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**Effect of Dietary Supplementation of Organic Acid and Mannan
Oligosaccharide on the Performance and Gut Health of Japanese Quail
(*Coturnix coturnix japonica*)**

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Abstract: An experiment was conducted to determine the influence of organic acid salts and mannan oligosaccharides on the performance and gut health of Japanese Quail. Day old chicks of Japanese Quail (n = 280) were randomly assigned into seven dietary treatments replicated four times with 10 chicks per replicate. Control (C₀) birds were given a standard basal diet, C₁ birds were provided with basal diet having antibiotic (Bacitracin methyl disalisylate-BMD) at 0.5 g kg⁻¹; T₁ birds having diet with sodium butyrate at 5 g kg⁻¹; T₂ birds were provided with basal diet having Mannan Oligosaccharide (MOS) at 1 g kg⁻¹; group T₃ birds were fed basal diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹ and sodium butyrate 5 g kg⁻¹; T₄ birds having diet with sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹; T₅ birds having diet with ammonium formate at 1 g kg⁻¹, calcium propionate at 1 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of the diet. Organic acid salt supplementation increased (p<0.05) live weight, live weight gain compared to control (C). Cumulative feed intake was not significantly affected due to dietary treatments. Superior results in terms of Feed Conversion Ratio (FCR) and Performance Index (PI) were found in organic acid salt combination (T₃) and organic acid salts combination (OASC) with MOS (T₅) supplemented groups compared to others. OASC and OASC with MOS improved (p<0.05) gut health by reducing bacterial load in gut compared to control (C₀) and other groups also. The result showed OASC and OASC with MOS supplementation significantly improved villus height compared to other groups. This experiment revealed that the use of OASC or OASC with MOS in the diet improved body weight gain, FCR, PI, villus height and reduced bacterial load in gut as antibiotic compared.

Key words: Japanese quail, mannan oligosaccharide, organic acid, performance

INTRODUCTION

Public disapproval for the use of antibiotics in the poultry production due to their residual effects in meat, concerns over the emergence of antibiotic resistant Superbugs (JETACAR, 1999). The problem is however much more complicated that just a simple withdrawal of this growth promoters overnight, it will not only affect feed efficiency but also increase mortality and morbidity of animals. Therefore, many parts of the world are experimenting alternative feed additives that may be used to alleviate the problems, associated with the withdrawal of antibiotic from feed. In poultry production organic acids like formic and propionic have mainly been used in order to sanitize the feed having problem with bacterial infection (Hinton and Linton, 1985; Berchieri and Barrow, 1996; Thompson and Hinton, 1997). Butyrate is considered to be responsible for normal development of epithelial cell

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(Pryde *et al.*, 2002). Mannan oligosaccharides (MOS), derived from yeast cell wall, maintain gut health by adsorption of pathogenic bacteria containing type-I fimbriae of different bacterial strains and remove the bacteria from gut (Oyofe *et al.*, 1989; Spring *et al.*, 2000) and increase villus height, uniformity and integrity (Loddi *et al.*, 2002). Under these circumstances, the present study was carried out to evaluate the efficiency of various organic acid salts, viz., formic acid, propionic acid and butyric acid salt, as well as MOS as growth promoter and performance enhancer of Japanese quail.

MATERIALS AND METHODS

Day old chicks of Japanese quail (n = 280) were randomly distributed into seven experimental groups consisting of four replicates in each. Each replicate was with 10 birds. The chicks were individually weighed and allocated to 28 cages of electrically heated battery brooder so that each cage had birds with similar (p>0.05) average weight. In control (C₀) birds were supplied with standard basal diet; group C₁ birds were provided with diet having antibiotic Bacitracin methyl disalisylate (BMD) at 0.5 g kg⁻¹ of diet; group T₁ birds having diet with sodium butyrate at 5 g kg⁻¹ diet; group T₂ birds were provided with diet having MOS 1 g kg⁻¹ diet; group T₃ birds were fed diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹ and sodium butyrate 5 g kg⁻¹ of the diet; group T₄ birds having diet with sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of the diet; group T₅ birds having diet with ammonium formate at 1 g kg⁻¹, calcium propionate at 1 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of the diet. Basal diet was starter (0 to 3 weeks) and finisher (4 to 6 weeks). The diets (Table 1) of the experiment were formulated to meet or exceed the nutrient requirement as per Bureau of Indian Standard (BIS, 1992).

Housing and Management

Chicks were housed in a electrically heated battery brooder which were disinfected thoroughly three days prior to arrival of birds. The day-old chicks were offered electrolyte solution upon arrival. Birds were maintained on a 24 h constant light schedule. Body weight was recorded at the initial day

Table 1: Composition of basal diets for starter and finisher for quail (By parts)

Item	Starter	Finisher
Maize	45.505	50.000
Deoiled rice bran	2.000	6.000
Soyabean oil	1.000	1.000
Soyabean meal	41.000	33.000
Fish meal	7.600	7.000
Dicalcium phosphate	1.400	1.400
Lime stone powder	1.000	1.000
Trace minerals+	0.045	0.045
Daily mix++	0.025	0.025
Briplex+++	0.025	0.030
Choline chloride (60%)	0.100	0.100
DL-Methionine	0.100	0.100
L-lysine	-	0.100
Common salt	0.200	0.200
Nutrient composition		
ME (kcal kg ⁻¹) (Calculated)	2800.660	2805.000
CP (%) (Estimated)	27.050	23.950
Lysine (%) (Calculated)	1.616	1.480
Methionine (%) (Calculated)	0.562	0.527

+: Composition of trace minerals. Manganese Sulphate 15 g, Copper Sulphate 2 g, Ferrus Sulphate 6 g and Zinc Carbonate 6 g. ++: Composition of DAILYMIX. Vitamin A 82,500 IU, Vitamin B₂ 50 mg, Vitamin D₃ 12,000 IU, Vitamin K 10 mg, Vitamin B₁₂ 15 mcg. +++ Composition of BRIPLEX. Vitamin B₁ 8 mg, Vitamin B₂ 4 mg, Vitamin B₅ 16 mg, Vitamin B₁₂ 80 mcg, Niacin 120 mg, Calcium Pantothenate 80 mg, Folic acid 3600 mcg and Vitamin E 80 mg

followed by at weekly interval up to 6 weeks before offering feed. FCR, feed intake and live weight of birds were recorded at weekly intervals. PI for each group was calculated from total body weight gain and feed efficiency ratio.

Dietary Ingredients of Feeds

The dietary ingredients were in Table 1. Total protein was analyzed according to AOAC (1995).

Measurement of pH of Different Parts Gastrointestinal (GI) Tract

To determine the pH of gut of birds, 10 g of gut content from crop, proventriculus, gizzard, duodenum, jejunum and ileum were collected aseptically in 90 mL sterilized physiological saline (1: 10 dilution) (Al-Natour and Alshawabkeh, 2005) and pH was determined.

Measurement of Villus Height

For measurement of villus height, parts of small intestine were taken during slaughter. Then slides were prepared by sectioning and staining of tissues from parts of intestine. Heights of intestinal villus were measured by ocular micrometer under 10x objective of microscope. The reading was taken from ocular micrometer and the actual villus height was obtained by multiplying the ocular micrometer reading by conversion factor derived from stage micrometer (Lillie, 1965). The heights of intestinal villus were expressed as micrometer (μm).

Microbial Count in Intestinal Content

Two birds from each replicate of different group were electrically stunned and sacrificed at 6th week of age. Intestine including duodenum, jejunum, ileum were removed and ligated at both sides and placed into 50 mL tubes in normal saline and then kept at 4°C until used for intestinal sampling. Serial dilution of collected samples from different parts of intestinal contents were made upto fifth dilution with normal saline and different bacterial loads of content were enumerated by pour plate method (Quinn *et al.*, 1992).

Statistical Analysis

The analysis was done by using General Linear Model of SPSS programme (SPSS, 1997) with replicates as experiment units for studying the effect of organic acid on broiler performance and gut health. Levels of significance were calculated by Duncan Test (Duncan, 1995) whenever any effect was found significant.

RESULTS AND DISCUSSION

Effect on Performance

0-3 Weeks

The average body weight and cumulative feed intake (Table 2) of Japanese quail upto 3 weeks were not varied ($p>0.05$) due to dietary treatments but significant variation was found only in FCR and Performance Index (PI). Combination of acid salts with MOS (T_3) showed highest ($p<0.05$) PI over other groups. Group C_1 , T_3 and T_1 were also comparable with T_5 . Even group C_0 showed better performance index than MOS supplemented group. FCR was not affected ($p>0.05$) due to supplementation of OAS or in combination form of OAS and MOS when compared to control. However, supplementation of individual MOS in the diet (T_3) increased ($p<0.05$) FCR.

3-6 Weeks

It was observed that supplementation of only acidifier combination and acidifier combination with MOS showed better ($p<0.01$) body weight gain, FCR and PI (Table 2) compared to others. No difference ($p>0.05$) in body weight gain was seen in control (C_0) and MOS (T_2) supplemented group.

Table 2: Effect of feeding organic acid salts and MOS on the performance traits of Japanese quail†

Attributes	Treatment groups							SEM	p-value
	C ₀	C ₁	T ₁	T ₂	T ₃	T ₄	T ₅		
0-3 week									
Initial body weight (g/bird)	7.57	7.58	7.56	7.57	7.58	7.57	7.57	0.007	0.439
Live body weight (g/bird)	105.60	105.92	105.69	105.44	105.92	105.24	105.90	0.424	0.881
Cumulative feed intake (g/bird)	204.02	201.98	202.23	207.93	202.36	201.89	201.18	1.942	0.258
Feed conversion ratio (FCR)	2.08 ^b	2.05 ^b	2.06 ^b	2.12 ^a	2.06 ^b	2.07 ^b	2.05 ^b	0.013	0.007
Performance index (PI)	47.12 ^b	47.88 ^{ab}	47.61 ^{ab}	46.07 ^c	47.80 ^{ab}	47.26 ^b	48.07 ^a	0.237	0.000
3-6 week									
Body weight gain (g/bird)	90.56 ^d	101.76 ^{bc}	107.86 ^{ab}	97.73 ^{cd}	111.41 ^a	108.79 ^{ab}	113.41 ^a	3.032	0.000
Cumulative feed intake (g/bird)	487.09	482.84	500.13	474.39	481.32	509.36	485.11	7.988	0.077
Feed conversion ratio (FCR)	5.38 ^a	4.76 ^b	4.64 ^b	4.87 ^b	4.32 ^c	4.70 ^b	4.28 ^c	0.103	0.000
Performance index (PI)	16.85 ^c	21.49 ^b	23.26 ^{ab}	20.21 ^b	25.79 ^a	23.31 ^{ab}	26.52 ^a	1.103	0.000
0-6 week									
Live body weight (g/bird)	196.16 ^d	207.68 ^{bc}	213.54 ^{ab}	203.17 ^{cd}	217.33 ^{ab}	214.03 ^{ab}	219.32 ^a	3.289	0.001
Cumulative feed intake (g/bird)	691.11	684.82	702.36	682.32	683.68	711.25	686.29	8.793	0.207
Feed conversion ratio (FCR)	3.67 ^a	3.42 ^b	3.41 ^b	3.49 ^b	3.26 ^c	3.49 ^b	3.24 ^c	0.039	0.000
Performance index (PI)	51.47 ^d	58.49 ^c	60.41 ^{bc}	56.15 ^c	64.36 ^{ab}	59.98 ^{bc}	65.34 ^a	1.48	0.000

† Values bearing no common superscript in a row differs significantly ($p < 0.05$). C₀ birds supplied with basal diet; C₁, T₁ and T₂ provided with diet having BMD at 0.5 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of diet, respectively. T₃ birds were fed diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹ and sodium butyrate 5 g kg⁻¹, T₄ birds having diet with sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ and T₅ birds having diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of the diet

0-6 Weeks

Average live body weight (Table 2) after 6 weeks varied due to dietary treatments showing highest ($p < 0.05$) body weight in acidifier combination with MOS supplemented group followed by T₃ T₄, T₁ and C₀. Control group attained lowest ($p < 0.05$) body weight than all other treatment groups. FCR and PI were found better ($p < 0.01$) in acidifier combination and acidifier combination with MOS supplemented group compared to others. Individual MOS supplemented group showed significantly better FCR than control group (C₀).

In some of the previous studies it was found that organic acids as a substitute of AGPs improved the performance of birds even in absence of antibiotic (Mairoka *et al.*, 2004). Benedetto (2003) also observed mixture of organic acids can replace Antibiotic Growth Promoters (AGPs) and acid salts improved production performance along with other beneficial effects. Present study also showed that T₃ group (combination of acid salts) attained better performance in live weight gain, FCR and PI compared to others groups except T₅. Stanley *et al.* (2000) found that addition of 0.1% MOS in the Tom's diet significantly increase the body weight gain than negative control. Similarly Parks *et al.* (2001) reported from a study in turkey supplemented with MOS, utilized as alternative to AGPs to improve turkey performance. In this experiment MOS supplemented result, partially support the earlier studies. Live weight gain of Japanese quail in MOS supplemented group (T₃) after 6 week of age was comparable to antibiotic but no significance difference with control group (C₀). On the other hand FCR and PI of T₃ group had significant difference with control group. Data on live weight gain showed any marked difference with MOS in the diet of Japanese quail which complies with the report of Elangovan *et al.* (2005). Vander Wielen *et al.* (2000) reported birds previously fed butyrate can better withstand the stress of coccidial challenge. In the present study Japanese quail received butyrate showed better result in live weight gain, FCR and PI than control as well as consistent result with antibiotic. Butyrate + MOS and mix of acid salts + MOS gave good result in live weight gain. OASC + MOS attained highest body weight gain after 6 weeks period with best FCR and PI. Organic acids in un-dissociated form kill the pathogenic bacteria (Nursey, 1977) and on the other hand MOS being

Table 3: Effect of Organic acid salts and MOS on the gut microbial count of Japanese quail†

Attributes	Treatment groups							SEM	p-value
	C ₀	C ₁	T ₁	T ₂	T ₃	T ₄	T ₅		
Coliform ($\times 10^4$ c.f.u g ⁻¹ intestinal content)	10.75 ^a	8.25 ^b	8.63 ^{ab}	8.38 ^{ab}	6.75 ^{bc}	7.38 ^{bc}	5.50 ^c	0.785	0.005
<i>Escherichia coli</i> ($\times 10^3$ c.f.u g ⁻¹ intestinal content)	8.88 ^a	7.88 ^{ab}	8.13 ^{ab}	7.75 ^{ab}	6.50 ^{bc}	6.38 ^{bc}	5.38 ^c	0.564	0.004
<i>Clostridium perfringens</i> ($\times 10^4$ c.f.u g ⁻¹ intestinal content)	6.0 ^a	2.13 ^c	2.75 ^{bc}	3.38 ^b	2.38 ^{bc}	2.88 ^{bc}	2.50 ^{bc}	0.378	0.000

† Values bearing no common superscript in a row differs significantly ($p < 0.05$). C₀ birds supplied with basal diet, C₁, T₁ and T₂ provided with diet having BMD at 0.5 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of diet, respectively. T₃ birds were fed diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹ and sodium butyrate 5 g kg⁻¹, T₄ birds having diet with sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ and T₅ birds having diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of the diet

undigested in GI tract act as a ligand, bind with the type I fimbriae of pathogenic bacteria and thus increase the population of helpful bacteria in the gut. So it may be inferred that the synergistic effect of OASC with MOS resulted superior performance in Japanese quail.

Effect on Gut Microbial Count

The intestinal content of experimental Japanese quail was subjected to gut microbial count like Coliform, *Escherichia coli* (*E. coli*) and *Clostridium perfringens* (*Cl. perfringens*) were counted and presented in Table 3. It was seen that acidifier combination with MOS supplementation reduced ($p < 0.01$) coliform count followed by T₃, T₄, T₂, C₁, T₁ and C₀. The coliform count in C₁, T₂, T₃ and T₅ group showed significant difference ($p < 0.05$) with control (C₀) group. In case of *E. coli* the treatment group T₅ (acidifier combination + MOS) possessed lowest bacterial count followed by T₄, T₃, T₂, C₁, T₁ and C₀. AGP supplementation in diet resulted reduced ($p < 0.05$) *Cl. perfringens* in the intestine. However, dietary treatment with OASC and MOS resulted to reduced ($p < 0.05$) *Cl. perfringens* than control group (T₀). Here MOS and organic acid salts supplementation were comparable with AGP group (T₁). From the trend of the analysis it was indicated that MOS and organic acid salts supplementation groups had an influence to reduce coliforms, *E. coli* and *Clostridium* in the gut of experimental birds which was in accordance with the findings of Lon (1995), Spring *et al.* (2000), Fairchild *et al.* (2001) and Sims *et al.* (2004).

The reduced microorganism in the G.I. tract of Japanese quail with the treatment of MOS might be due to block type-I fimbriae which prevent pathogens from attaching to the intestinal lining and passes them out of gut (Dawson and Pirvulescu, 1999). On the other hand organic acids in their undissociated forms pass through the cell membrane of the bacteria and dissociate to form H⁺ ions which lower the pH of bacterial cell, causing the organism to use its energy, trying to restore the normal balance. Whereas R_{coo}⁻ anions produced from the acid can disrupts DNA, hampering protein synthesis and putting the organism in stress. As a result the organism can not multiply rapidly and decrease in number (Nurse, 1997).

Effect on Villus Height

The result showed that only OASC and OASC with MOS supplementation significantly improved villus height of duodenum, jejunum, ileum (Table 4) compared to other groups. However, the villus height of ileum did not significantly differ in acidifier combination and butyrate+ MOS supplementation group. Earlier workers Macari and Mairoka (2000) and Pelicano *et al.* (2005) reported higher villus height in small intestine with most organic acidifier and MOS in diet of broiler. The increase of villus height of different segment of small intestine may be attributed due to intestinal epithelium acts a natural barrier against pathogenic bacteria and toxic substances that are present in the

Table 4: Effect of organic acid salts and MOS on the villus height in different parts of intestine of Japanese quail†

Parameter	Treatment groups							SEM	p-value
	C ₀	C ₁	T ₁	T ₂	T ₃	T ₄	T ₅		
Duodenum (µm)	597.33 ^d	599.77 ^d	631.72 ^c	634.05 ^c	688.60 ^a	677.75 ^b	697.30 ^a	3.133	0.000
Jejunum (µm)	326.04 ^d	327.59 ^d	343.12 ^c	340.25 ^c	395.28 ^a	360.82 ^b	393.90 ^a	2.581	0.000
Ileum (µm)	280.16 ^d	278.46 ^d	296.03 ^c	298.11 ^c	317.37 ^b	317.26 ^b	325.37 ^a	1.833	0.000

† Values bearing no common superscript in a row differs significantly ($p < 0.05$). C₀ birds supplied with basal diet; C₁, T₁ and T₂ provided with diet having BMD at 0.5 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of diet, respectively. T₃ birds were fed diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹ and sodium butyrate 5 g kg⁻¹, T₄ birds having diet with sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ and T₅ birds having diet with ammonium formate at 1 g kg⁻¹, calcium propionate 1 g kg⁻¹, sodium butyrate at 5 g kg⁻¹ and MOS at 1 g kg⁻¹ of the diet

intestinal lumen. Organic acidifier may reduce the growth of many pathogenic or non-pathogenic intestinal bacteria, therefore, reduce intestinal colonization and infectious process, ultimately decrease inflammatory process at the intestinal mucosa resulting improved villus height and function of secretion, digestion and absorption of nutrients (Iji and Tivey, 1998; Pelicano *et al.*, 2005).

CONCLUSION

From the present study it may be inferred that the organic acid combination along with MOS have better performance compared to antibiotic supplementation in the diet. The improved villus height in duodenum, jejunum and ileum in the respective group also supports the finding. Thus, it may be concluded that organic acid combination along with MOS can be a potential tool as an alternative to antibiotic in the diet of Japanese Quail for its optimum production.

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