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Evaluation of mannanoligosaccharide as prebiotic functional food for dogs: effect on nutrient digestibility, hind gut health and plasma metabolic profile

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ABSTRACT

The present study was designed, using 5 adult dogs in complete crossover design, to assess the effect of dietary supplementation of mannanoligosaccharide (MOS) on nutrient digestibility, hind gut health indices and plasma metabolic profile. Accordingly, the dogs were fed on a homemade diet alone or that supplemented with MOS (at 1% level). A digestion trial, conducted at the end of each period, revealed that the intake of feed DM and other nutrients increased (P<0.05) when supplemented with MOS. Digestibility of fibre was improved (P<0.05) in MOS supplemented group, while that of other nutrients were not affected. The concentration of lactate and short-chain fatty acids (SCFA) in faeces, viz. propionate and butyrate showed increasing trend which resulted in higher faecal concentration of total SCFAs due to MOS supplementation. Addition of MOS tended to reduce faecal coliforms with an associated elevation (P<0.05) in lactobacilli count compared to control. The plasma triglyceride was reduced while that of inorganic phosphorous increased in dogs supplemented with MOS. Overall, supplementation of MOS @ 1% of diet DM positively influenced feed intake, fiber digestibility and indices of hindgut health with subtle influence on certain blood biochemical parameters.

Key words: Digestibility, Dogs, Functional food, Metabolic profile

Nutritionally equitable diet and a balanced microbial ecology are required for a well functioning healthy GI tract. The dietary use of prebiotics as functional food has gained significance as a strategy to manipulate the intestinal microflora in a beneficial manner, improving intestinal metabolism and health (Farnworth *et al.* 1992). Functional food contains one or a combination of components in adequate concentration which affects functions in the body so as to have positive cellular or physiological effects (Roberfroid 1993). Mannanoligosaccharides (MOS), a prebiotic derived from cell wall of yeast *Saccharomyces cerevisiae* (Spring *et al.* 2000) proved as a potential functional food supplement in various species, including dogs (Pawar *et al.* 2008).

Although prebiotics are non-digestible by digestive enzymes in GI tract, they are easily fermented in the lower gut to stimulate selective growth of probiotic like bacteria such as *Lactobacillus* and *Bifidobacteria* (Montesi *et al.* 2005), and suppress the growth of pathogens (Flickinger *et al.* 2003), thus improving the nutritive value of food through increased digestibility and utilization of nutrients. Prebiotics are also demonstrated to exert potential positive effect on metabolic profile by helping to reduce hyperlipidemia (Delzenne and Kok 2001) and to enhance mineral absorption (Delzenne *et al.* 1995). With more than 90% pet dogs being reared on home cooked diets (Pattanaik and Sharma 2006, Shakhar *et al.* 2011) as compared to processed diets in western countries, the response to prebiotics may vary with source and type of basal diet (Swanson *et al.* 2002). In this perspective, the present study was aimed at ascertaining the effect of supplementation of prebiotic MOS as a functional food on nutrient digestibility, hind gut health and metabolic profile of dogs.

MATERIALS AND METHODS

Animals and experimental protocol: The study was conducted by using 5 adult spitz dogs (~16 months, 6.60±0.21 kg BW) in two periods in a complete crossover design. Each period consisted of a 10-days preliminary feeding period followed by digestion trial of 4 days. The dietary treatments consisted of feeding the basal diet alone (control) or that

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supplemented with prebiotics mannanoligosaccharide at 1% level (MOS). The basal diet was pressure cooked at 15 psi for 20 minutes and thoroughly mixed with mineral-vitamin premix and whole milk (250 g/head/d) before feeding to the animals. The nutrient requirements of dogs were calculated as per AAFCO (2003) recommendations. The acceptability of the experimental diets was recorded by assessment of 1–4 point palatability score as detailed elsewhere (Shakhar *et al*. 2007).

Sample collection and handling

Faecal consistency score (1–5 point scale) was recorded every day throughout the experimental period adopting standard protocol (Strickling *et al*. 2000). On day 11 of each period, feed and faeces collection was started ending on day 15. All faeces composited during preceeding 24 h were individually weighed for each dog to know the faecal excretion. Pooled fresh faecal samples of individual dog were mixed thoroughly before recording the pH with digital pH meter and subsequent aliquoting. Three different aliquots were taken each for estimation of nitrogen (2–3 g preserved in 1: 4 H_2SO_4), lactate (2g diluted with equal volume of distilled water, mixed, centrifuged at 1000 rpm, and supernatant recovered and stored) and short chain fatty acids (SCFAs; 1 g of fresh faeces was mixed with 1 ml of 25% metaphosphoric acid and centrifuged at 10,000 rpm; the resulting supernatant was stored at –20° till further analysis). Rest of the faecal samples was used for determination of DM.

For faecal microbial enumeration, about 5g fresh faeces was collected from rectum through digital manipulation at the end of each period, and brought to the laboratory under aseptic condition for further analysis. Blood samples (5 ml) were collected via cephalic vein into heparinized vials, centrifuged (3000 rpm for 10 min), plasma recovered, and frozen (–20°) for further analysis to assess the clinical chemistry indices.

Chemical and statistical analysis

Samples of food offered, ort and faeces were dried at 70°C in a forced draft oven and ground through 2-mm screen in a Wiley mill and analyzed for proximate principles as per AOAC (1995). Nitrogen/crude protein, ether extract, crude fibre and ash were determined by methods 954.01, 920.39, 962.09, and 942.05 respectively (AOAC 1995), . The nitrogen-free extract (NFE) was determined as the difference between 100 and the sum of CP, EE, crude fibre and ash (all expressed as % DM basis). The calcium of the samples was estimated titrimetrically (Talapatra *et al.* 1940). Phosphorus of the feed and faecal samples was determined spectrophotometrically adopting the metavanadate method (AOAC 1995). Faecal lactate (Baker and Summerson 1941), ammonia (Chaney and Marbach 1962) and SCFAs (Barnett and Reid 1957, by using gas liquid chromatography) were determined by adopting standard protocols.

Metabolic profile via, haemoglobin and haematocrit of the whole blood was determined as per Dacie and Lewis (1968). The plasma concentrations of glucose, triglycerides, plasma proteins, urea, creatinine, alkaline phosphatase (ALP), alanine aminotransferases (ALT) was estimated using commercial diagnostic kits.

Faecal bacterial populations were enumerated by 3 sets of serial 10-fold dilutions (10^{-1} to 10^{-7}) with the total volume of 10 ml including 1 g faeces and 9 ml normal saline (0.9% NaCl). Collected faecal samples were inoculated onto sterile petri dishes containing Rogosa agar medium (Rogosa *et al.* 1951) for lactobacilli, and on Mac Conkey agar medium (Wilson and miles 1964) for coliforms enumeration. After incubation at 37°C for 48 h, bacterial colonies were counted and expressed as colony forming units (cfu)/per gram of faeces.

The experimental data were analyzed using statistical package (SPSS 11.0) adopting standard statistical procedures (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Intake and digestibility of nutrients

The chemical composition of the experimental diets is presented in Table 1. The nutrient composition of the basal diet was in conformity with the stipulated requirements for adult dogs. The mean daily DM intake showed an improvement ($P<0.05$) upon MOS supplementation compared to control; consequently, intake of OM, protein, fat and fibre also enhanced significantly ($P<0.01$) in MOS supplemented group (Table 2). Similar to the present findings, dietary supplementation with MOS alone or along with chicory have been reported to increase food intake in senior

Table 1. Ingredient and chemical composition (% DM basis) of the experimental diet

Ingredients composition		Chemical composition	
Attribute	Quantity	Attribute	Quantity
Pearl millet	72.0	Crude protein	20.60
Extruded soya meal	23.0	Ether extract	10.50
Soya oil	2.0	Crude fibre	2.55
Salt	0.4	Total ash	4.66
Dicalcium phosphate	1.3	Gross energy (MJ/kg)	1.97
Calcium carbonate	1.0	Metabolizable energy	1.63
Mineral premix[†]	0.12	(MJ/kg)[#]	
Vitamin premix[‡]	0.10		

[†]Supplied per kilogram of diet: Mn 14.2 mg; Fe 110 mg; Cu 9 mg; Co 1.8 mg; Zn 150 mg; I 1.6 mg; Se 0.3 mg.[‡]Provided per kilogram of diet: vitamin A 11000 IU; vitamin D 910 IU; vitamin E 57.5 IU; vitamin K 0.65 mg; thiamin 7.56 mg; riboflavin 11.89 mg; pantothenic acid 18.50 mg; niacin 93.16 mg; pyridoxine 6.60 mg; biotin 12.42 mg; folic acid 1,142.10 mg; vitamin B_{12} 164.87 mg.[#] Calculated.

Table 2. Intake and digestibility of nutrients by the experimental dogs fed diet with or without MOS

Attribute	Control	MOS
Palatability score†	1.16	1.22
Intake (g/d) of nutrients		
Dry matter	152.46±1.72[a]	169.86±0.00[b]
Organic matter	146.14±1.65[a]	161.07±0.00[b]
Crude protein	31.45±0.36[a]	34.94±0.00[b]
Ether extract	15.95±0.18[a]	17.93±0.00[b]
Crude fiber	3.61±0.041[a]	4.65±0.00[b]
Nitrogen free extract	95.13±1.07[a]	103.55±0.00[b]
Digestibility (%) of nutrients		
Dry matter	81.75±0.45	80.91±1.87
Organic matter	83.27±0.46	82.22±1.72
Crude protein	79.50±0.44	79.22±1.62
Ether extract	77.51±1.56	81.43±1.64
Crude fiber	31.83±2.24[a]	43.92±4.39[b]
Nitrogen-free extract	82.85±0.93	79.95±2.10

†Based on a 1–4 point scale;[ab]Means bearing different superscripts in a row differ significantly *P<0.05.

dogs (Grieshop *et al.* 2004). On the contrary, no significant influence on intake of DM was observed by Flickinger *et al.* (2003) when diet of dogs was supplemented with oligofructose, another prebiotic. It could be substantiated form the palatability score that MOS supplementation helped boosting the acceptability of the diet, thereby inducing greater food consumption which is in agreement with similar observations in dogs (Swanson *et al.* 2002).

Although, the intake of various nutrients was improved, the digestibility of DM, OM, CP and NFE did not vary between the 2 groups. No improvement in digestibility of DM, OM and CP in dogs supplemented with fructo-oligosaccharides (FOS) or MOS was reported (Swanson *et al.* 2002, Strickling *et al.* 2000 and Flickinger *et al.* 2003). Unlike DM or CP, the digestibility of EE tended (P, 0.121) to increase in MOS diet and, that of fibre improved significantly (P<0.05) due to MOS supplementation. The marked improvement in the digestibility of fibre could possibly be attributable to greater fermentability of the dietary fibre in the hind gut. The results were in line with Pawar *et al.* (2008) who observed improved fibre digestibility in dogs supplemented with MOS in nutritionally balanced homemade diet.

Hindgut health indices

Assessment of faecal characteristics can be used as an indicator of overall health status of the hindgut. In the present study, although the mean faecal output and DM was slightly higher in MOS supplemented group, there was no significant (P>0.05) difference between the 2 groups in terms of faecal score, wet faeces voided and pH (Table 3). Similar to the present findings, Swanson *et al.* (2002) have concluded that supplementation of MOS or FOS did not affect the faecal score, faecal output as well as faecal DM %. No discernable variation was recorded in the ammonia concentration of the wet (data not shown) or dry faeces between the two groups in the present experiment similar to the observations of Flickinger *et al.* (2003) using FOS as prebiotic source. The lactate concentration of the faeces tended to increase upon MOS supplementation, confirming the earlier results of greater faecal lactate concentration due to feeding of FOS (Swanson *et al.* 2002) and MOS (Pawar *et al.* 2008). Lactate, produced mainly by saccharolytic bacteria (*Lactobacillus, Bifidobacteria, Streptococcus etc.*) during the fermentation of prebiotics, may serve to protect the animal from pathogenic bacteria by decreasing the pH of the hind-gut (Barrow 1992). The increased concentration of lactate in the present study correlates well with the observed higher population of lactobacillus (Table 3) in MOS group. While there was no influence of the dietary treatments on the acetate content of the faeces, that of propionate tended (P,0.127) to show higher values in MOS group; this, coupled with a significantly (P<0.05) higher concentration of butyrate, resulted in higher

Table 3. Faecal characteristics, hind gut health indices and blood metabolic profile of dogs fed with or without MOS

Parameters	Control	Treatment
Faecal score†	3.80±0.20	3.86±0.02
Faeces voided (g/d)	94.03±9.19	111.10±9.91
Faecal DM (g/d)	27.81±0.66	32.43±3.17
Faecal pH	5.70±0.12	5.48±0.14
Gut health indices‡		
Ammonia	13.75±1.54	12.56±1.34
Lactate	11.96±1.04	14.59±0.85
Acetate	165.67±3.81	183.79±17.63
Propionate	23.03±1.97	48.12±8.13
Butyrate	85.19±6.27[a]	105.05±9.82[b]
Total SCFAs	273.89±7.16	336.96±33.62
Blood metabolites		
Haemoglobin (g/dl)	15.40±0.49	15.46±0.99
Haematocrit (%)	49.60±1.57	51.60±0.68
Glucose (mg/dl)	106.59±5.95	115.58±3.73
Triglycerides (mg/dl)	38.30±3.58[b]	26.98±2.95[a]
Total protein (g/dl)	5.81±0.35	6.30±0.22
Albumin (g/dl)	3.52±0.19	3.49±0.11
Globulin (g/dl)	2.30±0.39	2.81±0.16
A:G ratio	1.77±0.40	1.26±0.06
ALP (U/L)	45.78±6.38	41.44±1.87
ALT (U/L)	44.31±9.08	43.41±4.160
Urea (mg/dl)	21.09±2.47	19.77±2.90
Creatinine (mg/dl)	1.53±0.08	1.30±0.20
Calcium (mg/dl)	11.51±0.58	11.02±0.24
Phosphorous (mg/dl)	2.50±0.30	3.22±0.20
Sodium (mmol/L)	125.75±1.45	124.67±3.74
Potassium (mmol/L)	3.858±0.19	3.816±0.17

†Based on a 1–5 point scale; [ab]means bearing different superscripts in a row differ significantly *P<0.05; ‡µmol/g dry faeces, SCFAs: short chain fatty acids.

(P, 0.104) total faecal SCFAs in MOS supplemented dogs than control. Similar increase in propionate has been recorded by Flickinger *et al.* (2003) in faeces of dogs supplemented with oligofructose. The SCFAs decrease luminal pH and create an environment less favorable for pathogenic species. Significantly (P<0.05) higher proportion of butyrate observed in the present study is supportive of similar results observed with increased butyrate concentra-tions *in vitro* (Gibson and Wang 1994) and *in vivo* (Le Blay *et al*. 1999) when diets were supplemented with FOS. Overall, increased total SCFA concentrations in the faeces could be attributed to an increase in the amount of readily fermentable substrate provided as MOS, as have been suggested by Flickinger *et al* (2003). Increase in production of individual SCFAs is known to help in maintaining the optimal gut health by serving as energy sources for the colonocytes among other mechanisms. Strictly speaking, the appearance and concentration of SCFAs in faeces may not be a true reflection of their production at the hind gut level, which is known to be strongly influenced by the rate and site of fermentation of the dietary fibre in the colon. However, concentration of SCFAs, and for that matter that of lactate and ammonia, in faeces have traditionally been used as a fair indicator of the type of fermentation undergoing in the hind gut.

The use of prebiotics in animal nutrition has attracted considerable interest recently, primarily because they might act as modulator of colonic bacterial population besides modulating fermentation end-products to improve host health (Czarnecki-Maulden 2000, Flickinger and Fahey 2002). The faecal enumeration of coliform and lactobacilli indicated that the mean coliform count (6.94±0.30 vs. 6.29±0.25 log cfu/g) tended to reduce (P,0.128) with a concomitant increase (P<0.01) in that of lactobacilli (7.12±0.14 vs. 8.02±0.17) upon MOS supplementation of the control diet (Fig. 1). Similar to the present observations, Swanson *et al.* (2002) reported that dogs fed FOS tended to have greater lactobacilli concentrations in faeces. This increase correlates well with the observed increase in faecal lactate concentration as discussed in the preceding section (Table 3). Lactobacilli constitute one of the major health positive bacterial populations in the hind gut, and in increase in their population could be attributable to the increased provision of substrates in the form of fermentable fibre MOS. This could also explain the reduction observed in the coliform population, known for their health negative attributes. Overall, the favourable shifts evident in the bacterial population coupled with the perceptible increase in SCFAs indicated that supplementation of MOS is beneficial for the maintenance of the gut health.

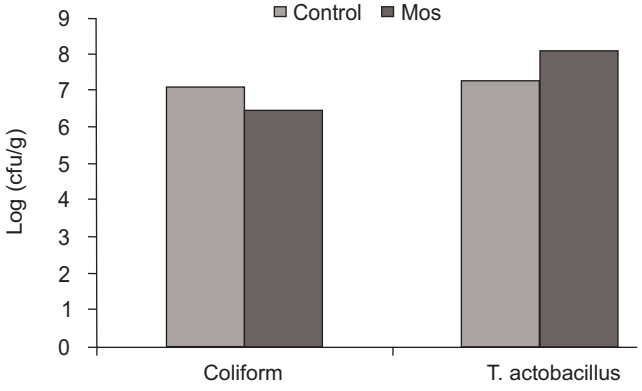

Fig. 1. Faecal microbial count (Lactobacillus (P<0.01) and Coliform (P=0.128)) of the experimental dogs

Metabolic profile

The concentrations of various metabolites in plasma are given in Table 4. Plasma levels of total protein, albumin, and calcium were apparently not affected by MOS supplementation, as was reported in lambs by Demirel *et al.* (2007). Glucose concentration in blood plasma is a balance between its hepatic mobilization and peripheral uptake (Rovira *et al.* 2007). FOS has been shown to reduce post-prandial glycemia and insulinemia in rats (Roberfroid and Delzemme 1998) thought to be mediated by decreased gastric emptying or changes in hepatic metabolism. However, no apparent variation was observed in plasma glucose concentrations in the present study similar to the findings of Strickling *et al.* (2000). The plasma triglycerides exhibited a significant (P<0.05) reduction upon MOS supplementation of the control diet which is in line with the results of Pawar *et al.* (2008) who reported lower plasma triglycerides in dogs fed a homemade diet supplemented with MOS. Feeding of chicory fructans has been shown to induce hypotri-

Table 4. Plasma metabolic profile of dogs fed diet with or without MOS

Attribute	Diet	
	Contol	MOS
Haemoglobin (g/dl)	15.40±0.49	15.46±0.99
Haematocrit (%)	49.60±1.57	51.60±0.68
Glucose (mg/dl)	106.59±5.95	115.58±3.73
Triglycerides (mg/dl)	38.30±3.58[b]	26.98±2.95[a]
Total Protein (g/dl)	5.81±0.35	6.30±0.22
Albumin (g/dl)	3.52±0.19	3.49±0.11
Globulin (g/dl)	2.30±0.39	2.81±0.16
A:G ratio	1.77±0.40	1.26±0.06
ALP (U/L)	45.78±6.38	41.44±1.87
ALT (U/L)	44.31±9.08	43.41±4.160
Urea (mg/dl)	21.09±2.47	19.77±2.90
Creatinine (mg/dl)	1.53±0.08	1.30±0.20
Calcium (mg/dl)	11.51±0.58	11.02±0.24
Phosphorous (mg/dl)	2.50±0.30	3.22±0.20
Ca:P	4.82±0.53	3.49±0.29
Sodium (mmol/L)	125.75±1.45	124.67±3.74
Potassium (mmol/L)	3.858±0.19	3.816±0.17
Na:K	32.91±1.63	32.87±1.35

[ab]Means bearing different superscripts in a row differ significantly *P<0.05.

glyceridaemia in rats (Delzenne *et al.* 1993), mostly due to a decrease in the plasma VLDL (Fiordaliso *et al.* 1995). The triacylglycerol-lowering action of prebiotics (oligofructose) is suggested to be due to a reduction of de novo fatty acid synthesis in the liver through inhibition of all lipogenic enzymes, namely acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, ATP citrate lyase and glucose-6-phosphate dehydrogenase (Delzenne and Kok 2001). This hypothesis could have mediated the reduction in plasma triglycerides, in the present study, upon MOS supplementation.

The mean concentration of plasma electrolytes remained apparently uninfluenced by the dietary treatments as indicated from the statistically similar values between the two groups. Delzenne *et al*. (1995), based on mineral balance measurement, have shown that prebiotics enhance the mineral absorption, but elucidation of the effect of enhanced electrolytes absorption in terms of increased plasma concentrations obviously require long-term studies, which was outside the scope of the present experiment. Nonetheless, higher (P,0.080) plasma level of inorganic phosphorous observed in the MOS supplemented dogs in the present study highlights the potential role of prebiotics (MOS) in increasing mineral absorption from hind gut. Overall, although there were variations in some of the metabolic indices between the two groups, all were within the normal physiological limit specified for healthy dogs (Kaneko *et al.* 1997) which signifies that there is no adverse effect of prebiotics supplementation on metabolic health of the animals.

The results obtained from the experiment indicated positive influence of MOS supplementation @ 1% of dry diet on food and nutrient intake, fiber digestibility and indices of hindgut health with some potential effects on plasma metabolic profile. This in turn highlights the functional property of MOS as a supplement for overall health in pet dogs.

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