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Influence of feeding species specific probiotic in combination with prebiotic on serum lipid profile and carcass characteristics of broiler chicken

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Abstract

Lactobacillus salivarius, species specific probiotic (SSP) isolated from chicken gut was freeze dried and fed with combination of prebiotic - mannan oligosaccharides (MOS) was evaluated for serum lipid profile and abdominal fad pad thickness in broiler chicks. The basal diet was supplemented with species specific probiotic (SSP) and mannan oligosaccharides (MOS) at two lower and higher levels and was compared with control diet. The dose for probiotic and prebiotic was obtained by in vitro tests. The broiler feeding trial was conducted for five weeks. At the end of the feeding trial blood sample was collected for lipid profile analysis and carcass characteristics was also studied. On supplementation with SSP MOS significantly (P<0.05) reduced the total serum cholesterol, LDL cholesterol and triglycerides. Carcass and carcass parts yield percentage, abdominal fat pad and meat to bone ratio was not influenced by SSP MOS treatments. It is concluded that L.salivarius in combination with mannan oligosaccharide in ration reduced the serum cholesterol level in broilers.

Keywords: ***Lactobacillus salivarius, Mannan oligosaccharides, Broiler chicken, Lipid profile***

1. Introduction

Probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). Probiotics should be species specific for better performance, but the commercially available probiotic microflora is not species specfic. The pre-condition for probiotic microbe to favour animal's performance is colonization in the gut which is best attained if the organism being administered originates from the gut of same species (Gibson and Fuller, 2000). In view of consumer awareness and restriction or total ban on the use of antibiotic as growth promotant in poultry industry, probiotic has been introduced as an alternative. Prebiotics have been defined as non digestible feed ingredients, which are growth substrates, specifically directed towards potentially beneficial bacteria already existing in caecum and colon. Pelicano *et al.* (2004) have shown that addition of prebiotics to the diet of broiler, layer and pig leads to improved performance through improving gut microflora. Santoso *et al.* (1995) found that supplemented *B. subtilis* to broiler chicks did not significantly lower the serum total cholesterol.

In this perspective, a study was performed with identified species specific probiotic - *Lactobacillus salivarius* in combination with prebiotic - MOS on serum lipid profile and carcass characteristics in broiler chicken.

2. Materials and method

2.1 Experimental birds and diet

Probiotic organisms were isolated from chicken gut, and subjected to probiotic property assay. It was then assessed for required optimum dietary concentration and synchronized with commercially available prebiotic, whose optimum dietary concentration was also arrived through an *in vitro* test. The optimum dose of species specific probiotics that synchronises with prebiotics was assessed by *in vitro* test at different levels against *Escherichia coli*. Based on the antimicrobial activity against *E.coli* in a well diffusion assay, dose for feeding broiler chicken was fixed. (Gunasekaran *et al*. 2012) It was validated through an *in vivo* trial in broiler chicken with the ration as given in table 1.

Table 1: Percent ingredient composition of broiler chicken ration

Ingredients	Inclusion level (%)
Maize	58
Ground nut oil cake	9
Soyabean meal	17
Fish meal	8
Deoiled rice bran	6
Mineral mixture	2

The trial was conducted in Department of Animal Nutrition, Madras Veterinary College with one hundred and eight unsexed straight run day-old broiler chicks

(VENCOBB) obtained from a commercial hatchery. On day one, the chicks were wing banded, weighed and distributed randomly to the six experimental groups with three replicates of six chicks each. The experimental chicks were housed in five tiered, well-ventilated battery cages provided with artificial lighting. The management practices adopted were as per the standards and were uniform for all the treatments. The birds were vaccinated with the thermo stable live Newcastle disease vaccine developed at the Department of Veterinary Microbiology, Madras Veterinary College, Chennai using D 58 isolate of Newcastle disease vaccine. The vaccine was given to chicks at the age of 7^{th} day as nasal and ocular drops at a dose of $10^{6.5}$ EID $_{50}$ /0.1 ml.(EID-Embryo infective dose) The chicks were fed *ad libitum* quantity of their respective experimental rations in separate feed troughs. Clean drinking water was provided *ad libitum* throughout the day. The six experimental groups are given in table 2.

Table 2 Experimental groups involved in feeding broilers

Treatment	Dietary level of probiotics and prebiotics
T1	Basal diet +Without probiotics and p rebiotics
T2	Basal diet +20% less dose of probiotics ($2.0x10^9$ cfu/ kg)and prebiotics (2.4 g/kg) than *in vitro* results
T3	Basal diet +10% less dose of probiotics ($2.25x10^9$ cfu/ kg)and prebiotics (2.7 g/kg) than *in vitro* results
T4	Basal diet +Dose of probiotics ($2.5x10^9$ cfu/ kg)and prebiotics (3.0 g/kg) than *in vitro* results
T5	Basal diet +10% more dose of ($2.75x10^9$ cfu/ kg)and prebiotics (3.3 g/kg) than *in vitro* results
T6	Basal diet +20% more dose of probiotics ($3.0x10^9$ cfu/ kg)and prebiotics (3.6 g/kg) than *in vitro* results

2.2 Serum lipid profile

At the end of 35 days of feeding trial, blood samples were collected from the wing vein of five birds per treatment and were centrifuged at 1400 g for 10 minutes to separate serum. The serum was transferred into vials, labelled and stored at -20 º C until used. Serum samples were analysed for total cholesterol, HDL cholesterol (HDL-C) and serum triglyceride (TG) by enzymatic diagnostic kit (Agappe & Co., India) measured colorimetrically. The LDL cholesterol (LDL-C) was determined as,

LDL-C (mg/dl) = Total Cholesterol - [HDL - C + TG / 5]

For serum cholesterol, HDL cholesterol, serum triglycerides the method of Wybenga and Pileggi's, Enzymatic and GPO/POD enzymatic methods were followed respectively.

2.3 Slaughter studies

In order to correlate the effect of species specific probiotics and prebiotics on the carcass characteristics, all the live birds used were subjected to carcass composition. Birds were starved for 12 hours and were slaughtered by cervical dislocation then carcass standard characteristics were determined.

Abdominal fat pad thickness of five birds per treatment was examined in the birds. The abdominal fat pad from each bird was removed and weighed. The percentage of abdominal fat pad was calculated as that of Kalavathy *et.al.* (2003)

$$\frac{\text{Abdominal fat pad (g)}}{\text{Body weight (g)}} \times 100$$

Deboning was performed and the ratio between bone and meat was calculated as meat weight in grams, divided by bone weight in grams.

3. Results

3.1 Serum lipid profile

The effect of SSP MOS on serum total cholesterol, HDL, LDL and triglyceride in broiler chicken of 35 days of age are furnished in table 3.

The total cholesterol in the control birds had highest level significantly ($P<0.05$) than SSP and MOS supplemented birds. The lowest level ($P<0.05$) of total cholesterol was observed in T3, T1, T4, T5. This study shows a consistent reduction in serum cholesterol irrespective of the level of probiotic and prebiotic supplementation and comparable to the findings of Kalavathy *et.al.* (2003) and Jin *et. al.* (1998) for multiple probiotic.

Non significant difference were recorded for HDL values among the treatments experimented. Many workers reported non significant increase of serum HDL for single probiotic (De Rodas *et al.* 1996; De Smet *et al.* 1998), multiple probiotic (Kalavathy et al. 2003) and Prebiotic (Kannan *et .al.* 2005) supplemented birds. However, (Usman and Hosono, A., 1999) observed decrease in serum HDL in pigs fed with single probiotic. Increase in serum HDL rats fed with multiple probiotics was observed (Fukushima *et al.* 1995).

Table 3 Species specific probiotic and prebiotic - mannanoligosaccharide (MOS) on serum lipids such as total cholesterol, HDL, LDL and triglycerides

concentrations (mg/dl) of broiler chickens at 35 days of age (Mean *± SE)

SERUM LIPIDS (mg/dl)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Total Cholesterol	250.00^{c} ± 0.00	231.25^{b} ± 3.95	215.62^{a} ± 4.97	211.75^{a} ± 5.10	208.12^{a} ± 1.97	200.62^{a} ± 5.92
HDL ^{NS}	123.33 ± 12.01	105.00 ± 9.57	106.66 ± 6.66	106.66 ± 6.83	103.33 ± 3.33	106.66 ± 4.21
LDL	101.00^{c} ± 3.77	92.75^{b} ± 5.21	86.12^{ab} ± 11.52	81.75^{a} ± 5.16	80.12^{a} ± 4.54	79.62^{a} ± 4.40
Triglycerides	170.00^{b} ± 6.32	130.00^{a} ± 6.32	110.00^{a} ± 6.32	110.00^{a} ± 6.32	110.00^{a} ± 6.32	115.00^{a} ± 10.32

*Mean of six observations
Means bearing different superscripts in a row differ significantly (P<0.05) NS - non significant

Significant (P<0.05) reduction in LDL was observed with SSP MOS supplemented groups. This study shows a consistent reduction in serum LDL irrespective of the level of probiotic and prebiotic supplementation. The observation made in this study is agreeable to the findings for single probiotic (Usman and Hosono, A., 1999) and for multiple probiotic (Kalavathy *et. al.* 2003). However, did not observe much reduction when probiotic was supplements to broiler chicks (Kannan *et. al*. 2005).

There was a significant (P<0.05) reduction in triglycerides of broiler chicken of 1-35 days of age supplemented wth probiotic and prebiotic at various levels compared unsupplemented control birds. The per cent reduction in serum triglyceride among probiotic and prebiotic supplemented birds varied from 23.5 to 35.3 when compared to control birds. Similair reduction in serum triglycerides for probiotic (Santoso *et al.* 1995) and prebiotics (Kannan *et al*. 2005) supplemented broilers have been reported.

3.2 Slaughter studies

The yield as per cent of carcass weight was studied for thigh, wing, breast muscle, back muscle and drumstick are furnished in table 4.

A non significant difference was observed among the treatments and the control groups for carcass yield, carcass parts yield, abdominal fat pad and meat to bone ratio.

The highest carcass yield of 77.2% was observed in T2, T1, and T3. The per cent increase in carcass yield compared to control for T3, T2, T1, T4 and T5 were 4.0, 1.9, 2.96, 2.43, and 0.81 respectively. However, per cent carcass yield did not significantly (P>0.05) differ across the treatments. This shows that addition of probiotic had no effect on the carcass yield of broilers (Maiorka *et al.* 2001). But this results differ from that birds fed with *Enterococcus* (10^{6} cfu/ g) and MOS 0.85 g/g had higher carcass yield (P<0.05) compared to control (Pelicia *et al.* 2004).

Control birds had lower per cent of abdominal fat pad. The per cent abdominal fat pad in various SSPMOS treatments ranged from 1.33 to 1.62. The per cent abdominal fat pad among the treatments did not significantly (P > 0.05) differ from each other. Pelicia *et al.* (2004) observed that combination of probiotic and prebiotic supplementation to broilers had no effect on abdominal fat pad.

Contrary to this, Kannan *et al.* (2005) observed decrease in abdominal fat pad in MOS supplemented broilers. But it differs from (Santoso *et al.* 1995) reported that a reduction in abdominal fat content in female broilers supplemented with *B.subtilis* at 45 days of age. Also, Hood (1984) suggested *B.subtilis* decreased the activity the acetyl Co A carboxylase which is the rate limiting enzyme in fatty acid synthesis.

The carcass parts yield as per cent of carcass weight was studied for thigh, wing, breast muscle, back muscle and drumstick are furnished (Table 4). Per cent thigh yield in control bird was 15.4 and its per cent yield indifferent levels of SSP MOS varied from 13.7 to17.1. There was no significant difference (P>0.05) among all the treatment with regard to per cent yield of thigh. The highest wing yield was noted in T1 (1.13%) and lowest wing yield in SSP MOS+20. However, the per cent wing yield was comparable among the treatments. A higher per cent of the breast muscle in SSP MOS +10 and lower per cent of breast muscle in SSP MOS-10 was recorded in this study. The per cent breast muscle yield did not significantly vary (P>0.05) between control and SSP MOS treatments. Similarly, there was no difference (P>0.05) in per cent back muscle in control birds (22.2%) T1 birds wherein it varied from 23.5% to 24.3%. The per cent drumstick yield was higher in control (16%) than SSP MOS (12.9% to 14.8%) which did not differ significantly (P>0.05) among the treatments. The highest meat to bone ratio was noted in T4 (4.31%) the lowest value in T3 and T1 (3.78%). However, none of the treatments differed significant (P>0.05) from each other with regard to the meat to bone ratio.

Table 4 Species specific probiotic (SSP) and prebiotics - mannanoligosaccharide (MOS) on carcass (%), abdominal fat pad (%) and carcass parts yield (%), meat to bone ratio of broiler chickens at 35 days of age (Mean *± SE)

CARCASS VARIABLES *	T1	T2	T3	T4	T5	T6
Carcass yield (%)	74.20 ±1.24	77.20 ±1.31	75.6 ±5.00	76.4 ±8.71	76.0 ±7.07	74.80 ±1.15
Abdominal fat pad (%)	0.97 ±0.22	1.62 ±0.35	1.62 ±0.28	1.33 ±0.33	1.47 ±0.28	1.45 ±0.30
Meat to Bone ratio	4.00 ±0.09	3.73 ±0.22	3.79 ±0.13	3.73 ±0.13	4.31 ±0.21	4.02 ±0.25
1. Carcass parts yield						
Thigh (%)	15.40 ±0.22	13.90 ±0 .12	16.00 ±0.53	17.10 ±0.88	15.20 ±0.52	13.70 ±0.46
Wing (%)	0.86 ±0.11	1.03 ±0.12	1.07 ±0.20	1.13 ±0 .65	1.01 ±0.33	0.84 ±0.43
Breast muscle (%)	23.80 ±0.31	22.50 ±0.16	26.40 ±0.47	26.50 ±0.15	26.60 ±0.52	25.90 ±0.14
Back muscle (%)	22.20 ±0.26	24.10 ±0.19	23.50 ±0.07	23.90 ±0.12	24.30 ± 0.23	23.90 ±0.77
Drumstick (%)	16.00 ±0.21	13.30 ±0.84	14.80 ± 0.54	14.80 ±0.13	13.20 ±0.47	12.90 ±0.59

*NS - non significant Mean of six observations
Carcass yield and abdominal fat were expressed as (%) of the live weight
Cut up parts were expressed as (%) of the carcass weight.

Maiorka *et al.* (2001) noted that probiotic fed to birds had no effect on breast muscle yield as reported in the study. In line with this finding, Pelicia et al. (2004) also observed that no effect on carcass and breast yield of broilers fed with probiotic diet. Takahashi *et al.* (2005) did not observed significant difference in carcass yield of control (67.88%) and prebiotic and probiotic treated birds (68.38%) and back yield of control (24.16%) and probiotic and prebiotic treated birds (25.05%).

4 DISCUSSION

4.1 Serum lipid profile

The results from this study indicated that *Lactobacillus* species and mannanoligosaccharides have a hypocholesterolemic effect on the host. *Lactobacilli* species are able to incorporate cholesterol into the cellular membrane of the organism and hence less cholesterol will be absorbed from the intestine into blood, Gilliland et al (1985). The hypocholesteroleamic effect may also be due to the ability of *Lactobacilli* to produce bile salt hydrolase (EC 3.5.1.2.4), the enzyme responsible for bile salt deconjugation (Klaver *et al.* 1993). Deconjugated bile salts are less soluble at low pH and poorly absorbed in the intestine and are more likely to get excreted in the faeces (Klaver *et al.* 1993).In order to maintain the homeostasis, more bile needs to be synthesized and this in turn will reduce the cholesterol in the body pool as cholesterol is the precursor for bile acids.

Probiotics are able to inhibit hydroxy methyl glutaryl co enzyme A (HMGCoA), an enzyme involved in the cholesterol synthesizing pathway. Kiriyama *et al.* (1974) reported that mannan can induce hypocholesterolemia. The hypocholesterolemia activity of the mannan oligosaccharide is proportional to the molecular weight of its constituent glucomannans and its viscosity Ebihara *et al.* (1981). MOS probably decreases intestinal absorption of bile salts by interfering with their actives transport mechanism.

4.2 Slaughter studies

The non significant difference observed among the treatment groups, indicate that the additional nutrients generated through SSP MOS were evenly distributed to target organs.

Statistical analysis

The data collected were subjected to analysis of variance procedure of Statistical Analytical System (SPSS version 10.0 for Windows). When significant difference ($P<0.05$) were noticed the Duncan multiple range test was used to separate its mean values.

Conclusion

Broiler chicken supplemented with *L.salivarius* at the concentration of 2.75 x 10^9 cfu/kg and mannan oligosaccharides at the concentration of 3.3 g/kg reduces serum total cholesterol, LDL and triglycerides in broiler chicken of 35 days of age.

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