

Effect of dietary supplementation of mannan oligosaccharides on hydrogen ion concentration of the digestive tract and microbial populations of the ceca of Japanese quail (*Coturnix japonica*)

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Abstract: The effect of the dietary supplementation of mannan oligosaccharides (MOS) on hydrogen ion concentration of the digestive tract and microbial populations of the ceca of the Japanese quail (*Coturnix japonica*) was investigated in this experimentation. Two hundred twenty-five 1-day old Japanese quail divided into 3 groups, with 3 subgroups each, were fed a basal diet that served as control, or a basal diet containing 1 g MOS/kg or 2 g MOS/kg. On days 21 and 42, hydrogen ion concentrations of the quail crop, proventriculus, gizzard, duodenum, jejunum, ileum, large intestine, and ceca were measured in situ. Furthermore, on the same days, the total aerobic bacteria, lactic acid bacteria, Enterobacteriaceae, and coliform counts of the cecal content were recorded. The results of the experimentation showed that the dietary supplementation of MOS in the feed of growing quail did not significantly affect the hydrogen ion concentration in various parts of the digestive tract, the bacterial counts in the ceca and the mortality of growing healthy Japanese quail reared in unstressed conditions.

Key words: Mannan oligosaccharides, Japanese quail, digestive tract, hydrogen ion concentration, microflora

Introduction

To maintain the intestinal microflora balance in animals, it is important to prevent diseases and carcass contamination by controlling the overgrowth of potentially pathogenic bacteria. For this reason, antibiotic substances have been added to livestock

animal feeds as growth promoters since 1950 because it was found that their use improved the performance and health of the animals. Nevertheless, in recent years, the public disapproval for antibiotic growth promoters, due to their residual effects, has created a growing interest in the identification and evaluation of alternative natural feed additives (1-3).

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One such additive that is being tested as growth promoter is the mannan oligosaccharides (MOS) of the cell wall of the yeast *Saccharomyces cerevisiae*. When MOS are incorporated in the animal feed, they can adhere to pathogenic bacteria that have type-I fimbriae and so limit their ability to adhere to the mucosa of the digestive tract and multiply. In addition, MOS can benefit the intestinal function by improving the height, uniformity, and integrity of the intestinal villi (4-7). Moreover, they can exert a positive effect on the immune response of the animal and the production of IgA antibodies. As a result, the replication of many pathogens is being limited and the health of the gut improves (5,7).

The objective of this study has been to examine whether diets supplemented with MOS can exert an effect on the hydrogen ion concentration of the digestive tract and the native cecal bacteria populations of growing Japanese quail (*Coturnix japonica*).

Materials and methods

Animals and diets

Two hundred twenty-five 1-day-old Japanese quail (*Coturnix japonica*) hatchlings were individually weighed and assigned randomly to 3 treatment groups with 25 birds each, which were housed in separate wire suspended cages until 42 days of age.

To meet the nutrient requirements of growing quail, a complete basal diet in mash form was formulated (8,9). Table 1 presents the ingredients and composition of this diet, which was analyzed according to AOAC (10). Furthermore, the calcium, total phosphorus, lysine, methionine plus cystine, starch, sugar, and metabolizable energy content were calculated from the composition of the feed ingredients following Novus (11), NRC (9), and Spais et al. (12).

The basal diet was given to controls (group CON). The birds of the other 2 groups were fed the above

Table 1. Ingredients and composition of basal diet.

Ingredients	g/kg	Chemical analysis	g/kg
Maize	452.6	Dry matter	914
Soybean meal	320.0	Crude protein	238
Wheat	100.0	Crude fat	28
Corn gluten meal	79.7	Crude fiber	36
Calcium carbonate	14.3	Ash	62
Dicalcium phosphate	11.4		
Soybean oil	10.8	Calculated analysis	
Lysine	3.7	Calcium	8.5
Vitamin and trace mineral premix ¹	3.5	Total phosphorus	6.5
Salt	2.1	Lysine	13
Sodium bicarbonate	1.9	Methionine & Cystine	8.7
		Starch	350
		Sugar	44.5
		Metabolizable energy, kcal/kg	2950

¹ Supplying per kg feed: 14,000 IU vitamin A, 5000 IU vitamin D₃, 30 mg vitamin E, 13 mg vitamin K, 3 mg vitamin B₁, 8 mg vitamin B₂, 3 mg vitamin B₆, 20 µg vitamin B₁₂, 85 mg vitamin niacin, 20 mg pantothenic acid, 2 mg folic acid, 200 µg biotin, 10 mg vitamin C, 960 mg choline chloride, 100 mg Zn, 116 mg Fe, 120 mg Mg, 20 mg Cu, 0.2 mg Co, 1 mg I, 0.3 mg Se.

diet with the addition of 1 g MOS/kg (group MOS1) or 2 g MOS/kg (group MOS2). The MOS used was "MOS 500" (Ultra Bio-Logics Inc., Canada). Feed and drinking water were offered to birds ad libitum. Mortality was recorded daily. Conventional breeding and management procedures were applied throughout the experimental period. The quail were handled according to the principles of the Greek Directorate General of Veterinary Services for the care of animals in experimentation.

pH measurements

On days 21 and 42 of the experimentation, the hydrogen ion concentration values of the main parts of the quail digestive tract including the crop, proventriculus, gizzard, duodenum, jejunum, ileum, rectum, and ceca were determined using a WTW, model pH 330/SET-2, pH meter, equipped with a pH-electrode SenTixSp probe (Weilheim, Germany). One bird from each subgroup was randomly selected and sacrificed by cervical dislocation following anesthetizing with chloroform. Just after sacrifice, the birds were laparotomized, the digestive tract was opened in situ at the middle of the mentioned parts, and the tip of the pH-electrode probe (shaft diameter 4 mm) was directly inserted into the content of the corresponding part (13).

Microbiological analyses

On days 21 and 42 of the experiment, one quail from each subgroup was sacrificed as explained above and the cecal content of each bird was transferred under aseptic conditions into a sterile glass vial. After recording its weight, the material was diluted 1:10 with sterile diluent peptone water (Conda, Spain). Subsequently, the glass vial was shaken 25 times in 7 s over a 30 cm arc. The interval between mixing and removing the test portion did not exceed 3 min (14). Ten-fold serial dilutions for each sample were made with sterile diluent peptone water until they were diluted to 10^{-7} and plated in duplicate to enumerate the following microorganisms: (1) Total Aerobic Bacteria were enumerated by the pour-plate method using Standard Methods Agar (Conda, Spain). Plates were incubated at 36 °C for 2-3 days (15,16). (2) Lactic acid bacteria (LAB) were enumerated by the pour-overlay method using MRS agar (Conda, Spain). Plates were incubated anaerobically at 30 °C for 3 days. (3) *Enterobacteriaceae* were enumerated by the

pour-overlay method using Violet Red Bile Glucose agar (Conda, Spain). Plates were incubated at 37 °C for 24 h. Purple colonies surrounded by the purple zone were enumerated and recorded as *Enterobacteriaceae* (17). (4) Coliforms were enumerated by the pour-overlay method using Violet Red Bile Lactose agar (Conda, Spain). Plates were incubated at 37 °C for 24 h. Purple colonies surrounded by the purple zone were enumerated and recorded as coliforms (17). Average results of duplicate measurements are presented as \log_{10} colony forming units (CFU)/g of the cecal content (18).

Statistical analysis

The statistical analyses were performed using SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) for the 3 groups of the experimentation was performed. In addition, one-way ANOVA was performed to compare the measurements on day 21 with those on day 42 for each group. Furthermore, regression analysis of the effect of MOS was performed using the curve estimation function of SPSS. Mortality was analyzed with the chi-square test. A value of $P \leq 0.050$ was considered significant and a value of $0.050 < P \leq 0.100$ was considered a tendency. Levene's test was applied to test the homogeneity of the variances. Tukey's test was applied to determine statistical differences between the means.

Results

The results concerning the effect of the dietary MOS on hydrogen ion concentration in the main parts of the digestive tract on days 21 and 42 of the experimentation are given in Table 2. Statistical analysis showed that the recorded pH values were not influenced significantly ($P > 0.100$) by the addition of MOS in the diets. Moreover, regression analysis of the data showed that the increase of MOS incorporation in the feed did not result in any significant ($P > 0.100$) effect. Moreover, in the comparison between days 21 and 42, significant ($P \leq 0.050$) increase of pH was noticed in the jejunum, ileum, and large intestine for the CON group, tendency ($0.050 < P \leq 0.100$) for decreased pH in the ceca for the MOS1 group, and tendency ($0.050 < P \leq 0.100$) for increased pH in the ileum and large intestine for the MOS2 group.

Table 2. Effect of dietary MOS on hydrogen ion concentration in various parts of the quail digestive tract, on days 21 and 42 (mean \pm standard deviation).

Day 21 of the experiment								
Group	Part of digestive tract							
	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Large intestine	Ceca
CON	5.11 \pm 0.46	4.61 \pm 0.43	3.77 \pm 0.15	6.07 \pm 0.14	6.25 \pm 0.21*	6.57 \pm 0.31*	6.96 \pm 0.16*	6.51 \pm 0.55
MOS1	5.44 \pm 0.55	3.96 \pm 1.13	3.53 \pm 0.35	6.21 \pm 0.24	6.44 \pm 0.18	7.00 \pm 0.31	6.98 \pm 0.75	7.04 \pm 0.13**
MOS2	5.13 \pm 0.67	4.08 \pm 0.92	3.49 \pm 0.51	5.91 \pm 0.30	6.41 \pm 0.41	6.62 \pm 0.39**	6.88 \pm 0.34**	6.83 \pm 0.65
P value	0.732	0.646	0.633	0.359	0.697	0.299	0.961	0.467
Day 42 of the experiment								
Group	Part of digestive tract							
	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Large intestine	Ceca
CON	5.35 \pm 0.40	3.87 \pm 1.26	3.36 \pm 0.50	6.23 \pm 0.10	6.76 \pm 0.17	7.38 \pm 0.11	7.57 \pm 0.19	6.96 \pm 0.17
MOS1	5.09 \pm 0.81	3.39 \pm 0.92	3.36 \pm 0.36	6.20 \pm 0.06	6.53 \pm 0.17	7.26 \pm 0.24	7.50 \pm 0.03	6.70 \pm 0.22
MOS2	5.58 \pm 0.21	3.23 \pm 0.19	3.50 \pm 0.71	6.19 \pm 0.12	6.73 \pm 0.36	7.34 \pm 0.40	7.55 \pm 0.28	6.96 \pm 0.47
P value	0.556	0.681	0.939	0.857	0.518	0.861	0.913	0.533

Groups: CON = Control, MOS1 = 1 g MOS/kg, MOS2 = 2 g MOS/kg.

*Measurements on days 21 and 42 differ significantly ($P \leq 0.050$) for the same group and digestive tract part.

** Measurements on days 21 and 42 tend to differ ($0.050 < P \leq 0.100$) for the same group and digestive tract part.

Table 3 presents the counts of total aerobic bacteria, lactic acid bacteria, *Enterobacteriaceae*, and coliforms in the quail ceca on days 21 and 42 of the experimentation. On day 21, there was a tendency ($P \leq 0.100$) for group MOS1 to have a higher total aerobic bacteria count compared to group CON, but there was no significant difference ($P > 0.100$) for the other bacterial populations. Moreover, on day 42, there was a tendency ($P \leq 0.100$) for group CON to have a higher *Enterobacteriaceae* count compared to group MOS1, but no significant difference ($P > 0.100$) was noticed for the other bacterial populations. In addition, regression analysis of the data showed that the increase of MOS incorporation in the feed did not result in any significant ($P > 0.100$) effect. Furthermore, in the comparison between days 21 and 42 tendency ($0.050 < P \leq 0.100$) for increased *Enterobacteriaceae* and coliform counts was noticed in the CON group, but no significant ($P > 0.100$) effect was found in the other 2 groups.

No significant ($P > 0.100$) difference was noticed in mortality between the groups (CON: 17.3 \pm 2.3%, MOS1: 14.7 \pm 2.3%, MOS2: 18.7 \pm 6.1%).

Discussion

In this experiment, no significant difference between the groups was noted on the hydrogen ion concentration of the digestive tract of quail on days 21 and 42. The pH profiles found in the digestive tract parts agree well with those observed in the avian digestive tract (13,19). In a previous study, Sarica et al. (20) reported no significant difference in the hydrogen ion concentration in the small intestine of quail fed MOS. A possible change of the pH value may be the result of modification of the digestive tract function or a change of its microflora balance. Lactic acid bacteria, such as lactobacilli, can produce lactic acid and other volatile fatty acids and therefore can influence the pH of the digestive tract (21).

Furthermore, in this experimentation, a tendency for a significant difference between groups CON and MOS1 on total aerobic bacteria counts on day 21 and *Enterobacteriaceae* on day 42 was noted. In previous studies involving quail, Sarica et al. (20) reported no significant difference in birds fed MOS compared to

Table 3. Effect of dietary MOS on quail cecal total aerobic bacteria, lactic acid bacteria, *Enterobacteriaceae*, and coliforms (log CFU / g) on days 21 and 42 (mean \pm standard deviation).

Day 21 of the experiment				
Group	Total aerobic bacteria	Lactic acid bacteria	<i>Enterobacteriaceae</i>	Coliforms
CON	9.50 \pm 0.24	9.36 \pm 0.22	8.58 \pm 0.45*	8.71 \pm 0.44*
MOS1	10.41 \pm 0.31	9.70 \pm 1.14	9.33 \pm 1.05	9.03 \pm 0.86
MOS2	9.76 \pm 0.59	9.15 \pm 0.96	9.31 \pm 0.42	9.34 \pm 0.93
P value	0.083	0.750	0.389	0.635
Day 42 of the experiment				
Group	Total aerobic bacteria	Lactic acid bacteria	<i>Enterobacteriaceae</i>	Coliforms
CON	9.85 \pm 0.75	9.70 \pm 0.79	9.43 \pm 0.49	9.72 \pm 0.57
MOS1	9.52 \pm 0.94	8.86 \pm 1.16	8.64 \pm 0.30	9.11 \pm 0.60
MOS2	9.85 \pm 0.08	9.51 \pm 0.48	9.35 \pm 0.33	9.48 \pm 0.21
P value	0.804	0.494	0.080	0.377

Groups: CON = Control, MOS1 = 1 g MOS/kg, MOS2 = 2 g MOS/kg.

* Measurements on days 21 and 42 tend to differ ($0.050 < P \leq 0.100$) for the same group and microbial count.

controls on total aerobic bacteria or *Escherichia coli* counts in the small intestine. Ghosh et al. (5), as well, found no significant difference on coliforms or *E. coli* counts in the small intestine of quail, but a decrease of *Clostridium perfringens*. Baurhoo et al. (22,23) reported an increase of lactobacilli and bifidobacteria in the ceca of broilers due to dietary MOS, while Spring et al. (24) noted a decrease of *Salmonella* in the ceca of broilers, but no difference in lactobacilli, coliforms, enterococci, and anaerobic bacteria. Sims et al. (25) found no significant difference on lactobacilli, coliforms, and *E. coli* in turkeys fed MOS, whereas Song and Li (26) reported a decrease in *E. coli* in the small intestine and feces in broilers.

The dietary supplementation of MOS can limit the growth of some pathogens or potential pathogens, such as *Salmonella* and *E. coli* (4-7). In addition, it can increase the counts of beneficial bacteria, such as lactobacilli and bifidobacteria. Several mechanisms have been proposed to explain this modification in

the microflora balance: competition for receptor sites, production of antimicrobial products (e.g., bacteriocins), production of volatile fatty acids, or stimulation of the host immune system (27,28). This beneficial change of the microflora can affect the length of the intestinal villi and the number of the villi goblet cells (23). Long and uniform villi are considered indicative of good gut health and function (25).

It was reported that the most dramatic responses to the dietary supplementation of feed additives are seen in stressed animals that are not offered good quality feed or are reared in non-optimum conditions (22,25,27). The effect of stress on the intestinal microflora may be seen as a reduction in the number of anaerobic microorganisms. A rise in endogenous corticosteroid levels due to stress may decrease the secretion of mucin. Under these conditions, the balance in the gut microflora is adversely affected resulting in the overgrowth of coliforms and other pathogenic bacteria (21).

The differences in the hydrogen ion concentration and the bacterial counts between days 21 and 42 can be attributed to age differences of quails. Birds at a younger age have not completed the development of their digestive tract and the bacterial populations of their digestive tract have not stabilized yet. On the other hand, adult birds have a mature digestive tract and the bacterial populations are much more stable and not so easily influenced by different diets (25,29).

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Conclusions

The dietary supplementation of MOS in the feed of growing quail did not significantly affect the hydrogen ion concentration in various parts of the digestive tract, bacterial ceca population counts, and mortality in growing healthy Japanese quails reared in unstressed conditions.

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