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Jerusalem Artichoke as a Potential Prebiotic: Influence on Nutrient Utilization, Hindgut Fermentation and Immune Response of Labrador Dogs

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ABSTRACT

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In order to explore the potential of Jerusalem artichoke (JA) as a prebiotic, four groups (CON, PRE-1, PRE-2 and PRE-3) of adult Labrador dogs (n=4 in each) were fed for 90d on a basal diet supplemented with pulverized JA tuber at 0, 1, 2 and 3% levels, respectively. Cell-mediated immunity was assessed on d56 by measuring skin indurations following intra-dermal phytohaemagglutinin-P inoculation. At d60, peripheral lymphocyte sub-populations in blood were assessed by flow cytometry. Humoral immunity was assessed by quantifying the antibody titre against sheep-RBC (SRBC). A digestion trial of 6d duration was conducted after 80d of feeding. Results revealed an increase (P=0.016) in skin indurations in PRE-1 and PRE-2 compared to CON and PRE-3. There was no impact of JA on circulating peripheral CD3+ and CD8+ populations, however, CD4+ populations enhanced (P=0.005) in JA supplemented groups than CON. The antibody titre (log_2) against SRBC was also higher (P=0.007) in treatment groups than control. There was an increase (P<0.05) in faecal concentration of lactate and short-chain fatty acids with a concomitant reduction in ammonia. The faecal counts (log_{10} cfu/g) of health-positive *Bifidobacterium* (P=0.035) and *Lactobacillus* (P=0.062) were higher in treatment groups while health-negative coliforms and *Clostridium* counts remained unaltered. The digestion trial indicated that JA supplementation improved the digestibility of fibre (P=0.003) along with that of calcium and phosphorus. Overall findings revealed that JA may constitute a perspective prebiotic additive because of its apparent positive impacts on immune status as well as hindgut fermentation.

Key words: Jerusalem artichoke, Digestibility, Hindgut fermentation, Immune response, Dogs.

INTRODUCTION

Inulin and fructo-oligosaccharides (FOS) are plant-derived prebiotics with the benefits of soluble dietary fibres which are not digested or absorbed in the small intestine,

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but fermented in the large intestine resulting in recovery of a part of the chemical energy from these carbohydrates. They may target selected groups of the hindgut microbiota, thus having the ability to alter the composition towards a more 'beneficial' community, i.e. selectively increasing populations of *Bifidobacterium* and/or *Lactobacillus*, which have been proposed to be connected with health-promoting functions (Macfarlane *et al.,* 2006). The structural complexity of these prebiotics may allow maintenance of high fermentation activity throughout the hindgut.

Inulin belongs to the fructan family; naturally occurring fructans are important storage carbohydrates, widespread in various flowering plants. The FOS is always present in inulin and is a product of partial enzymatic hydrolysis of inulin. It differs from inulin in regard to its degree of polymerization (Watzl *et al.,* 2005). Chemically, inulin consists of one α-glucopyranosyl units linked to a varying number of α-fructosyl units. Because no α-fructosidase has so far been reported in the small intestine, hydrolysis of the fructosyl-fructosyl linkages is probably low in the upper digestive tract and the fructans reaching the hindgut are readily metabolized by the digestive microbiota, resulting in the production of organic acids (volatile fatty acids and lactic acid) which are extensively absorbed. The Jerusalem artichoke (JA) plant contains 16-20% inulin and 12-15% FOS (Moshfegh *et al*., 1999) and therefore possesses prebiotic potential to be used in the diets of human and animals. In addition to their conventional use for the production of FOS syrups, JA has been investigated for its role as gut modulators. Besides, the use of JA tuber as such rather than using the derived prebiotic compound (inulin and FOS) would constitute a cost-effective approach, especially in Indian conditions. Although few reports are available concerning the health-effects of JA, still there is a dearth of literature characterizing the detailed studies on the potential of JA. Hence, the present experiment was undertaken to assess the diet digestibility, hindgut fermentation and immune response of adult Labrador dogs fed graded levels of JA in the diet.

MATERIALS AND METHODS

Animals and management

Sixteen adult Labrador dogs with average initial BW of 21.92 ± 0.56 kg were divided into 4 equal groups (CON, PRE-1, PRE-2 and PRE-3) in a randomized block design after deworming and vaccination. The dogs were housed in well ventilated kennel with facilities for individual feeding under hygienic and uniform managemental conditions. They were fed to satisfy the nutrient requirement (NRC, 2006). The basal diet (rice-30%, sorghum-19%, Bengal gram-25%, soybean meal-18%, soya oil-4% and skimmed milk powder-4% on as-fed basis) was pressure cooked and thoroughly mixed with mineral-vitamin premix before feeding. Pulverized JA tuber powder was supplemented @ 0, 1, 2 and 3% levels in the diets of CON, PRE-1, PRE-2 and PRE-3 groups, respectively. The experimental feeding lasted for 90 days. The animals had access to clean and fresh drinking water twice a day, during morning and afternoon hours.

Sampling and processing

The DM consumption of individual dogs under each dietary group was monitored daily. The dogs were offered weighed amount of food twice a day; any amount which

was not consumed within 30 min of offering was removed and taken as refusal. Body weight was recorded at weekly intervals before feeding and watering. A digestion trial of 6d duration (2 days of adaptation and 4 days of collection) was conducted after 80 days of experimental feeding to determine nutrient utilization. During the digestion trial, the daily food offered and refused, and faeces voided were collected and recorded on a 24h basis. Faecal consistency score was assigned at each collection by adopting standard protocol (Lewis *et al.,* 1994; Strickling *et al.,* 2000). A 1-4 point palatability score was adopted for subjective assessment of the acceptability of the experimental diets as detailed elsewhere (Shakhar *et al*., 2007). A suitable aliquot of fresh faeces was drawn for DM determination and another aliquot was preserved in 10 ml of dilute 1:4 sulphuric acid for subsequent determination of nitrogen. Samples were drawn for the faecal estimation of ammonia, lactate and short-chain fatty acids (SCFAs) as per Kore *et al*. (2009). The pooled samples of food, refusals and faeces were dried in a forced-air oven (80^{o}C), grounded through 1-mm sieve and preserved for chemical analysis.

Microbial populations were enumerated by two sets of serial 10-fold dilutions (10^{-1} to 10^{-9}) with the total volume of 10 ml including 1 g homogenized faecal content and 9 ml normal saline (0.9% NaCl) and plated in duplicate onto selective media: Rogosa agar (BBL Difco) for *Lactobacillus*, MacConkey agar (BBL-Difco) for coliforms, Reinforced clostridial agar (Himedia) for *Clostridium* and Bifidobacteria agar for *Bifidobacterium* (Himedia). For *Lactobacillus* and coliforms, relevant agar plates were incubated aerobically at 37°C for 24h and 48h respectively; bifidobacteria and clostridial agar plates were incubated anaerobically at 37°C for 24-48h. The bacterial colonies were counted as colony forming units (cfu)/per g faeces and expressed as $\log_{10}$ cfu/g.

On 56th day of experimental feeding, all animals were inoculated intra-dermally with phytohaemagglutinin-p (PHA-P) mitogen (0.5 mg/ml saline solution) to monitor the *in vivo* cell mediated immune response as a delayed-type hypersensitivity (DTH) reaction. The DTH response was assessed by measuring skin indurations at 0, 12, 24, 48 and 72h post-injection and was expressed as per cent increase in skin thickness compared with 0h. Subsequently, after 60 days of experimental feeding, the immune-phenotyping of T-lymphocyte subtypes was determined in peripheral blood by flow cytometry. For humoral immune response, dogs were intravenously injected with 1ml of 10% SRBC suspension in 0.15M NaCl. Sera for antibody determinations were collected at weekly intervals to measure the antibody titre ($\log_2$) by the microtitre haemagglutination procedure (Wegmann and Smithies, 1966).

Chemical and statistical analyses

The food, left over residues and faecal samples were analyzed for their proximate composition (AOAC, 1995). The calcium content was estimated titrimetrically as per Talapatra *et al*. (1940) and phosphorus content was determined spectrophotometrically adopting the metavandate method (AOAC, 1995). Faecal samples were analyzed for ammonia (Chaney and Marbach, 1962), lactate (Baker and Summerson, 1941) and SCFAs (Barnett and Reid, 1957).

The experimental data were analysed by one-way ANOVA (Snedecor and Cochran, 1994), followed by a post-hoc test, with SPSS version 16.0 Statistics software (SPSS Inc., Chicago, Illinois, USA). Means were compared with Duncan's multiple range test, and significance declared at $P<0.05$.

RESULTS AND DISCUSSION

There was no difference in the chemical composition of the composite diets offered to the four groups of dogs (Table 1). The dogs under the four dietary groups consumed the diet readily and maintained their BW throughout the feeding trial.

Apparent total tract digestibility of nutrients

The intake (g/d) and digestibility (%) of all nutrients except that of crude fibre were comparable among the four groups (Table 2), which was higher ($P<0.05$) in JA-supplemented groups than control. These are consistent with the earlier reports on FOS or MOS supplementation (Strickling *et al.,* 2000; Swanson *et al*., 2002). Nevertheless, a reduction in nutrient digestibility was reported due to inulin and FOS (Propst *et al.,* 2003), FOS (Flickinger *et al.,* 2003) and MOS (Zentek *et al*., 2002) supplementation. Increased ($P=0.003$) crude fiber digestibility in JA-supplemented groups was in agreement with the findings of Pawar *et al*. (2008) and Kore *et al*. (2012) who used MOS as a prebiotic source. Zentek *et al*. (2002) also recorded increased ($P<0.05$) CF digestibility, when diet was supplemented with MOS, TGOS and lactulose. The marked improvement in the fibre digestibility could possibly be attributable to greater fermentability of the dietary fibre in the hindgut. Significantly higher digestibility of calcium ($P=0.003$) and phosphorus ($P=0.003$) were encountered in JA-supplemented groups. Pawar (2007) have also reported better ($P=0.059$) apparent absorbability of calcium in MOS supplemented group than control group but no effect on phosphorus absorption. Similar to the present observations, many investigators have demonstrated that rats fed with FOS and/or inulin absorbed more calcium than control rats, despite an increase in total faecal mass (Ohta *et al.,* 1994; Delzenne *et al.,* 1995). Contrary to the present findings, Rideout and Fan (2004) reported that, inulin had no effect on phosphorus absorption but reduced urinary phosphorus excretion in growing pigs.

Hindgut fermentation characteristics

Assessment of various faecal characteristics can be used as an indicator for the overall hindgut fermentation. The data of the physical, chemical and microbiological attributes of the faeces are listed in Table 3. The mean faecal score, faecal output as well as faecal DM% were found to be similar ($P>0.05$) among the four dietary groups highlighting no influence of JA supplementation on faecal quality. This was supported by the similar findings by Swanson *et al*. (2002), Flickinger *et al*. (2003) and Kore *et al*. (2012).

The faecal pH was apparently uninfluenced ($P>0.05$) by the dietary treatments. Likewise, Hesta *et al*. (2003) and Kore *et al*. (2012) did not observe any significant variation in faecal pH due to prebiotics supplementation. However, a decreased faecal pH and increased concentration of total SCFAs was observed by Hesta *et al*. (2001)

Table 1. Chemical composition (% DM basis) of the experimental diets

Attributes	Dietary treatments			
	CON	PRE-1	PRE-2	PRE-3
Organic matter	93.50	93.90	93.93	94.03
Crude protein	33.29	21.71	27.77	26.53
Ether extract	3.14	3.71	2.88	3.06
Crude fibre	1.69	1.77	1.82	1.87
Nitrogen-free extract	52.06	63.77	58.47	59.22
Total carbohydrates	57.07	68.47	63.29	64.44
Calcium	1.42	1.95	2.80	3.41
Phosphorous	0.76	0.76	0.75	0.72

Table 2. Nutrient intake and digestibility in dogs in response to dietary supplementation of JA

Attributes	Dietary treatments				SEM	P value
	CON	PRE-1	PRE-2	PRE-3		
Palatability score[s]	1.75	1.72	1.82	1.72	0.35	0.997
Nutrient intake (g/d)						
Dry matter	306.71	297.25	300.65	341.85	30.63	0.722
Digestibility (%)						
Dry matter	82.79	83.68	83.06	80.99	1.85	0.762
Organic matter	86.35	87.08	86.65	84.49	1.54	0.657
Crude protein	81.32	80.63	82.96	78.79	2.37	0.668
Ether extract	82.57	85.99	84.71	82.75	2.96	0.820
Crude fibre	49.19[a]	54.81[b]	57.52[b]	56.96[b]	1.33	0.003
Nitrogen-free extract	86.88	89.98	87.38	85.81	1.61	0.348
Total carbohydrates	85.77	89.11	86.67	85.14	1.67	0.394
Calcium	37.71[a]	44.96[b]	43.49[b]	45.79[b]	1.26	0.003
Phosphorous	29.17[a]	35.03[b]	38.52[b]	35.28[b]	1.35	0.003

[s]Based on a 1-4 point scale; [a,b]Means with different superscripts in a row differ significantly ($P<0.05$).

using FOS or inulin as prebiotic in cats but only when higher concentrations were used and not even with 3% level of supplementation. The faecal ammonia concentration varied significantly ($P=0.001$) being more in CON compared to JA-supplemented groups of dogs. In contrast, the faecal ammonia content was higher due to feeding of MOS (Pawar, 2007) and FOS or inulin (Propst *et al.,* 2003). Corroborating the present results, Zentek *et al*. (2002) and Flickinger *et al*. (2003) noted decreased ammonia concentration due to MOS or FOS supplementation, respectively. Nevertheless, no influence of prebiotics supplementation on the faecal ammonia concentration was observed (Strickling *et al.,* 2000; Swanson *et al.,* 2002; Kore *et al.,* 2012). There was a significant increase ($P<0.05$)

in the faecal lactate content (μmol /g dry faeces) in PRE-1 as well as PRE-3 compared to CON and PRE-2. Similar to the present observations, the faecal lactate concentration was significantly ($P<0.01$) higher in dogs supplemented with MOS in nutritionally balanced homemade diet (Pawar *et al.,* 2008). Swanson *et al.* (2002) also found greater faecal lactate ($P=0.06$) concentration in dogs supplemented with FOS. The total SCFAs showed significant ($P<0.01$) variation among the different groups being lowest in CON compared to treatment groups. Similar to the present results, total SCFAs increased linearly ($P<0.01$) with increasing concentrations of prebiotic as a dietary supplement, with the greatest increase noted for the 0.3% FOS treatment (Propst *et al.,* 2003). Kore *et al.* (2012) reported apparently higher content of total faecal SCFAs ($P=0.104$) in the MOS-supplemented dogs.

The data on faecal enumeration of *Bifidobacterium* population revealed significant ($P=0.035$) variation among the dietary groups, with increased values in JA-supplemented groups i.e. PRE-1 and PRE-2 compared to CON group while PRE-3 was comparable to both. The present findings corroborated those of Flickinger *et al.* (2000) who reported an increased ($P=0.130$) concentration of *Bifidobacterium* upon GOS supplementation. Similar were findings of Howard *et al.* (1995) in mice and Gibson *et al.* (1995) in human beings due to FOS supplementation. There was concomitant increase ($P=0.062$) in faecal *Lactobacillus* count in JA-supplemented groups compared to CON. The increase in

Table 3. Physical, chemical and microbiological characteristics of faeces of dogs in response to dietary supplementation of JA

Attributes	Dietary treatments				SEM	P value
	CON	PRE-1	PRE-2	PRE-3		
Physical						
Faecal score†	2.44	2.60	2.54	2.63	0.116	0.671
Faeces voided (g/d)	228.33	217.35	207.28	335.48	53.15	0.334
Defecation frequency	2.08	2.48	2.38	2.44	0.343	0.840
Faecal DM (%)	25.83	23.54	25.92	19.70	1.98	0.145
Chemical						
pH	5.60	5.46	5.42	5.25	0.11	0.217
Ammonia (μmol/g)	9.37^{b}	6.59^{a}	5.64^{a}	6.91^{a}	0.40	0.000
Lactate (μmol/g)	33.20^{a}	46.96^{c}	40.90^{b}	46.35^{c}	1.05	0.000
Total SCFAs (μmol/g)	316.05^{a}	404.62^{c}	440.46^{d}	356.29^{b}	9.88	0.000
Microbial count ($\log_{10}$ cfu/g)						
Lactobacilli	8.61^{a}	9.32^{b}	9.42^{b}	9.26^{b}	0.21	0.062
Bifidobacteria	9.20^{a}	9.75^{b}	9.92^{b}	9.52^{ab}	0.16	0.035
Coliforms	6.57	6.19	5.36	6.20	0.39	0.225
Clostridia	9.84	9.45	9.14	9.72	0.21	0.138

†Based on a 1-5 point scale; [abcd]Means bearing different superscripts in a row differ significantly ($P<0.05$).

Lactobacillus population is in confirmation with the observed increase in the lactate concentration of the faeces. Similar to the present findings, Swanson *et al.* (2002) reported that dogs fed FOS tended to have greater *Lactobacillus* concentrations in their faeces. No discernable variation was recorded in the faecal coliforms and *Clostridium* counts among the groups. Likewise, Strickling *et al.* (2000) and Flickinger *et al.* (2003) have found no influence of MOS or FOS addition in canine diets on the concentration of coliforms.

Immune response

Both the cell mediated and humoral immune responses were improved in treatment groups than control group. The data in the form of absolute and comparative increase in skin indurations after 12, 24, 48, 72 and 96h of post inoculation of the mitogen, showed that there was significant ($P<0.05$) increase in skin indurations in PRE-1 than CON, PRE-2 and PRE-3 (Table 4; Fig. 1). While there seems to be no impact ($P>0.05$) of feeding JA to dogs in terms of their circulating peripheral CD3+ populations, it significantly ($P<0.01$) altered the CD4+ populations; the CD4+ population was found to increase in PRE-3 and PRE-2 groups as compared to CON and PRE-1 groups. Additionally, although non-significant, the population of CD8+ tended to increase in PRE-1 and PRE-2 groups than CON and PRE-3 groups. Consequently, there was significant increase in CD4+:CD8+ ratio in PRE-3 than CON, PRE-1, and PRE-2 (Table 4), confirming the earlier similar results of Pawar (2007) due to dietary supplementation of prebiotics in the form of MOS. Other studies conducted with recognized prebiotic fibres have shown an alteration of the CD4+ and CD8+ T cell proportions and increased lymphocyte and leukocyte numbers in the gut-associated lymphatic tissue in canines (Field *et al.,* 1999) and calves (Kaufhold *et al.,* 2000; Fleige *et al.,* 2009). The mean antibody titre against SRBC was significantly ($P=0.007$) increased in all JA-

Table 4. Immune response attributes of dogs fed graded levels of JA

Attributes	Dietary treatments				SEM	P value
	CON	PRE-1	PRE-2	PRE-3		
Peripheral lymphocyte sub-population (%)						
CD3+	76.90	77.19	75.70	71.71	3.39	0.653
CD4+	34.11[a]	35.76[a]	40.58[b]	42.75[b]	1.52	0.005
CD8+	21.16	25.00	25.65	21.44	1.68	0.177
CD4+:CD8+	1.62[a]	1.45[a]	1.60[a]	2.07[b]	0.17	0.053
Cell mediated immune response						
DTH response[†]	127.4[a]	144.1[b]	130.2[a]	125.5[a]	4.61	<0.001
Humoral immune response						
HA titre[‡]	3.94[a]	5.13[b]	5.69[b]	4.88[ab]	0.34	0.007

[a,b]Means with different superscripts in a row differ significantly ($P<0.05$).

[†]Comparative skin thickness (%); mean of five post-inoculation measurements compare to 0h value (100%).

[‡]Mean antibody titre (log_2); mean of five post-inoculation measurements.

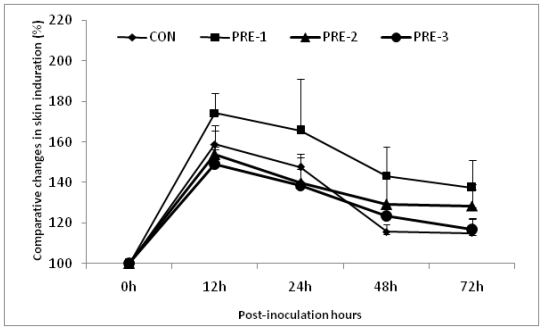

Fig. 1. DTH response to PHA-p in dogs fed graded levels of JA.

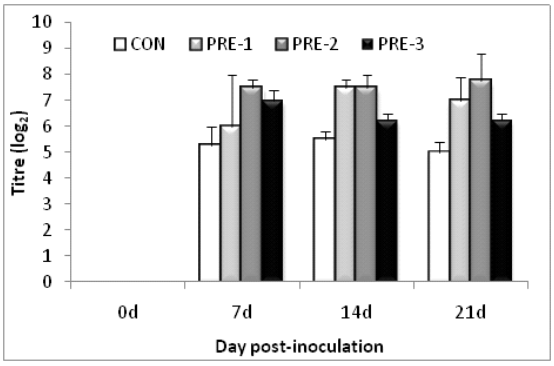

Fig. 2. Antibody response to SRBC in dogs fed graded levels of JA.

supplemented groups than control group (Table 4). A more critical appraisal of the curves points to the fact that it was more sustained in the PRE-2 and PRE-1 groups as compared to PRE-3 group (Fig. 2). The present findings consolidates the implications that supplementation of JA helped to improve both the cell-mediated as well as humoral immune status of the dogs.

CONCLUSION

The study envisaged that Jerusalem artichoke may constitute a novel prebiotic additive for dogs because of its positive influence on fibre utilization which was translated into better apparent absorption of calcium and phosphorus. The positive impacts on gut health indices and immune status were also apparent besides positive shifts in colonic microbiological attributes. However, further in depth investigations are warranted for establishing its efficacy as a prebiotic in dogs.

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