Pakistan J. Zool., vol. 47(3), pp. 641-648, 2015.

Egg Quality and Egg Geometry Influenced by Mannan-Oligosaccharides (MOS), a Prebiotic Supplementation in Four Closebred Flocks of Japanese Quail Breeders (*Coturnix coturnix japonica*)

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Abstract.- In the present study 960 birds (12 week old) of four closebred flocks (CBF) of Japanese quail breeders were used to explore effects of mannan-oligosaccharides (MOS) on egg geometry and egg quality characteristics. Diet given to birds comprised of corn-based or corn supplemented with 0.25%, 0.50% and 1.0% MOS for 15 weeks. Collected data was analyzed by two-way ANOVA utilizing SAS 9.1 for windows. Birds fed MOS supplemented diets had significantly ($p<0.05$) higher eggs' weight and lower egg shell weight (g) than control. No significant differences, in yolk index, were recorded in MOS supplemented and control groups, however, significant differences were observed among CBF, having highest value in Kaleem flock. In the present study, MOS supplementation did not significantly affect shell thickness, albumin index, albumin and yolk pH, Haugh unit score and shape index. Surface area and volume were non-significantly ($p>0.05$) differed in MOS supplemented and control groups, however, a significant change was recorded among CBF and interaction of CBF and MOS levels. From obtained results, it is concluded that MOS did not significantly affect the egg quality and egg geometry in laying quails except eggs' weight.

Key words: Mannan-oligosaccharides, Japanese quail, egg quality, egg geometry, closebred flocks.

INTRODUCTION

The European Union has impended ban on the sub-therapeutic use of antibiotics growth promoters (AGP) in animal feeds on January 1, 2006, due to resistance of microorganisms and some residues in food products related to human (Hayes and Jensen, 2003). Strict public and governmental scrutiny resulted in prohibition of use of AGP in diets of poultry and other farm animals (Cervants, 2006; Michard, 2008). Regarding consumers concern, an urgent industrial requirement for the alternative to AGPs urged researchers and scientists to sort out the alternatives. Current study is also one of the similar efforts for alternatives.

Probiotics, prebiotics and organic acids are considered as possible alternatives to AGPs. Prebiotics are feed ingredients that are not digested by host enzymes and enhance growth performance of host by increasing the growth or activity of selected gut microbiota (Gibson *et al.*, 2004). The exact mechanism of action of prebiotics is not clearly understood, however, it is considered that it promote gut health through alteration of gut pH, keeping protected gut mucus, promoting fermentation, increasing nutrient absorption, altering the population of pathogenic bacteria with beneficial ones that ultimately modulates the immune system of host (Cakir *et al.*, 2008; Bauhroo *et al.*, 2009; Sadeghi *et al.*, 2013).

Mannan-oligosaccharides (MOS) are one of the important prebiotics, derived from outer cell wall of the yeast *Saccharomyces cerevisiae* and is being used in poultry diet. It competes with pathogens for attachment to intestinal wall (Baurhoo *et al.*, 2012) and binds with mannose specific type-1 fimbriae of pathogens, thus eliminates them from the host gut without colonization (Ferket *et al.*, 2002). Mannan-oligosaccharide is not used as nutrient, neither for microbiota, nor for host, but has some growth promoting effects on farm animals (Adam, 2000). The MOS enhance intestinal function by improving the height, integrity and uniformity of intestinal villi and stimulate immune system of host to produce antibodies like IgA. These effects result

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0030-9923/2015/0003-0641 $ 8.00/0

in reduction of pathogen colonization in gut and thus health and performance of animal improves (Hooge, 2004; Ghosh *et al.*, 2007; Rehman *et al.*, 2009).

Japanese quail breeders are important alternatives to poultry production and numerous factors like genetics, age, male/female ratio, nutritional status, fertility rate, hatchability and egg quality traits affects its performance (Seker, 2003). Egg quality relates to the general characteristics of eggs that give quality of egg parts like eggshell (weight, thickness, shape, freshness and color), yolk and albumin. Numerous detailed investigations have been performed throughout the world on composition and egg quality of chickens (Dimitrov *et al.*, 2007; Gerzilov, 2011). Heavier eggs are considered of best quality, so most preferable to consumers (Genchev, 2012). In quails, egg weight is usually increased by the age of birds (Hussain *et al.*, 2011), by sexual maturity (Kumar *et al.*, 2000) and the stage of production cycle (Yanakopolous and Tserveni-Gousi, 1986).

Available information indicates that no research was conducted to find the effects of dietary supplementation of MOS on egg quality traits of four closebred flocks of Japanese laying quails. Therefore, present study was also conducted to explore influence of MOS supplementation on egg internal and external quality traits and egg geometry of four closebred flocks of laying Japanese quail.

MATERIALS AND METHODS

Experimental birds and feeding

A trial was conducted on 960 birds (12 weeks old) of four closebred flocks (CBF) of Japanese quail, naming, Kaleem, Major, Sadat and Zahid, placed at Avian Research and Training (ART) Center, University of Veterinary & Animal Sciences (UVAS), Lahore, Pakistan. Each flock was consisting of 240 birds and divided in four supplemental groups (A, B, C and D). Each feeding group (n=60) was further replicated into three groups (n=20) randomly (15 female + 5 male). The birds were placed in multi-deck cages for 15 weeks and fed a corn-based basal diet prepared according to NRC (1994) standards.

Ingredients and chemical composition of experimental basal diet is maize, 30.0%; rice polish, 8.00%; canola meal, 10.0%; soybean meal, 25.0%; corn gluten 60%, 5.00%; rice tips, 14.0%; lime stone, 1.00%; D-L methionine, 0.10%; L-lysine, 0.20%; threonine, 0.15%; soy oil, 1.85%; DCP, 1.50%; vitamin premix, 0.20% and molasses, 3%.

Nutrient composition of basal diet is ME Kcal/kg, 2900%; CP, 20.00%; Ca, 3.00%; Available P, 0.40%; phytate P, 0.34 %; total P, 0.74%; crude fiber, 4.38%; linoleic acid, 1.00%; methionine, 0.50%; and lysine, 1.30%;

Birds of A, B and C groups were fed corn-based diet added with 0.25%, 0.50%, and 1.0% MOS, respectively, whereas, birds of D group were fed only basal diet (control group). Birds could feed and drink *ad libitum* throughout the experimental period. Quails were exposed to 16 hrs lighting program.

Egg quality characteristics

Egg quality test was performed at the end of 15^{th} week (27 weeks old birds) of experiment. At last day of 15^{th} week of trial, two eggs from each replicate (96 eggs) were collected randomly and weighed on the China made electronic analytical balance, Yueke, sensitive up to 0.001 g. Egg was broken in a glass Petri dish and shell was separated carefully. Egg shell weight was recorded using electrical weighing balance; sensitive up to 0.001 g. Measurement of shell thickness was taken from middle, top and bottom using a micrometer screw gauge (Chowdhury, 1987). Recorded values were used to calculate average shell thickness.

Albumin height and diameter were measured using vernier caliper, sensitive up to 0.1mm (Froning and Fank, 1958) for the calculation of albumin index and Haugh unit (HU) using following formulae:

$$\text{Albumin index: AI} = H / [(0.5(D+d)], \text{ HU} = 100[\log \{(H+7.57) - (1.7)(W\ 0.37)\}]$$

(Haugh, 1937) Where, H = height, W= egg weight, d = long diameter and D = short diameter of albumin. Yolk index was calculated by vales of yolk height and width, measured by vernier caliper, using following formula:

$$\text{Yolk index (YI)} = \text{Yolk height (YH)} / \text{Yolk width (Yw)}$$

A digital pH meter was used to record albumin and yolk pH. Width and length of eggs were recorded using vernier caliper for calculation of shape index by the following formula (Zeidler, 2002; Kırıkc *et al.*, 2007).

Shape index = Breadth / Length × 100

Surface area (S) and Volume (V) of eggs were calculated using formulae

$$S = k\,(\pi LB^2/6)^{0.67}$$

where k, 4.85; L, length of egg; B, breadth of egg.

$$V = k\,\pi LB^2/6$$

where k = 0.929 (Sreenivasiah, 2006)

Statistical analysis

Two-way Analysis of Variance (Steel *et al.*, 1997) was used to analyze collected data and interpreted by General linear Model (GLM) procedures. The means were compared by Duncan's Multiple Range (DMR) test (Duncan, 1955) using SAS 9.1 for windows (2002-03).

RESULTS

Eggs weight was significantly ($p<0.05$) higher in MOS supplemented groups compared to control group (Table I). The highest eggs weight was recorded in group B; however, it did not vary significantly among closebred flocks. Birds fed MOS supplemented diets had significantly ($p<0.05$) lower egg shell weight (g) than control. However, egg shell weight did not vary significantly in different CBF and in interaction of CBF and MOS levels. Non-significant ($p>0.05$) difference in yolk index was recorded in MOS supplemented and control groups. However, significant ($p<0.05$) change was recorded among CBF having highest

Table I.- Shell weight and thickness, albumin and yolk pH of quail parent flocks fed with different levels of MOS.

CBF	MOS levels (%)	Egg weight (g)	Shell weight (g)	Shell thickness (mm)	Albumin pH	Yolk pH
CBF						
Major		13.05 ± 0.05	1.87 ± 0.03	0.20 ± 0.002	7.55 ± 0.04	6.13 ± 0.03
Kaleem		13.11 ± 0.09	1.80 ± 0.03	0.20 ± 0.002	7.58 ± 0.05	6.10 ± 0.03
Sadat		13.01 ± 0.08	1.88 ± 0.03	0.20 ± 0.001	7.63 ± 0.03	6.04 ± 0.04
Zahid		13.05 ± 0.11	1.89 ± 0.04	0.20 ± 0.002	7.60 ± 0.04	6.01 ± 0.04
MOS levels (%)	0.25	13.08 ± 0.07^{a}	1.83 ± 0.02^{b}	0.20 ± 0.002	7.61 ± 0.04	6.09 ± 0.04
	0.50	13.19 ± 0.05^{a}	1.82 ± 0.03^{b}	0.20 ± 0.002	7.56 ± 0.04	6.05 ± 0.04
	1.00	13.18 ± 0.05^{a}	1.84 ± 0.03^{b}	0.20 ± 0.002	7.60 ± 0.05	6.09 ± 0.04
	0.00	12.77 ± 0.08^{b}	1.95 ± 0.05^{a}	0.20 ± 0.002	7.59 ± 0.05	6.04 ± 0.03
CBF ×MOS levels (%)						
Major	0.25	12.91 ± 0.04^{cde}	1.86 ± 0.04	0.20 ± 0.001	7.57 ± 0.10	6.11 ± 0.08
	0.50	13.20 ± 0.10^{abc}	1.88 ± 0.04	0.19 ± 0.005	7.57 ± 0.07	6.09 ± 0.06
	1.00	13.02 ± 0.05^{abcde}	1.86 ± 0.05	0.20 ± 0.006	7.64 ± 0.08	6.16 ± 0.04
	0.00	13.07 ± 0.11^{abc}	1.89 ± 0.06	0.20 ± 0.003	7.42 ± 0.10	6.15 ± 0.06
Kaleem	0.25	13.30 ± 0.10^{ab}	1.79 ± 0.05	0.20 ± 0.005	7.63 ± 0.08	6.08 ± 0.07
	0.50	13.28 ± 0.12^{abc}	1.73 ± 0.04	0.20 ± 0.004	7.56 ± 0.13	6.19 ± 0.08
	1.00	13.20 ± 0.01^{abc}	1.79 ± 0.05	0.20 ± 0.006	7.49 ± 0.12	6.05 ± 0.05
	0.00	12.67 ± 0.12^{ef}	1.90 ± 0.10	0.20 ± 0.004	7.66 ± 0.06	6.07 ± 0.05
Sadat	0.25	12.92 ± 0.19^{bcde}	1.84 ± 0.05	0.19 ± 0.004	7.65 ± 0.06	6.09 ± 0.10
	0.50	13.18 ± 0.03^{abcd}	1.79 ± 0.03	0.21 ± 0.003	7.56 ± 0.02	5.96 ± 0.08
	1.00	13.15 ± 0.11^{abcd}	1.91 ± 0.06	0.20 ± 0.004	7.67 ± 0.10	6.18 ± 0.06
	0.00	12.81 ± 0.20d^{ef}	1.97 ± 0.12	0.20 ± 0.001	7.65 ± 0.06	5.95 ± 0.08
Zahid	0.25	13.21 ± 0.04^{abc}	1.84 ± 0.04	0.20 ± 0.007	7.62 ± 0.05	6.07 ± 0.06
	0.50	13.09 ± 0.18^{abcd}	1.90 ± 0.04	0.21 ± 0.004	7.56 ± 0.07	6.00 ± 0.06
	1.00	13.34 ± 0.16^{a}	1.79 ± 0.06	0.20 ± 0.003	7.62 ± 0.08	5.98 ± 0.12
	0.00	12.54 ± 0.03^{f}	2.03 ± 0.11	0.20 ± 0.004	7.62 ± 0.12	5.99 ± 0.08

In column, values (Mean ± SEM) with different superscripts on means are differ significantly at P<0.05.
CBF, closebred flocks.

Table II.- Egg weight, albumin and yolk index and haugh Unit Score of quail parent flocks fed with different levels of MOS.

CBF	MOS levels (%)	Albumin index	Yolk index	Haugh Unit score
Major		24.55 ± 0.52	37.96 ± 0.41[b]	99.06 ± 0.38
Kaleem		24.33 ± 0. 37	39.54 ± 0.49[a]	98.85 ± 0.27
Sadat		24.81 ± 0.42	36.88 ± 0.74[b]	99.25 ± 0.35
Zahid		25.07 ± 0.47	37.88 ± 0.46[b]	99.49 ± 0.40
MOS Levels (%)				
	0.25	24.26 ± 0.51	38.06 ± 0.52	98.81 ± 0.41
	0.50	24.39 ± 0.41	37.94 ± 0.54	99.01 ± 0.30
	1.00	25.19 ± 0.35	37.81 ± 0.77	99.49 ± 0.26
	0.00	24.92 ± 0.51	38.45 ± 0.39	99.34 ± 0.41
CBF × MOS Levels (%)				
Major	0.25	24.73 ± 1.42	37.53 ± 0.39	99.31 ± 1.08
	0.50	24.44 ± 1.08	39.08 ± 0.98	98.98 ± 0.56
	1.00	25.08 ± 0.93	37.51 ± 0.64	99.52 ± 0.82
	0.00	23.96 ± 0.88	37.74 ± 1.11	98.44 ± 0.64
Kaleem	0.25	24.08 ± 0.69	40.50 ± 1.17	99.75 ± 0.42
	0.50	24.66 ± 0.36	39.88 ± 0.90	98.90 ± 0.33
	1.00	25.56 ± 0.71	39.28 ± 1.25	99.71 ± 0.30
	0.00	23.00 ± 0.87	38.48 ± 0.49	98.02 ± 0.85
Sadat	0.25	23.91 ± 0.39	36.64 ± 0.77	98.60 ± 0.73
	0.50	23.67 ± 1.05	35.72 ± 1.00	98.39 ± 0.78
	1.00	25.59 ± 0.78	36.29 ± 2.59	99.89 ± 0.44
	0.00	26.06 ± 0.71	38.88 ± 0.78	100.10 ± 0.66
Zahid	0.25	24.33 ± 1.43	37.58 ± 1.04	98.58 ± 1.10
	0.50	24.80 ± 0.69	37.07 ± 0.73	99.77 ± 0.67
	1.00	24.51 ± 0.26	38.18 ± 1.18	98.83 ± 0.37
	0.00	26.66 ± 0.93	38.70 ± 0.77	100.80 ± 0.72

For statistical details and abbreviations see Table I.

value in Kaleem flock, compared to other treatment groups and no difference was recorded among interaction of CBF and MOS levels. In current study, shell thickness, albumin index, albumin and yolk pH, Haugh unit score and shape index (Tables I, II) were non-significantly ($p>0.05$) different in MOS supplemented and control groups and among CBF and interaction of CBF and MOS levels.

Surface area (cm^2) and volume (cm^3) were non-significantly ($p>0.05$) differed in MOS supplemented and control groups (Table III). However, surface area and egg volume were significantly ($p<0.05$) higher in Sadat and Zahid flocks compared to other flocks. In interaction of CBF and MOS levels, egg surface area and egg volume were significantly ($p<0.05$) higher in C and D groups of Zahid flock compared to other groups, however lowest value was recorded in group A of Kaleem flock.

DISCUSSION

In the present study, significantly ($p<0.05$) higher egg weight in MOS supplemented groups was might be due to reduction of pathogens and their stress on intestinal mucosa, increased absorption and utilization of the dietary nutrients that resulted in higher egg weight. This increased egg weight is in agreement with the findings of Gracia *et al.* (2004), who observed significant increase in egg weight from 54 to 58 week of laying hens fed by MOS supplemented diet. Tarasewicz *et al.* (2004) and Hannan (2010) also observed that birds fed with oligosaccharides supplemented diet showed highest eggs weight. In another study, Abd El-Samee *et al.* (2012) observed that MOS supplemented diet has improved egg weight in quails. In contrast, various researchers reported that MOS supplementation did not show any significant

Table III.- Egg geometry parameters of quail parent flocks fed with different levels of MOS.

CBF	MOS levels (%)	Shape index (%)	Surface area (cm^2)	Volume (cm^3)
Major		78.31 ± 0.47	25.81 ± 0.10^{b}	12.16 ± 0.07^{b}
Kaleem		78.45 ± 0.40	25.53 ± 0.17^{b}	11.98 ± 0.13^{b}
Sadat		78.50 ± 0.29	26.39 ± 0.13^{a}	12.57 ± 0.09^{a}
Zahid		78.74 ± 0.29	26.67 ± 0.14^{a}	12.76 ± 0.10^{a}
MOS Levels (%)				
	0.25	78.75 ± 0.34	25.82 ± 0.14	12.17 ± 0.10
	0.50	78.27 ± 0.43	26.09 ± 0.18	12.36 ± 0.13
	1.00	78.37 ± 0.33	26.22 ± 0.18	12.45 ± 0.11
	0.00	78.62 ± 0.37	26.28 ± 0.16	12.49 ± 0.11
CBF × MOS Levels (%)				
Major	0.25	78.42 ± 0.71	26.00 ± 0.18b^{cde}	12.29 ± 0.13^{bcd}
	0.50	77.53 ± 1.51	25.77 ± 0.21^{def}	12.13 ± 0.15^{cde}
	1.00	78.91 ± 0.72	25.55 ± 0.07^{ef}	11.97 ± 0.05^{de}
	0.00	78.39 ± 0.70	25.94 ± 0.27^{bcde}	12.25 ± 0.19^{bcd}
Kaleem	0.25	79.40 ± 0.72	25.03 ± 0.21^{f}	11.64 ± 0.14^{e}
	0.50	78.12 ± 0.49	25.86 ± 0.56^{cdef}	12.40 ± 0.43^{bcde}
	1.00	77.32 ± 0.59	25.74 ± 0.25^{def}	12.12 ± 0.18^{cde}
	0.00	78.97 ± 1.13	25.47 ± 0.17^{ef}	11.92 ± 0.12^{de}
Sadat	0.25	79.15 ± 0.46	25.98 ± 0.15^{bcde}	12.27 ± 0.11^{bcd}
	0.50	78.21 ± 0.40	26.19 ± 0.31^{abcde}	12.42 ± 0.22^{abcd}
	1.00	77.99 ± 0.68	26.67 ± 0.29^{abc}	12.77 ± 0.21^{ab}
	0.00	78.66 ± 0.72	26.74 ± 0.15^{ab}	12.81 ± 0.11^{ab}
Zahid	0.25	78.02 ± 0.78	26.25 ± 0.29^{abcde}	12.47 ± 0.20^{abcd}
	0.50	79.20 ± 0.61	26.53 ± 0.29^{abcd}	12.66 ± 0.20^{abc}
	1.00	79.27 ± 0.46	26.93 ± 0.19^{a}	12.95 ± 0.14^{a}
	0.00	78.46 ± 0.35	26.96 ± 0.26^{a}	12.97 ± 0.18^{a}

For statistical details and abbreviations see Table I.

effects on egg weight (Iscan and Guclu, 2000; Chen *et al.,* 2005; Fernando *et al.,* 2008; Bonos *et al.,* 2011; Güçlü, 2011).

It has been reported that egg shell quality of broilers improved (Berry and Lui, 2000) and egg shell deformation in layer hens decreased (Cabuk *et al.,* 2006) under MOS supplemented diets. However, in current study, birds fed MOS supplemented diets have significantly lower average egg shell weight (g) than control. Reduction in egg shell weight might be due to thinning of egg shell with age, as age increases, it causes less deposition of calcium ions in egg shell (Butcher *et al.*, 1991), however, Hussain *et al.* (2011) observed no effect of age on egg shell thickness of Japanese quail. The results of present study did not agree with results of Berry and Lui (2000) and Bozkurt *et al.* (2012), who recorded higher egg shell weight under diet supplemented with MOS and an essential oil blend (EOB), however, other egg quality traits were not affected.

Supplementation of MOS did not show any significant effect on albumin and yolk indices, Haugh unit score, pH of yolk and albumin**.** These results are in accordance with findings of some earlier researchers (Berry and Lui, 2000; Chen *et al.,* 2005; Güçlü, 2011; Bozkurt *et al.,* 2012) who concluded that egg quality traits were not significantly affected by MOS supplementation, except shell weight. Abd El-Samee *et al.* (2012) added that albumin and yolk indices, albumin%, yolk %, Haugh unit score, shell% of laying quails were not influenced by MOS supplementation.

Contrarily, some researchers reported that MOS supplementation increased shell thickness (Barca, 2002; Dimovelis *et al.,* 2004; Güçlü, 2011; Ozek, 2012). Furthermore, albumin weight (Stanley *et al.* (2004), shell and yolk percentage (Radu-Rusu,

2009) were increased by MOS supplementation. In another study, Ozek (2012) recorded significantly higher Haugh unit score and albumin height in EOM alone or combined with MOS supplemented groups.

Shape index was non-significantly ($p>0.05$) different in MOS supplemented groups, closebred flocks and in interaction of CBF and MOS levels. Similar results were reported by Abd El-Samee *et al*. (2012), who observed that MOS supplementation did not show any significant effect on shape index of Japanese quail eggs.

CONCLUSION

The results of present study indicate that Mannan-oligosaccharide supplementation of Japanese quails' diet improves the egg weight without influencing other egg quality traits except for egg shell weight. The lower egg shell weight at MOS supplemented groups did not associated with thinner shell; therefore likely not compromise the egg shell quality. In conclusion, MOS supplementation (0.25-1.0%) might be recommended in feeding of Japanese quails.

ACKNOWLEDGEMENT

The author is very thankful to Avian Research and Training Centre, Department of Poultry Production, UVAS, Lahore, Pakistan for provision of excellent research facilities and financial support.

Declaration of conflict of interest

There are no conflicts relating to the article.

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(Received 14 October 2014, revised 16 March 2015)