control with MOS @ 0.5%) following completely randomized design (CRD). Each dietary treatment was fed to four replicated groups of eight birds each besides one more replicate to study blood metabolites and HSP70 gene expression.

All birds were reared under uniform and standard managemental conditions. The birds were reared up to 42ndday of age on starter (14-21d, 21.44% CP and 2839 kcal ME/kg) and finisher (21-42d, 19.75% CP and 2891 kcal ME/kg) diets. The ingredient and chemical composition of control and experimental diet for starting and finishing phase are presented in Table 1.

Production attributes: Data regarding growth performance such as body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and livability/mortality were recorded every week from 0-42 days of age.

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 and Cu- 4 mg/kg diet. The vitamin premixes supplied vitamin A 8250 IU, vitamin D_3 1200 ICU; vitamin K 1mg; vitamin E 40 IU, vitamin B_1 2mg, vitamin B_2 4mg, vitamin B_{12} 10mcg; niacin 60mg; pantothenic acid 10mg and choline chloride 500mg/kg diet.

** Analyzed values as fed basis

***calculated values as fed basis.

Immune responses: For cell mediated immunity (CMI) and humoral immune response 8 birds (4 from each sex) from each treatment were used. The CMI measured as foot web index in response to mitogenphyto-hemagglutinin-P(PHA-P) (Corrier and Deloach, 1990) and humoral immune response measured as serum haemagglutination (HA) titer against sheep red blood corpuscles (Siegel and Gross, 1980) on 28th day post-hatch. Lymphoid organ weights (bursa of fabricius, spleen and thymus) were recorded on 28thand on 42nd day from 6 birds (three male and three female) in each treatment and was expressed as per cent of live weight.

Hematological analysis: Whole blood samples (1ml) were collected from the jugular vein in EDTA coated tubes on 28thand 42ndday of age from 6 birds (3 of each sex) of each treatment for hemoglobin concentration (g/dl) estimation. Also blood smears prepared from fresh blood were stained by Geimsa stain (1:9 Dilution for 45 min) to calculate H:L ratio.

Biochemical profile: Blood sample were collected from 6 birds (equal sex) of each treatment on 28th and 42ndday of age, serum was separated and subjected to blood biochemical test *viz*. total protein and total cholesterol using standard commercial kits (Cogent, SPAN diagnostic Ltd, India).

Serum Corticosterone: Corticosterone EIA Kit (Cayman Chemical Company, USA) was used for the estimation of serum corticosterone at 42nd day of age (eight samples per treatment). The intensity of the colour was determined spectro-photometrically 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

Absorbance α [Bound Corticosterone Tracer]

α 1/[Corticosterone]

Expression analysis of heat shock protein (HSP70) gene: At 24th and 42nd day of age, approximately two centimeter of the proximal portion of the jejunum was collected from four birds per treatment, then opened and flushed with normal saline and 50 mg of tissue was homogenized. The total RNA isolation from tissue and cDNA synthesis was carried out as per the procedure of Bhanja et al. (2014). The Oligonucleotide sequences of the primers used for the gene expression study are presented in Table 2. Expression of HSP70 gene was analyzed by real-time PCR, using an iQ5 cycler. The relative expression ratio (ER) of each target gene was 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 (Pfaffi, 2001) using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference housekeeping gene:

ER = (E target)ΔCt target (control – treatment)

(E ref) ΔCt ref (control – treatment)

Table 2: Oligonucleotide sequence of HSP-70 gene primers

Sr No.	Gene	Primer	Annealing Temperature	Length(bp)	Reference / Acc. number
1	HSP- 70	F- GGCACCATCACTGGGCTT R- TCCAAGCCATAGGCAATAGCA	56^0C	74	HM587997
2	GAPDH	F-CCGTCCTCTCTGGCAAAGTCC R-AGCCCCAGCCTTCTCCATG	57.5^0C	266	NM_204305

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

RESULT AND DISCUSSION

Production attributes: MOS either 0.3% or 0.5% significantly (P<0.05) improved body weight gain (BWG) of broilers at all growth phases compared to control (Table 3). MOS supplementation at 0.3% and 0.5% level lead to 4.53% and 10.49% increase in body weight gain respectively at 42nd day of age. Feed intake was 1.22% and 5.52% higher in 0.3% and 0.5% MOS fed groups respectively, during 42nd day of age compared to control (un-supplemented) birds (Table 3). Significant (P<0.01) improvement in FCR was observed in MOS fed birds than control during all growth phases. Feeding of MOS (prebiotic) can significantly improve BWG, FI, and FCR in heat stressed broilers (Chee *et al.* 2010). Decreased feed intake and body weight gain during heat stress can be attributed to a greater expenditure of energy for physiological adaptation instead for growth performance (Lei and Slinger, 1970). Less feed intake may be a defence strategy to reduce metabolic heat production during heat stress. Better performance due to MOS supplementation may be attributed to better microbial environment in the gut, disease resistance and higher enzymatic activities which in turn enhanced feed consumption, digestion, absorption and utilization of nutrients (Awad *et al.* 2008).

Immune responses: Supplementation of MOS lead to significant improvement in relative weights of thymus (P<0.01) and spleen (P<0.05) at 28th day of age while bursa (P<0.01) and thymus (P<0.05) at 42nd day of age (Table 4). Humoral and cell mediated immune response showed significant (P<0.05) improvement in MOS supplemented treatments (Table 4). The results are in accordance with Sohail *et al.* (2013) and Huang *et al.* (2007). Stress reduces the weight of lymphoid organs so it could be postulated that MOS supplementation reduces the heat stress in broilers leading to higher lymphoid organ weight (Houshmand *et al.* 2012).This improved immunity may be due to improved intestinal absorption of some minerals such as Zn, Cu and Se. Also defense cells in the gut-associated lymphoid tissue (GALT) detect the presence of microbes by recognizing molecules unique to microorganisms that are not associated with host cells. These unique molecules are called pathogen associated molecular patterns (PAMP). These include yeast cell wall components such as mannan and glucan along with peptidoglycan, lipopolysaccharide, and glycolipids. Mannan and glucan of the yeast cell wall may bind to pattern-recognition receptors on a variety of defense cells of the GALT and in turn activate immune defenses such as phagocytosis, the alternative complement pathway and the lectin pathway (Vijaya Janardhana *et al.* 2009).

Hematologicalanalysis: The percentage of hemoglobin was significantly (P<0.01) increased in MOS (both levels) fed broilers (Table 5). Also the H:L ratio was significantly (P<0.01) lower in MOS fed birds at 28th as well as 42nd day of age. Present result was in agreement with the earlier

Table 3: Effect of supplemental mannan-oligosaccharide on production performance in different growth phase during hot-dry summer.

Parameter	Phase(d)	Control	MOS 0.3%	MOS 0.5%	SEm	P value
Live wt gain (g)	14-21	231.5	250.1	258.7	10.24	0.588
	21-42	1050.2^a	1098.1^a	1178.5^b	19.51	0.007
	14-42	1281.7^a	1348.2^{ab}	1437.2^b	26.13	0.034
	0-42	1497.9^a	1565.9^{ab}	1655.0^b	26.68	0.034
Feed intake (g)	14-21	388.9	405.5	403.4	15.43	0.910
	21-42	2060.7	2074.1	2198.3	29.12	0.092
	14-42	2449.6	2479.6	2601.7	37.80	0.235
	0-42	2801.9	2836.2	2956.8	38.78	0.247
Feed Conversion ratio	14-21	1.68^c	1.62^b	1.56^a	0.015	0.000
	21-42	1.96^c	1.89^b	1.87^a	0.013	0.000
	14-42	1.91^c	1.84^b	1.81^a	0.013	0.000
	0-42	1.87^c	1.81^b	1.79^a	0.011	0.000

^{a,b}Values bearing different superscript differed significantly (P<0.05)

Table 4: Effect of supplemental mannan-oligosaccharide on immune response during hot-dry summer

Parameter	Sex	Control	MOS 0.3%	MOS 0.5%	Mean	SEm	P value
CMI	Male	0.22	0.29	0.32	0.27	0.023	0.632
PHA-P foot	Female	0.23	0.31	0.34	0.29	0.026	0.972
Web Index (mm)	Mean	0.22^{a}	0.30^{ab}	0.33^{b}	0.28	0.017	0.046
Humoral	Male	6.50	7.25	7.50	7.08	0.229	0.378
HA Titer Against	Female	6.25	7.00	7.25	6.83	0.207	1.000
SRBC (Log_2)	Mean	6.37^{a}	7.12^{b}	7.38^{b}	6.96	0.153	0.222
28d Lymphoid wt. (% of live wt.)							
Thymus	Male	0.26	0.31	0.31	0.29	0.010	0.254
	Female	0.26	0.28	0.31	0.28	0.009	0.329
	Mean	0.26^{a}	0.30^{b}	0.31^{b}	0.29	0.007	0.004
Bursa	Male	0.26	0.29	0.29	0.28	0.016	0.586
	Female	0.22	0.27	0.30	0.26	0.020	0.733
	Mean	0.24	0.28	0.29	0.27	0.013	0.221
Spleen	Male	0.12	0.16	0.17	0.15	0.010	0.509
	Female	0.13	0.14	0.16	0.14	0.006	0.314
	Mean	0.13^{a}	0.15^{ab}	0.16^{b}	0.15	0.006	0.031
42d Lymphoid wt. (% of live wt.)							
Thymus	Male	0.20	0.24	0.27	0.24^{M}	0.011	0.11
	Female	0.25	0.28	0.29	0.27^{N}	0.010	0.682
	Mean	0.23^{a}	0.26^{ab}	0.28^{b}	0.25	0.009	0.160
Bursa	Male	0.16	0.24	0.28	0.23^{M}	0.019	0.011
	Female	0.19	0.30	0.31	0.27^{N}	0.021	0.360
	Mean	0.18^{a}	0.27^{b}	0.30^{b}	0.25	0.015	0.000
Spleen	Male	0.13	0.14	0.16	0.14	0.010	0.165
	Female	0.14	0.17	0.17	0.16	0.008	0.634
	Mean	0.13^{a}	0.16^{ab}	0.16^{b}	0.15	0.007	0.084

^{abc} (Treatment), ^{MN} (Sex) values bearing different superscript differed significantly (P<0.05).

Table 5: Effect of supplemental mannan-oligosaccharide on hematological and biochemical parameter at 28th and 42nd day of age during hot-dry summer

Parameter	Sex	Control	MOS 0.3%	MOS 0.5%	Mean	SEm	P value
28 days							
Hemoglobin (g%)	Male	12.73	14.23	14.74	13.90	0.332	0.388
	Female	12.47	13.96	14.49	13.64	0.368	0.999
	Mean	12.60^{a}	14.09^{b}	14.62^{b}	13.77	0.242	0.000
H:L ratio	Male	0.43	0.33	0.31	0.36^{M}	0.018	0.011
	Female	0.44	0.36	0.34	0.38^{N}	0.016	0.729
	Mean	0.43^{b}	0.34^{a}	0.33^{a}	0.37	0.012	0.000
Total Protein (g/dl)	Male	5.98	7.19	7.30	6.82	0.184	0.290
	Female	5.95	6.94	7.10	6.66	0.154	0.142
	Mean	5.56^{a}	7.07^{b}	7.20^{b}	6.74	0.119	0.000
Total cholesterol (mg/dl)	Male	197.32	179.13	174.27	183.57^{M}	7.134	0.000
	Female	272.25	228.27	219.27	239.93^{N}	8.097	0.319
	Mean	234.78^{b}	203.70^{a}	196.77^{a}	211.75	7.898	0.004
42 days							
Hemoglobin (g%)	Male	13.33	14.90	15.33	14.52	0.362	0.242
	Female	13.13	14.48	14.84	14.15	0.311	0.919
	Mean	13.23^{a}	14.69^{b}	15.09^{b}	14.34	0.236	0.001
H:L ratio	Male	0.42	0.31	0.31	0.35^{M}	0.018	0.030
	Female	0.42	0.34	0.33	0.36^{N}	0.015	0.397
	Mean	0.42^{b}	0.33^{a}	0.32^{a}	0.36	0.012	0.000
Total Protein (g/dl)	Male	5.13^{x}	7.36^{Y}	7.78^{Y}	6.76^{M}	0.354	0.000
	Female	5.47^{X}	$6..63^{Y}$	6.48^{Z}	6.19^{N}	1.186	0.000
	Mean	5.30^{a}	7.00^{b}	7.13^{b}	6.48	0.204	0.000
Total cholesterol (mg/dl)	Male	313.16^{Y}	236.95^{X}	238.72^{X}	262.94^{N}	11.875	0.000
	Female	243.64^{Y}	224.65^{X}	190.47^{X}	219.58^{M}	8.222	0.025
	Mean	278.40^{b}	230.80^{a}	214.59^{a}	241.26	8.386	0.000

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

workers who attributed these change to the stimulating effects of MOS on hemopoitic organs (Hasan *et al.* 2014). The MOS supplementation might have stimulated folic and riboflavin acid production, which stimulated hematopoietic process of red blood cells (RBC). In different age groups of birds, a rising matured red blood cells led to the rise in the packed cells volume as there is a significant positive correlation between this two (Sturkie, 1986).The MOS stimulate the hemopoitic organs and causes erythropoiesis resulting into increase in hematological parameters during high environmental temperature (Sturkie, 1986).The H:L ratio is commonly used as an indicator of stress and under stress conditions the ratio tends to be wider (Dozier *et al.* 2006). Present results suggest that MOS supplementation enables the birds to overcome the stresses with less physiological responses (Ghareeb *et al.* 2008).

Biochemical profile: Supplementation of MOS significantly (P<0.001) improved serum total protein, while it significantly (P<0.001) reduced the serum cholesterol level (Table 5) at 28th and 42nd day. Increased serum total protein groups may be reflecting a more intensive metabolism of the proteins and effective protein utilization in the broilers (Taherpour *et al.* 2009). Mannan prevents cholesterol absorption from gastro intestinal tract and promotes the growth and activity of lactic acid bacteria (Gibson and Roberfroid,1995), which reduces the cholesterol level by producing enzymes disintegrating bile salts and making them un-conjugated, as well as by reducing the pH in the intestinal lumen (Dawkins *et al.* 2004). This mechanism leads to significant reduction of serum cholesterol concentration in MOS fed birds.

Serum corticosterone: Serum corticosterone reduced significantly (P<0.01) in MOS supplemented group at 42nd day of age (Fig.1). Dietary supplementation of MOS helps to stabilizes corticosterone level and reduced some of the detrimental effects of heat stress (Sohail *et al*. 2012; Houshmand *et al.* 2012). High environmental temperatures, results in activation of the hypothalamic-pituitary-adrenal axis which leads to elevated a plasma corticosterone concentration, causing deleterious effect (Munck *et al.* 1984). But in present study MOS supplementation leading to healthy and balanced microbial environment, might have helped in normalizing the adrenal gland activity. MOS feeding resulted in reduced serum cholesterol level which also subsequently reduced circulating corticosterone concentration as cholesterol is being considered as precursor of corticosterone synthesis (Rokade *et al.* 2016).

Expression of heat shock protein (HSP70) gene: Relative expression of HSP70 of jejunum tissue was significantly (P<0.01) down regulated in MOS fed birds either at 0.3% or 0.5% level in comparison to control (Fig 2). During heat stress, expression of heat shock proteins (HSP), by prokaryotic and eukaryotic cells is clearly observed (Wang and Edens, 1988). Similar to present study Zachary, 2012 also found that reduction in the level of expression of these biomarkers was more pronounced in MOS supplemented groups of heat-exposed chickens. It is believed to be associated with MOS-associated increased numbers of probiotic gut microbes, which potentially release bioactive substances that could prevent oxidative damage and ultimately lowers expression of HSP (Sohail *et al*. 2011).

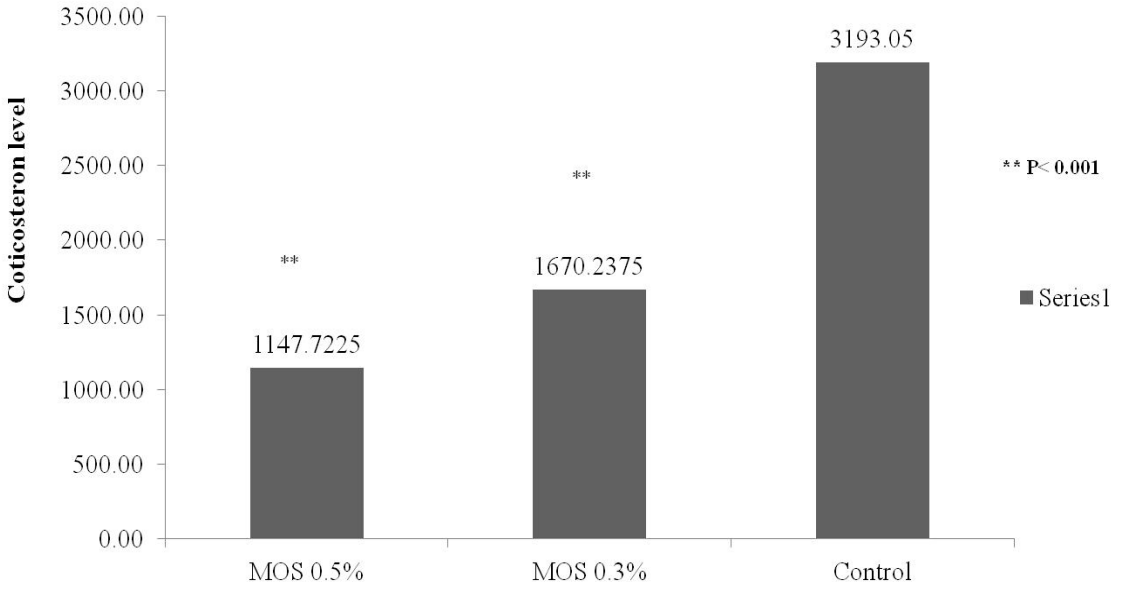

Fig 1: Effect of supplemental MOS on serum corticosterone level at 42nd day of age during hot-dry summer.

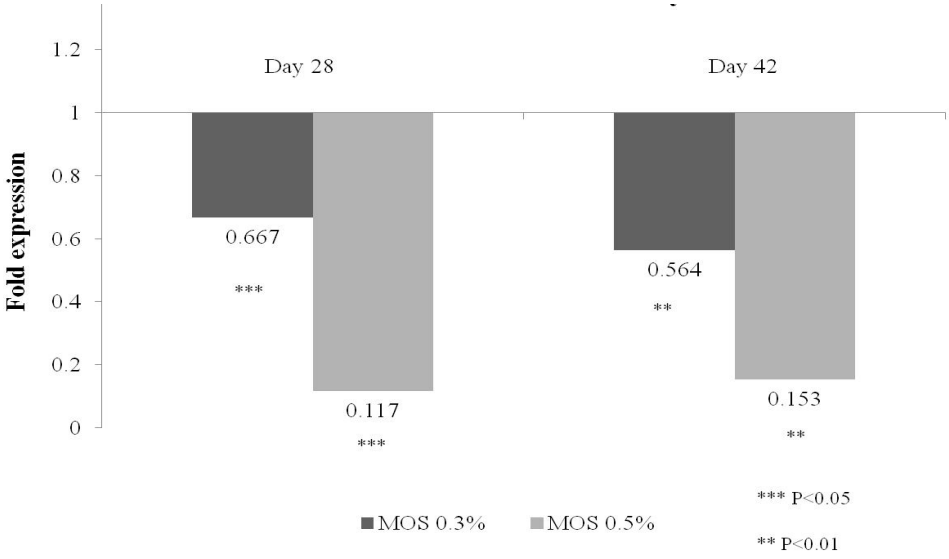

Fig 2: Effect of supplemental MOS on HSP70 expression in jejunum at 28th and 42ndday of age during hot summer

It is possible that local interaction of potentially pathogenic bacteria at the level of intestinal enterocyte receptor complexes might be sufficient to cause heat shock response, especially if bacterial toxins become systemic thereby inducing several HSP inductions. MOS supplementation, may act to bind bacterial mannose receptors, would effectively reduce the bacterial interaction resulting in decreased systemic stress response in MOS fed broilers and less induction in the jejunum of MOS fed broilers compared with control heat exposed broilers (Lowman *et al.* 2014).

Present study clearly established that supplementation of MOS in diet modulate physiological stress responses (H:L ratio, Corticosterone level, HSP70 expression) and improves production as well as immunity of heat stressed broiler chicken.

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