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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Influence of Prebiotics Supplementation on Lipid Profile of Broilers

M. Kannan, R. Karunakaran, V. Balakrishnan and T.G. Prabhakar
Department of Animal Nutrition, Madras Veterinary College,
Tamilnadu Veterinary and Animal Sciences University, Vepery, Chennai- 600 007, India

Abstract: The prebiotic - Mannanoligosaccharide (MOS) was extracted from yeast and copra meal and evaluated for their prebiotic potentiality on serum lipid profile and abdominal fat pad. The treatment groups were fed with basal diet (T_1). The basal diet was supplemented with extracted MOS from yeast [@ 0.5 g/kg (T_2); 1 g/kg (T_3)] and from copra meal [@ 1 g/kg (T_4); 1.5 g/kg (T_5)] and with MOS sources such as yeast [MOS equivalent of 0.5 g/kg (T_6); 1 g/kg (T_7)] and copra meal [MOS equivalent of 1 g/kg (T_8); 1.5 g/kg (T_9)]. A broiler trial was conducted for a period of five week. The results revealed that serum total cholesterol concentration was significantly ($P<0.01$) lowered by 10, 12 and 12 per cent in birds fed with T_2, T_6 and T_7 diet respectively when compared with control diet in broiler chicken at 5th week of age. There was no significant difference in serum HDL cholesterol of birds fed with extracted MOS / MOS equivalent source except T_6 which was found significantly ($P<0.05$) lower than control groups. LDL cholesterol level was similar in all treatment groups except higher value observed in T_5(91.69±13.54mg/dl). The abdominal fat pad (percentage of live weight) was found to be significantly ($P<0.01$) lowest in T_3. This study indicates that the supplementation of prebiotic extracted from yeast at 0.5 and 1g/kg and copra meal at 1.5g/kg and yeast source at 1g/kg level respectively reduced the abdominal fat content.

Key words: Prebiotics, broiler chicken, serum lipid profile, abdominal fat pad

Introduction

In the recent past, 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. However, it has some constraints like lack of viability, stability and inability to be established in intestinal eco-system due to the barriers like gastric acid, bile acid, during its transit etc., Alternatively, prebiotics have been suggested to acquire all probiotic mediated beneficial effects while overcoming all its constraints.

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. Several studies have shown that addition of prebiotics to the diet of broiler, layer and pig leads to improved performance through improving gut microflora (Xu *et al*., 2003; Spring *et al*., 2000 and Pelicano *et al*., 2004).

Research on poultry genetics, feeding and management for body weight gain, feed conversion ratio, etc. resulted in fast growth but decreased the quality of poultry products as modern fast growing broilers have been found to contain higher amount of abdominal fat (Chambers *et al*., 1981).

Most recently, considerable attention has been paid to test the potency of growth promotants on altering lipid metabolism, because, World Health Organization suggested that excess fat deposition is undesirable in human body which ended in fatal diseases like atherosclerosis. Now-a-days, consumers are also well aware of this fact and prefer lean meat. On the other way, excess fat is an economic burden to poultry producers, because fat is lost during processing of the carcass resulting in lower meat yields and further more the discarded abdominal fat and visceral fat increases waste management problems.

Several growth promotants have been tried in our laboratory. (Mohan *et al*., 1995; Mohan *et al*., 1996). Probiotic supplementation has been shown to reduce the cholesterol concentration in egg yolk (Mohan *et al*., 1995; Abdulrahim *et al*., 1996; Haddadin *et al*., 1996) and serum in chicken (Mohan *et al*., 1996; Jin *et al*., 1998). Recent report suggested that feeding of chicory beta fructans – an oligosaccharide, a prebiotic, reduced the serum cholesterol and abdominal fat of broiler chicken (Yusrizal and Chen, 2003). However, the effect of prebiotic is scanty, hence the present study was undertaken to study the effect of Mannanoligosaccharide (MOS), a prebiotic, extracted from various sources (yeast and copra meal) on abdominal fat, serum total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipo protein (LDL) cholesterol and triglyceride levels in broiler chicken with the objective of optimizing

Corresponding Author: Dr. M. Kannan, Department of Animal Nutrition, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Vepery, Chennai- 600 007, India.
Tel : 91-98411 10902.

Table 1: Percent ingredient composition of broiler ration

	T_1 [a]	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
Maize	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Broken rice	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Wheat bran	18.40	18.40	18.40	18.40	18.40	18.40	18.40	18.40	18.40
Casein	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
Fish meal	8.80	8.80	8.80	8.80	8.80	8.05	7.65	8.80	8.80
Test Mix[b]	1.80	1.80	1.80	1.80	1.80	—	—	0.60	—
DCP	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Yeast	—	—	—	—	—	0.75	1.15	—	—
Copra meal	—	—	—	—	—	—	—	1.20	1.80
CALCULATED:									
CP (%)	22.27	22.27	22.27	22.27	22.27	22.27	22.27	22.27	22.27
ME (Kcal/kg)	2912	2912	2912	2912	2912	2912	2912	2907	2905
MOS (g/kg) supplement	—	0.50 (yeast MOS)	1.00 (yeast MOS)	1.00 (copra MOS)	1.50 (copra MOS)	—	—	—	—

[a]The control diet was formulated in such a way that it contains negligible level of Mannan. The feed ingredients are selected in order to have a negligible level of Mannan as maize and broken rice have a maximal level of 0.0842 and 0.0606 per cent.

[b]A test mix was formulated with wheatbran and fish meal in order to arrive with 35 per cent crude protein level, so as to equally replace the copra meal with this mixture. Composition: Fish meal: 71.40 per cent and Wheat bran: 28.60 per cent.

Mineral Mixture: Vitamin – Mineral premix (200g/Quintal of feed)

1. Trace mineral mixture (45 g/Quintal)	Ferrous sulphate	2.00 g
	$CuSO_4$	1.90 g
	$ZnSO_4$	6.00 g
	$MnSO_4$	15.00 g
	KI	100 mg
2. Vitamin AB_2D_3K (10g/Quintal) each g contains	Vitamin A	82500 IU
	Vitamin B_2	50 mg
	Vitamin D_3	120000 IU
	Vitamin K	10 mg
3. Vitamin B Complex and E (25 g) each g contains	Vitamin B_1	4 ng
	Vitamin B_6	8 ng
	Vitamin B_{12}	12 µg
	Vitamin E	40 IU
	Niacin	60 ng
4. Choline chloride – 50 g		
5. Maize powder as carrier - qs		

the level of prebiotics supplementation on reducing the abdominal fat pad content.

Materials and Methods

Birds and diet: Ninety (day old), straight run, hatchery vaccinated (Mareks disease vaccine) chicks (COBB) were wing banded and weighed. They were randomly distributed on nine treatment groups with 5 triplicates per groups and each replicates had 2 birds. Birds were assigned to forty five numbers of 40x30x35 cm cages with 2 birds per cage. The different treatment groups were fed with basal diet (T_1) and each of them supplemented with extracted MOS from yeast [@ 0.5 g/kg (T_2); 1 g/kg (T_3)] and from copra meal [@ 1 g/kg (T_4); 1.5 g/kg (T_5)] or supplemented with MOS sources such as yeast [MOS equivalent of 0.5 g/kg (T_6); 1 g/kg (T_7)] and copra meal [MOS equivalent of 1 g/kg (T_8); 1.5 g/kg (T_9)]. (Table 1)

Housing and management: Birds were housed in well ventilated with electricity illuminated asbestos roofed house. They were maintained under uniform managemental condition and vaccinated as per recommended standard programme. Chicks had free access to antibiotic free respective feed (Table 1) and potable, whole some drinking water during the 5 week grow out period.

MOS extraction: MOS was extracted from autolyzed yeast by separating cell wall and cell contents and freeze dried. Similarly, from copra meal, MOS was extracted by sequential removal of fat, soluble carbohydrate, lignin and protein as per Saittagaroon *et al.* (1983).

Sample collection: A day prior to slaughter, blood samples were collected randomly from 5 birds in each treatment. At the end of the experiment (5^{th} week) 5 birds from each treatment, one bird from each replicate were weighed individually and transferred to a processing plant and were slaughtered, bled, scalded by hot water and mechanically defeathered. Then the dressed carcasses were measured for abdominal fat. After obtaining the measurement, carcass were eviscerated and the head, neck and feet removed for ready-to-cook

Table 2: The effect of different dietary levels of MOS on per cent abdominal fat pad, total cholesterol, triglycerides, HDL and LDL cholesterol of broiler chicken at 5 week of age (Mean ± SE)

Treatment	T_1	T_2	T_3	T_4
Abdominal fat pad				
As (%) carcass weight **	3.18^{a}±0.13	2.54^{bcd}±0.09	2.02^{a}±0.06	2.29^{de}±0.09
As (%) live weight **	2.28^{a}±0.09	1.81^{cd}±0.06	1.45^{e}±0.01	1.62^{de}±0.04
Total cholesterol (mg/dl)**	196.77^{a}±2.13	178.90^{bc}±3.42	188.91^{ab}±3.33	183.57^{abc}±3.00
Triglycerides (mg/dl) ^{NS}	82.52±4.55	69.87±6.74	61.44±4.67	59.03±5.43
HDL(mg/dl) *	111.31^{a}±3.90	93.15^{bc}±2.89	88.39^{c}±3.24	87.20^{c}±1.49
LDL(mg/dl) *	68.97^{abc}±3.45	71.79^{abc}±4.89	88.24^{ab}±5.49	84.56^{ab}±1.70

Treatment	T_5	T_6	T_7	T_8	T_9
Abdominal fat pad					
As (%) carcass weight **	2.47^{cde}±0.21	2.92^{abc}±0.26	2.66^{bcd}±0.19	2.88^{abc}±0.12	3.03^{ab}±0.10
As (%) live weight **	1.76^{cd}±0.13	2.02^{bc}±0.14	1.93^{bc}±0.14	2.14^{ab}±0.09	2.22^{ab}±0.09
Total cholesterol (mg/dl)**	183.51^{abc}±7.92	173.92^{c}±5.36	173.21^{c}±1.47	195.71^{a}±3.78	188.55^{ab}±4.95
Triglycerides (mg/dl) ^{NS}	61.44±7.90	60.84±6.48	68.07±7.12	60.24±6.37	65.06±9.74
HDL(mg/dl) *	91.37^{bc}±4.38	86.90^{c}±1.29	100.59^{abc}±5.64	99.20^{abc}±9.63	106.14^{ab}±11.44
LDL(mg/dl) *	91.69^{a} ± 13.54	74.86^{abc}±3.95	59.01^{c} ± 5.38	84.46^{ab}±7.69	68.19^{bc} ± 10.71

Mean of five observations. $^{abc\,def}$Means bearing different superscripts in a row differ significantly. * P<0.05; ** P<0.01; ^{NS} Non significant

(RTC)/dressed carcasses weight determination. Abdominal fat percentage calculated as follows:

Abdominal fat per cent of live weight = [Weight of abdominal fat (g) / Live weight (g)] X 100

Abdominal fat per cent of dressed weight = [Weight of abdominal fat (g) / Dressed weight (g)] X 100

Serum cholesterol assay: Blood samples were centrifuged at 2000 xg for 10 minutes and the serum was decanted into aseptically treated vials and stored at –20°C until further analysis. Serum samples were analyzed for total cholesterol, HDL cholesterol and triglycerides by enzymatic diagnostic kits (AGAPPE diagnostic kits). The LDL cholesterol calculated by difference between total cholesterol and HDL cholesterol.

Statistical analysis: The design for this experiment was completely randomized design (CRD) with five replications. Data were analyzed with analysis of variance (ANOVA) procedure of statistical analysis system (SAS/SPSS version 10.0 for Windows). When significant difference (P<0.05) were detected the multiple range test was used to separate the mean value.

Results

Abdominal fat pad content: The effect of different dietary levels of MOS on dressing percentage and abdominal fat percentage are presented in Table 2. The abdominal fat pad, (percentage live weight) was found to be significantly (P<0.01) lowest in T_3 (1.45 ± 0.01 per cent). With increased level of MOS extracted from yeast had significantly (P<0.01) lower abdominal fat pad percentage as expressed as carcass weight. However, the similar trend was not observed in MOS extracted from copra meal.

Serum lipid profile: The effect of MOS on total cholesterol, HDL and LDL cholesterol and triglyceride level is shown in Table 2.

Serum total cholesterol concentration were significantly lower (P<0.01) by 10, 12 and 12 per cent in broiler fed with T_2, T_6 and T_7 treatment diet when compare with control diet in broiler chicken at 5th of age, Adding Prebiotic from Yeast (0.5 and 1g/kg) and copra meal (1 and !.5g/kg) significantly (P<0.01) reduced the serum cholesterol. There was no significant difference in serum HDL cholesterol of birds fed with extracted MOS / MOS equivalent source except T6 which was found significantly (P<0.05) lower than control groups. The variable results were observed with regards to LDL cholesterol. It was similar in all treatment groups except higher value observed in T5 and T7. In contrary, Kalavathy *et al.* (2003), who found that probiotic (*Lactobacillus spp.*) supplementation increase serum HDL cholesterol, but serum LDL cholesterol was less. Serum triglycerides were also lower (P<0.05) in MOS extracted / MOS source fed broiler than in the control broiler at 5th week of age (Table 3).

Discussion

Abdominal fat pad content: The observation obviously indicates that abdominal fat pad per cent might be reduced due to increasing beneficial bacteria as result of supplementation with the prebiotics. In recent study, Kalavathy *et al.* (2003) found that supplementation of *Lactobacillus* culture reduces (P<0.05) abdominal fat pad. Similarly, Yusrizal and Chan (2003) reported that supplementation of beta fructans from chicory had produced low level (P<0.05) of abdominal fat pad. Santoso *et al.* (1995) also found that abdominal fat contents were reduced in female broilers supplemented with *B. subtilis* at 42 days of age and that *B.subtilis* culture decreased the activity of acetyl-CoA carboxylase. Acetyl-CoA carboxylase has been widely suggested as the rate limiting enzyme in fatty acids synthesis.

Serum lipid profile: The decrease in cholesterol level could be due to the cholesterol assimilation by *Lactobacillus*, (Gilliland *et al*., 1985) as the Prebiotic supplementation could have enhanced the Lactobacilli count. Similar results have been reported by Mohan *et al.* (1996) and Kalavathy *et al*. (2003) and a similar hypocholesterolaemic effect was observed in broiler chicken supplemented with beta fructans from chicory as a source of Prebiotic (Yusrizal and Chan, 2003).

The mechanism(s) involved in the overall hypocholesterogenic effect of MOS supplementation is not fully documented. However, MOS is considered as substrate for lactic acid producing bacteria like *Lactobacillus spp*. and *Bifidobacterium bifidum* (Van Loo, 2004). Increasing level of MOS also increase the CFU of this lactic acid producing bacteria (Xu *et al*., 2003). Gilliland *et al*. (1985) hypothesized that some *Lactobacillus spp*. are able to incorporate cholesterol into the cellular membrane of the organism, thus, cholesterol assimilation by *Lactobacillus* in turn reduce cholesterol absorption in the system.

The lower level of serum triglyceride might be due to increased level of lactic acid producing bacteria in the gut of broiler chicken. The results of Santoso *et al.* (1995) reported that supplementation of *B.Subtilis* in broiler diets decreased triglycerides in the serum.

Conclusion: The serum cholesterol concentration was significantly ($P<0.01$) reduced in prebiotic extracted from Yeast and Copra Meal as well in Yeast source, no such reduction noticed in Copra Meal fed birds. Likewise the percentage reduction in abdominal fat pad content was lowest among the group supplemented with extracted yeast

This study indicates that the supplementation of prebiotic extracted from Yeast at 0.5 and 1g/kg Copra Meal at 1 and 1.5/kg and the Yeast source at 1g/kg level helps in reduction of the Abdominal fat pad content.

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