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# Effect of Supplementation of Mannan-Oligosaccharides on Growth Performance, Viscera Development, Mineral Absorption and Caecal Microbiota of Japanese Quail (*Coturnix coturnix japonica*)

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**MAI designed the study and drafted the manuscript. HR supervised this work. AH edited this manuscript. FJ, IA and KA managed the quail chicks and formulated the feed. MIA analysed the data and OK helped in chemical analysis.**

**Key words**
**Animal feed formulation, Feed conversion ratio, Feed technology, Prebiotics.**

## ABSTRACT

This study was conducted to investigate the effect of mannan-oligosaccharides (MOS) as nutritional supplementation on zoo-technical parameters, visceral organ weights, serum mineral levels and caecal concentrations of *E. coli* and *Clostridium* spp. in Japanese quail (*Coturnix coturnix japonica*). Japanese quail (one-day old; n = 1320) were taken and randomly separated into four groups (n = 320), having 8 replicates (n = 40) in each group. Corn-based poultry feed (basal diet) was given to control group birds (Group A) and the same feed mixed with three different concentrations of MOS *i.e.*, 0.1, 0.5 and 1.0 %, was offered for 35 days to the birds of experimental groups, B, C and D, respectively. Results showed that the birds of group C were heavier ($P < 0.05$) during 2nd and 3rd weeks compared to the other groups. Feed conversion ratio was not affected by the dietary supplementation except on week 2, which was more ($P < 0.05$) in the group B liken with the group A. Birds of group B had higher relative weights of liver ($P = 0.57$) and gizzard ($P = 0.69$). The relative caecal weights of all MOS-supplemented groups were greater ($P = 0.10$) compared with the group A. Serum concentrations of calcium, magnesium, copper and iron were not influenced by the MOS supplementations. Serum phosphorus level was higher ($P < 0.05$) in the group B compared with the group A. The caecal *Clostridium* spp. counts were more ($P = 0.001$) in the group B compared with the other groups, while *E. coli* population remained unchanged in all groups. We found that supplementation of MOS partially influenced the growth performance of Japanese quails.

## INTRODUCTION

The sub-therapeutic use of antibiotics in livestock and poultry production is under severe scientific and public scrutiny, as antibiotic growth promoters (AGP) can promote antibiotic resistance in pathogens and halt therapeutic strategies. European Union banned sub-therapeutic usage of AGP in animal production in 2006. Due to impending ban of AGP in livestock and poultry feed, it was compulsory for the rapidly developing poultry industry to develop alternatives of AGP. The prebiotics and probiotics seemed to be alternate candidates for AGP. The prebiotics are believed to affect host health by affecting gut microbiota and stimulating mineral absorption (particularly magnesium, calcium and iron) in the host (Scholz-Ahrens *et al.*, 2001; Ferket, 2004). Sims *et al.* (2004) reported improvement in body weight and feed conversion efficiency in turkeys by the supplementation of these dietary ingredients in their feed.

Mannan-oligosaccharides (MOS), extracted from yeast cell wall, are not hydrolyzed by the host enzymes and are fermented by intestinal microbiota (Flickinger and Fahey, 2002). The MOS provide competitive

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binding sites for pathogens with mannose-specific type-1 fimbriae such as in *Salmonella* and *E. coli* decrease their attachment with intestinal wall and are ultimately excreted from the gut (Newman, 1994; Ferket *et al*., 2002). It has been documented that MOS supplementation constantly increases the ceacal populations of *Lactobacillus* spp. and *Bifidobacterium* spp. (Baurhoo *et al*., 2007; Yang *et al*., 2008). Hooge (2003) investigated significant improvement in body weight and feed conversion ratio in broilers without any effect on their mortality by the dietary supplementation of MOS.

Reasonable data are available on supplementation of MOS and improved body weight gain, feed conversion efficiency and nutrient digestibility in broilers and turkeys (Kumprecht *et al*., 1997; Parks *et al*., 2001; Ghasemian and Jahanian, 2016). However, very little data are available on mineral absorption and growth performance in Japanese quail by the dietary supplementation of MOS. The present study was, therefore, conducted to investigate effects of the MOS supplementation on growth performance, viscera development, mineral absorption and caecal microbiota in Japanese quail.

## MATERIALS AND METHODS

*Birds, diets and experimental design*

A day-old 1,280 Japanese quail broiler (*Coturnix coturnix japonica*) chicks, procured from the hatchery of Avian Research and Training Centre, University of Veterinary and Animal Sciences, Lahore, Pakistan, were randomly divided into 4 groups. Each group consisted of 320 birds replicated further eight times (n = 40) in a completely randomized design. The birds were housed in wire-bottomed batteries. The initial temperature of the house was maintained at 37°C during first week of the experiment and was gradually reduced according to normal management practice (1−2°C/week) to 32°C in the fifth week. The chicks were maintained on a 24 h constant light schedule during the 35-day experimental period. The birds were weighed weekly until end of the experimental trial.

Birds were fed *ad libitum*, a corn-based basal diet (control group), or the same basal diet supplemented with MOS either at 0.1 %, or 0.5 % or 1.0 % levels. The basal diet was formulated in accordance with the nutritional requirements of Japanese quails as specified in NRC (1994).

*Pre-slaughtering analyses*

The calculated amount of feed was offered to each replicate of birds. Residual feed and left over feed was recorded to determine weekly feed consumption of each group. At end of the experiment, the overall feed consumption was calculated by adding weekly feed consumption. Weekly body weight gain (BWG) was measured by subtracting the initial BW from the final BW. Weekly feed consumed was divided by BWG to get weekly feed conversion ratio (FCR).

*Post-slaughtering analyses*

On day 35, 16 birds (2/replicate/group) were randomly selected, weighed and slaughtered for sampling. Blood samples were collected in glass tubes, allowed to clot for eight hours and then centrifuged at 3000 rpm for 20 min to collect serum. Serum samples were stored at −20°C till further analysis. Small intestine was eviscerated to measure its length. The weights of gizzard, small intestine and caeca, with and without digesta were determined. Weights of the hearts and the livers were also measured. Caecal-digesta (0.5 g) were collected in sterile glass tubes and shipped on ice to the laboratory for enumeration of bacteria.

*Mineral estimation*

Serum levels of calcium, magnesium, copper and iron were estimated by atomic absorption spectrophotometer (Perkin Elmer, A Analyst-100). Phosphorous concentrations were determined spectrophotometerically.

*Microbial populations of the caecal-digesta*

The caecal-digesta collected thus, were utilized for the enumeration of bacteria. The caecal-digesta were diluted and spread on Clostridium Perfringens Agar and Eosin Methylene Blue Agar to count colony forming units (CFU) of *Clostridium perfringens* and *Escherichia coli*, respectively.

*Statistical analysis*

Statistical program, SPSS for windows (Version 13, SPSS Inc., Chicago, Illinois, USA) was used for the data analysis. The data were expressed as mean ± SE. The S-N-K Test was used to test the normal distribution of the data. The data were analyzed using one-way analysis of variance. The group differences were compared by the Duncan's Multiple Range Test (SAS, 2002). A probability value at $P < 0.05$ was considered significant.

## RESULTS

*Pre-slaughtering analyses*

Table I shows effect of MOS-supplementation on feed consumption, body weight and feed onversion ratios in quails. The results did not reveal any change in feed consumption in control and MOS-supplemented groups.

**Table I.- Effect of MOS-supplementation on mean feed consumption (g), mean body weight (g) and mean feed conversion.**

| Treatment group | Feed consumption (g) | | | | | |
|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Overall |
| Mean feed consumption (g) | | | | | | |
| Control | 33.00 ± 0.77 | 104.37 ± 4.74 | 132.62 ± 3.45 | 145.75 ± 6.93 | 168.87 ± 6.36 | 584.62 ± 18.28 |
| 0.1% MOS | 33.87 ± 1.34 | 100.75 ± 3.81 | 135.75 ± 5.57 | 137.87 ± 4.63 | 167.87 ± 4.05 | 576.12 ± 11.98 |
| 0.5% MOS | 35.37 ± 1.49 | 97.25 ± 4.76 | 134.75 ± 4.88 | 137.12 ± 3.88 | 171.12 ± 5.37 | 575.62 ± 12.38 |
| 1.0% MOS | 36.62 ± 1.36 | 104.00 ± 4.96 | 135.00 ± 3.83 | 141.12 ± 6.17 | 173.75 ± 6.24 | 590.50 ± 14.59 |
| Mean body weight during the experiment (g) | | | | | | |
| Control | 12.13 ± 0.18[a] | 32.91 ± 0.84 | 48.93 ± 0.85 | 46.73 ± 2.36 | 43.36 ± 1.98 | 184.08 ± 1.73[ab] |
| 0.1% MOS | 11.88 ± 0.35[a] | 34.86 ± 0.52 | 48.86 ± 1.78 | 44.83 ± 1.39 | 45.76 ± 1.88 | 186.21 ± 1.18[a] |
| 0.5% MOS | 11.68 ± 0.31[ab] | 32.20 ± 1.52 | 51.76 ± 2.03 | 44.11 ± 1.95 | 40.30 ± 1.95 | 180.06 ± 1.78[b] |
| 1.0% MOS | 10.92 ± 0.18[b] | 33.51 ± 0.93 | 49.22 ± 1.40 | 49.55 ± 2.23 | 40.87 ± 2.30 | 184.08 ± 2.19[ab] |
| Mean feed conversion ratio g feed/g BWG | | | | | | |
| Control | 2.72 ± 0.05[b] | 3.19 ± 0.21 | 2.71 ± 0.09 | 3.16 ± 0.18 | 3.95 ± 0.22 | 3.17 ± 0.09 |
| 0.1% MOS | 2.87 ± 0.16[b] | 2.89 ± 0.12 | 2.80 ± 0.16 | 3.10 ± 0.16 | 3.70 ± 0.16 | 3.09 ± 0.07 |
| 0.5% MOS | 3.05 ± 0.17[ab] | 3.04 ± 0.15 | 2.63 ± 0.14 | 3.14 ± 0.14 | 4.35 ± 0.33 | 3.19 ± 0.06 |
| 1.0% MOS | 3.37 ± 0.17[a] | 3.11 ± 0.16 | 2.76 ± 0.14 | 2.87 ± 0.15 | 4.37 ± 0.33 | 3.17 ± 0.07 |

Control, corn based diet; MOS, mannan-oligosaccharide.[a-b]Values within columns with no common superscripts are significantly different ($P < 0.05$). Values represent the mean ± S.E. of four groups of quail chicks. [a-b]Values within columns with no common superscripts are significantly different ($P < 0.05$).

**Table II.- Effect of total mean body weights (g) of control and MOS-supplemented on groups of quails.**

| Treatment group | Initial body weight(g) | Body weight(g) |
|---|---|---|
| Control | 7.8±0.12 | 191.25±2.28[ab] |
| 0.1% MOS | 7.97±0.10 | 193.87±1.34[a] |
| 0.5% MOS | 7.93±0.09 | 187.75±1.30[b] |
| 1.0% MOS | 8.08±0.18 | 193.18±1.46[a] |

Values represent the mean ± S.E. of four groups of quail chicks. [a-b]Values within columns with no common superscripts are significantly different ($P < 0.05$). For abbreviations, see Table I.

A significant difference ($P < 0.05$) in the body weight gain of quails was observed during the first week of age among control and MOS-supplemented groups. The body weight gain was significantly lower in group D (1.0% MOS) compared to control and group B (0.1% MOS). However, the body weight gain did not change among control and MOS-supplemented groups during rest of the experimental period. The overall body weight gain couldn't differ significantly among control and MOS-supplemented groups.

A significant difference ($P < 0.05$) in the FCR was observed during the first week of age among control and MOS-supplemented groups. The FCR of group D (1.0% MOS) was significantly higher compared to control and group B (0.1% MOS). However, overall FCR didn't change among control and MOS-supplemented groups during rest of the experimental trial.

*Post-slaughtering analysis*

The body weights of the MOS-supplemented quails were non-significantly different as compared to the control group. However, body weight of group C (0.5% MOS) was significantly lower compared to other MOS-supplemented groups (Table II).

Table III shos the effect of dietary supplementation of MOS on the relative weights of visceral organs and length of intestine. The relative weight of liver was significantly higher ($P < 0.05$) in group D (1.0% MOS) compared to control group (Table III). The supplementation of MOS could not significantly affect the relative lengths of small intestine and caeca compared to control group.

Table IV shows that dietary supplementation of MOS did not affect the calcium, magnesium, phosphorus, copper and iron concentrations of blood serum.

The results also revealed that MOS-supplementation did not affect microbial populations of the caecal-digesta (Table V).

## DISCUSSION

*Pre-slaughtering analyses*

*Feed consumption*

Our results of the present study showed that MOS supplementation didn't influence feed intake. Similar

**Table III.- Effect of MOS-supplementation on mean relative visceral organs weight (g) and length (cm) of quails.**

| | Control | 0.1% MOS | 0.5% MOS | 1.0% MOS |
|---|---|---|---|---|
| Relative weights (g/g BW) | | | | |
| Small intestine with digesta | 4.68 ± 0.18 | 4.87 ± 0.14 | 4.72 ± 0.14 | 4.80 ± 0.20 |
| Small Intestine without digesta | 2.39 ± 0.07 | 2.47 ± 0.06 | 2.57 ± 0.09 | 2.50 ± 0.10 |
| Caecum with digesta | 0.53 ± 0.04 | 0.57 ± 0.04 | 0.57 ± 0.04 | 0.59 ± 0.04 |
| Caecum without digesta | $0.27 \pm 0.01^{b}$ | $0.29 \pm 0.01^{ab}$ | $0.32 \pm 0.02^{a}$ | $0.31 \pm 0.01^{ab}$ |
| Gizzard with digesta | 3.43 ± 0.13 | 3.42 ± 0.14 | 3.54 ± 0.14 | 3.83 ± 0.21 |
| Gizzard without digesta | $2.65 \pm 0.11^{ab}$ | $2.38 \pm 0.09^{b}$ | $2.47 \pm 0.09^{ab}$ | $2.79 \pm 0.15^{a}$ |
| Heart | 0.85 ± 0.02 | 0.79 ± 0.02 | 0.84 ± 0.02 | 0.85 ± 0.04 |
| Liver | $2.35 \pm 0.09^{b}$ | $2.50 \pm 0.10^{ab}$ | $2.49 \pm 0.10^{ab}$ | $2.75 \pm 0.10^{a}$ |
| Relative lengths (cm/g BW) | | | | |
| Small intestine with digesta | 30.32 ± 0.63 | 29.93 ± 0.75 | 31.12 ± 0.65 | 30.69 ± 0.60 |
| Small intestine without digesta | 32.34 ± 0.78 | 31.37 ± 0.79 | 32.79 ± 0.63 | 32.11 ± 0.62 |

Values represent the mean ± S.E. of four groups of quail chicks. a-bValues within columns with no common superscripts are significantly different ($P < 0.05$). For abbreviations, see Table I.

**Table IV.- Effect of MOS-supplementation on mean serum mineral concentrations of quails.**

| Index | Control | 0.1% MOS | 0.5% MOS | 1.0% MOS |
|---|---|---|---|---|
| Ca (mg/dl) | 10.33±0.83 | 9.45±0.97 | 9.80±1.29 | 11.39±1.11 |
| P (mg/dl) | $5.10^{b}\pm0.15$ | $5.38^{ab}\pm0.13$ | $5.56^{a}\pm0.07$ | $5.61^{a}\pm0.08$ |
| Mg (mg/dl) | 3.81±0.32 | 3.51±0.20 | 3.27±0.31 | 3.92±0.34 |
| Fe (ppm) | 3.28±0.17 | 2.77±0.15 | 3.16±0.16 | 2.91±0.18 |
| Cu (ppm) | 0.41±0.02 | 0.36±0.02 | 0.41±0.03 | 0.35±0.02 |

Values represent the mean ± S.E. of four groups of quail chicks. a-bValues within columns with no common superscripts are significantly different ($P < 0.05$). For abbreviations, see Table I.

findings have been observed in broilers and quails (Cakir *et al*., 2008; Jung *et al*., 2008). However, outcomes of this study were not endorsed by the conclusions of Bonos *et al*. (2010) who reported significant feed intake in quails of MOS-supplemented groups. Similarly, Rosen (2007) observed low feed intake in birds, fed with MOS as compared to control. It is believed that unwanted microorganisms from the gastrointestinal tract were eliminated due to the presence of MOS that resulted in lessening of tenseness on the mucosa, made by pathogens and the dietary nutrients were absorbed in a normal way.

*Body weight gain analyses*

We observed a significant variation ($P < 0.05$) in quails' body weights gained after the first week of age among MOS-supplemented and control groups. Similarly, the overall body weight gain was statistically alike among control and MOS-supplemented groups. Flemming et al. (2004) also found that MOS had improved the BWG in broilers. Yang et al. (2007) reported that there were no significant changes in BWG among treatments, however, the MOS supplementation tended to improve BWG in the early lives of broiler chicks. In contrast with the present results, Ghosh et al. (2007) revealed that feed with MOS supplement didn't increase BWG in quails. Most probably, the supplementation of MOS eliminated pathogens from the gastrointestinal tracts of birds that resulted in the reduction of stress on the mucosa and hence assimilation and exploitation of the dietetic nutrients were increased.

*Feed conversion ratio*

We found a significant change in the FCR at first week of age among control and MOS-supplemented groups, however, during rest of the experimental period, FCR remained unchanged. Our results were in accordance with those of Bonos et al. (2010) who reported that the

**Table V.- Effect of dietary supplementation of MOS on microbial population of caecal-digesta of quails.**

| Index | Control | 0.1% MOS | 0.5% MOS | 1.0% MOS |
|---|---|---|---|---|
| *Escheria coli* × $10^{6}$ c.f.u /g | $7.72^{ab}\pm0.04$ | $7.66^{ab}\pm0.06$ | $7.83^{a}\pm0.05$ | $7.60^{b}\pm 0.06$ |
| *Clostridium perfringens*× $10^{6}$ c.f.u/g | $4.39^{b}\pm0.02$ | $4.36^{b}\pm0.04$ | $4.33^{b}\pm0.04$ | $4.56^{a}\pm 0.02$ |

Values represent the mean ± S.E. of four groups of quail chicks. a-bValues within columns with no common superscripts are significantly different ($P < 0.05$). For abbreviations, see Table I.

supplementation of MOS contributed for higher FCR in Japanese quails during the fourth week.

*Post-slaughtering analysis*

*Body weight*

In the present study, body weights of the MOS-supplemented quails were found non-significantly different as compared with control group. Our findings were in accordance with those of Ghosh *et al*. (2007) and Sarica *et al*. (2009) who didn't found any considerable variation in body weights of quails among MOS-supplemented and control groups. In contrast to the findings of the present study, Bonos *et al*. (2010) found higher body weights in quails for consumption of MOS, while Frittis and Waldroup (2003) observed greater weight of body in turkeys that consumed MOS. Similar findings were highlighted in broilers by Flemming *et al*. (2004).

*Relative weights and lengths of the visceral organs*

We observed that feed supplemented with MOS didn't influence the relative weights of caecum, small intestine (both filled and empty) heart and gizzard, while relative weight of liver was significantly higher in MOS-supplemented group D in comparison with control group. Our results were in accordance with the findings of Mohammed *et al*. (2008) who found that in broilers, dietary MOS didn't influence the relative weights of heart and gizzard. Similarly, Rehman *et al*. (2008) revealed that inulin had not any influence on the mean length of small intestine and weight in broilers. In the present study, the results of the relative weight of liver were not compatible with the results of Mohammed *et al*. (2008) who revealed that MOS supplementation couldn't considerably affect relative weight of liver in broilers. Our results of the relative length of small intestine were similar with the study of Juśkiewicz *et al*. (2004) who reported that in turkeys, diverse oligosaccharides had not any influence on the small intestine in the weight or length.

*Mineral estimation*

We found that dietary supplementation of MOS didn't affect the calcium, magnesium, phosphorus, copper and iron concentrations in blood serum. Prebiotics like inulin, oligofructose or other non-digestible oligosaccharide (NDO) didn't affect calcium or iron absorption in humans (López-Huertas *et al*., 2006). Similar findings has been reported previously in healthy human adults and added that inulin increased calcium absorption and had no effect on the metabolism of the other minerals like magnesium, phosphorous, copper and iron. Reporting of conflicting results regarding mineral absorption might be due to design of the experiment, the impact of non-digestible oligosaccharide depended on the dosage, given time, calcium content in the diet and the age of the animals (Coudray *et al*., 1997).

*Microbial populations of the caecal-digesta*

Our results revealed that MOS supplementation had not affected the population of *Escherichia coli* and *Clostridium perfringens* in the caecal-digesta of quails. Similar findings were highlighted by Yang *et al.* (2007) who stated that supplementation of MOS had no influence on the population of *Clostridium perfringens* in broilers. Ceylan *et al*. (2003) reported that MOS didn't reduce the concentrations of caecal pathogens in broilers significantly. There was no substantial variation in the level of *Clostridium* spp. in caecal contents of turkeys. Baurhoo *et al*. (2009) reported that reduction of the caecal concentration of total *Escherichia coli* due to MOS supplementation was more pronounced in *Escherichia coli*-challenged birds. Fairchild *et al*. (2001) also revealed that MOS supplement provided safety to chicks due to reduction in the pathogenic bacteria like *Escherichia coli.* Yang *et al*. (2008) also reported that dietary MOS also reduced the counts of *Clostridium perfringens* in the caeca of birds.

## CONCLUSION

Results of current study revealed that the birds fed diet supplemented with 0.5% MOS were significantly heavier during 2nd and 3rd weeks compared to the other groups. Similarly, Birds feeding with 0.1% MOS were having higher relative weights of liver and gizzard. Serum concentrations of calcium, magnesium, copper and iron were not influenced by the MOS addition to diet. However, Serum phosphorus level was significantly higher in 0.1% MOS supplemented group than control group. From the findings of the current study, we can conclude that supplementation of MOS partially influenced the growth and production performance of Japanese quails.

*Statement of conflict of interest*

The authors declare that no conflicts of interest, and financial or other, exists. The work described has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to this journal.

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