J.Natn.Sci.Foundation Sri Lanka 2018 46 (4): 477 - 485
DOI: http://dx.doi.org/10.4038/jnsfsr.v46i4.8623

RESEARCH ARTICLE

Prebiotic carbohydrate profile and *in vivo* prebiotic effect of pumpkin (*Cucurbita maxima*) grown in Sri Lanka

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Revised: 14 March 2018; Accepted: 25 May 2018

Abstract: This study was aimed at analysing the dietary fibre (DF) content and prebiotic carbohydrate profile of two local pumpkin varieties (Villachchi, Moragollagama) and five imported hybrid varieties (Arjuna, Suprima, Abishek, Lara, Pragathi) grown in Sri Lanka, and investigating the prebiotic potential of pumpkin powder *in vivo* using the pig as an animal model for human. High performance liquid chromatography (HPLC) technique was performed to identify the prebiotic carbohydrate profile. In the feeding trial, pigs were fed with standard diet (SD), pumpkin powder + SD (PD), SD + probiotics, and PD + probiotic. Freeze dried *Bifidobacterium animalis* subsp. *lactis* (500 $mgday^{-1}$) and *Lactobacillus acidophilus* (500 $mgday^{-1}$) were used as probiotic organisms. At the end of the experimental period, faecal microbial compositions of the pigs were analysed. Results revealed that the total DF content of pumpkin pulp did not vary ($p > 0.05$) among varieties. However, the local varieties had higher ($p < 0.05$) soluble DF (11.75 – 12.21 % dry matter) than the hybrid varieties. Mean concentrations of sorbitol, mannitol, stachyose + raffinose, verbascose + kestose and nystose of pumpkin were 106.7, 41.4, 76.5, 294.5 and 37.5 mg/100 g dry matter, respectively. Pumpkin powder fed pigs had higher ($p < 0.05$) counts of faecal lactic acid bacteria and bifidobacteria in comparison to SD fed pigs. In conclusion, this study revealed that pumpkin contains prebiotic carbohydrates, namely, sorbitol, mannitol, stachyose, raffinose, verbascose, kestose and nystose, and the feeding trial reflected prebiotic activity of pumpkin powder indicating a possibility to be used in functional foods.

Keywords: Bifidobacteria, lactic acid bacteria, pig, prebiotic carbohydrates, pumpkin, Sri Lanka.

INTRODUCTION

Obesity is a major public health and economic problem throughout the world and the prevalence is rising each year. The prevalence of obesity in the world is estimated to be over 13 % among adults, which has doubled between 1980 and 2014 (WHO, 2015). Sri Lanka is no exception in this regard where percentages of overweight, obese, and centrally obese adults are 25.2 %, 9.2 % and 26.2 %, respectively, comparable with the values in regional countries (Katulanda *et al*., 2010). As Sri Lanka is a middle-income country, which is undergoing a rapid change in dietary habits of adolescence and youth, changes in diet and lifestyles may have an influence on obesity risks. According to previous studies, positive correlation between obesity and type-2 diabetes, cardiovascular diseases, and certain cancers have been reported (Wilson *et al*., 2002; Bell *et al*., 2014). Since these non-communicable diseases are serious public health issues, sustainable preventive measurements are of utmost importance. Particularly, healthy eating habits and regular physical activities play vital roles in weight loss. With respect to the dietary pattern, intake of a prebiotic rich, low-calorie diet has been suggested to protect against obesity and related non-communicable diseases (Dumas *et al*., 2006; Wu *et al*., 2011).

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Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health (Gibson & Roberfroid, 1995). Health benefits of prebiotics include better mineral absorption, alleviation of constipation and irritable bowel syndrome, protection against colon cancer, enhancement of the immune system, anti-carcinogenic effect, reduction of the risk of obesity and lowering of cholesterol level (Yuan *et al*., 2004; Yasuda *et al*., 2006; Roberfroid, 2007). The definition of a prebiotic itself indicates that these health benefits are achieved through supporting the growth of health promoting microbiota. In the human gut, beneficial bacteria called the Bacteroidetes and the Firmicutes are dominant, and the relative proportion of Bacteroidetes is lower in obese people compared to lean people (Ley *et al*., 2006). Therefore, altering the gut microbial ecology using prebiotics would be helpful to promote health. A recent study has shown that dietary intake of prebiotics decreases Firmicutes and increases Bacteroidetes in obese mice (Everard *et al*., 2011). Furthermore, as reported by Parnell and Reimer (2012), prebiotic diet has the ability to increase the level of an appetite suppressing, endogenous hormone called glucagon-like peptide-1 (GLP-1), which leads to loss of weight.

Apart from that, prebiotics have gained much attention as naturally occurring substances for the prevention of colorectal cancer (CRC), since short chain fatty acids (SCFA) and products of prebiotic fermentation provide a preventive effect against CRC (Fotiadis *et al*., 2008). Fermented products of the dietary fibre such as acetate, butyrate and propionate increase pH in the large intestine and thereby mineral absorption is enhanced through increasing the solubility of minerals, specially of calcium (Scholz-Ahrens & Schrezenmeir, 2002).

Cereals, pulses, fruits and vegetables are good sources of prebiotics with high nutrient value. Naturally present prebiotic carbohydrates are categorised under dietary fibre according to the AACC definition (AACC, 2001). Nevertheless, all the dietary fibres do not show prebiotic properties. Only fermentable fibres can act as substrates for health promoting microorganisms such as lactobacilli and bifidobacteria (Roberfroid, 2007). Although the association between dietary fibre and health benefits are revealed by studies, the current intake level of Sri Lankans (18 $gday^{-1}$ $person^{-1}$) is well below the recommended level (25 $gday^{-1}$ $person^{-1}$) (Medagama *et al*., 2015). Therefore, adequate knowledge on dietary fibre rich foods would help to reduce the risk of obesity and associated chronic diseases.

Pumpkin belonging to the family Cucurbitaceae has experienced increased interest in recent years because of its nutritional and health protective effects such as antihypertension, antitumor, antibacterial, antihypercholesterolemia, intestinal antiparasitia, antiinflammation and antalgic properties (Caili *et al*., 2007; Yadav *et al*., 2010). Moreover, a recent study has shown that the edible portion of pumpkin supports the growth of *Lactobacillus fermentum*, *Bifidobacterium breve* and *Clostridium acetobutilicum* species, which express its *in vitro* prebiotic effect (Sreenivas & Lele, 2013). However, no study has examined the prebiotic property of pumpkin *in vivo* and the prebiotic carbohydrate profile of pumpkin grown in Sri Lanka. Hence, the aims of the current study were to characterise the prebiotic carbohydrate profiles of four pumpkin varieties and dietary fibre contents of seven pumpkin varieties grown in Sri Lanka and to evaluate the effects of pumpkin on faecal microflora composition, persistence of probiotic *in vivo* using the pig (*Sus scrofadomesticus*) as an animal model for human.

METHODOLOGY

Materials

Standards, reagents, and high-purity solvents used for high performance liquid chromatographic (HPLC) analyses and enzymatic assays were purchased from Sigma-Aldrich (St. Louis, MO, USA) and VWR International (Radnor, PA, USA). Regular maize starch (Megazyme International Ireland Ltd., Bray, Ireland) was used as an external reference sample. Water, distilled and deionised (ddH_2O) to a resistance of ≥18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA) was used for pumpkin sample extraction and preparation. Standard diet (pig fattener) used for the feeding experiment was purchased from CIC Feed (Pvt) Ltd., Ekala, Sri Lanka, and freeze dried probiotic cultures of *Bifidobacterium animalis* Subsp. *lactis* (nu-trish BB-12) and *Lactobacillus acidophilus* (LA-5) were obtained from CHR HANSEN holding A/S (BoegeAlle 10-12, 2970 Hoersholm, Denmark). Culture media used for microbial enumerations were bought from HiMedia Laboratories Pvt Ltd. (Mumbai, Maharashtra 400086, India). Termamyl / α-amylase (Sigma A3306), protease (Sigma P3910), and amyloglucosidase (A9913) used for the determination of dietary fibre were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Pumpkin samples

Seven varieties of pumpkin including two local varieties (Villachchi, Moragollagama) and five hybrid varieties (Pragathi, Lara, Arjuna, Abishek, Suprima) were used for the analyses. Three subsamples of pumpkin were randomly taken from each of the three replicated fruits harvested from three locations, Kuda Vilachchiya (8.49102 ºN, 80.1472 ºE), Makandura (7.3225 ºN, 79.9773 ºE) and Mannar junction (8.5066 ºN, 80.13034 ºE) in 2015. Soon after collection of the fruits, the pulp was cut into small pieces and oven dried at 60 ºC using YAMATO IC 600 drying oven (Yamato Scientific Co, Ltd., Japan) until a constant weight was achieved. The dried pumpkin pulp was finely ground using a grinder (MX- 15ISGS, Panasonic Co, Ltd., China) to obtain pumpkin powder for the compositional analyses and feeding experiment. In addition, samples used for the prebiotic carbohydrate analysis were shipped to Clemson University, USA.

Animals and diets

Ten-week old, healthy male pigs (initial body weight, 16.66 ± 0.97 kg) were selected for the feeding experiment (Swine Unit, Livestock Field Station, University of Peradeniya). Four weaned animals from four crossbred (Duroc × Large white) litters were separated and managed under same conditions until the beginning of the feeding experiment. One animal caged in an individual pen was considered as one replicate, therefore there were four replicates in one treatment. Pigs in the first treatment were fed with a standard diet (SD); the pigs in the second treatment received SD with probiotic; the third group was fed SD which was partly replaced with pumpkin powder (100 gkg^{-1}) (PD); and PD with probiotics was supplied to the fourth group. The Villachchi variety, which was highlighted by the highest content of prebiotic carbohydrates was used to prepare pumpkin powder in the feeding trial. With the aim of analysing the synbiotic effect of pumpkin, probiotic organisms were included in two treatments. It was decided to use *Bifidobacterium animalis* subsp. *lactis* (500 $mgday^{-1}$) and *Lactobacillus acidophilus* (500 $mgday^{-1}$) as probiotic microorganisms. The diets, provided *ad libitum*, were fed for 3 wks, followed by a period of 1 wk during which they were fed a standard diet containing an antibiotic (doxycycline) to achieve similar initial microbial level in their gut. All diets were isocaloric and isonitrogenous (Table 1).

Table 1: Chemical composition of experimental diets (dry matter basis)

Components	SD	PD
Energy ($kcalkg^{-1}$)	4041.8 ± 33.2	4034.0 ± 3.9
Ash (%)	9.6 ± 0.1	9.8 ± 0.2
Protein (%)	24.3 ± 0.1	24.7 ± 0.5
Fat (%)	13.1 ± 2.0	11.5 ± 1.2
Fibre (%)	8.9 ± 0.2	17.1 ± 1.1

Standard diet (SD); standard diet partly replaced with pumpkin powder (100 gkg^{-1}) (PD)

Analysis of water soluble prebiotic carbohydrates

Water soluble prebiotic carbohydrates for each triplicated pumpkin pulp sample were extracted using a method described by Muir *et al*. (2009). Five hundred milligrams of ground sample was weighed into a 15 mL polystyrene conical tube and dissolved in 10 mL of ddH_2O. Then, samples were incubated in a water bath at 80 ºC for 1 h followed by centrifugation at 3000 × g for 10 min (Beckman GPR centrifuge, Fullerton, CA, USA). An aliquot (1 mL) of the supernatant was diluted with 10 mL of ddH_2O and sent through a 13 mm × 0.45 µm nylon syringe filter (Fisher Scientific, USA). According to the method described by Feinberg *et al.* (2009), prebiotic carbohydrate concentrations were measured using a high performance anion exchange chromatograph with a pulsed amperometric detector (ICS-5000 Dionex, Sunnyvale, CA, USA). A CarboPac PA1 column (250 × 4 mm; Dionex, Sunnyvale, CA, USA) with a CarboPac PA1 guard column (50 × 4 mm) was used to separate oligosaccharides and sugar alcohols at 1 $mLmin^{-1}$ flow rate in mobile phase (100 mM sodium hydroxide/ 600 mM sodium acetate, 200 mM sodium hydroxide and ddH_2O). Linear gradient of mobile phase was maintained as described by Johnson *et al*. (2013). Oligosaccharides and sugar alcohols were detected using a pulsed amperometric detector (PAD), which contained a gold electrode with a silver-silver chloride electrode at 2.0 µA. Carbohydrates were identified and quantified using pure standards of sorbitol, mannitol, glucose, fructose, sucrose, raffinose, stachyose, verbascose, kestose, and nystose. The concentrations of the carbohydrates were detected within a linear range of 3 – 100 $µgg^{-1}$ with a minimal detectible limit of 0.2 $µgg^{-1}$. CRD Redberry sample was used as an external lab reference and oligosaccharide peak areas for this sample were routinely analysed with an error of less than 5 %. Concentrations of oligosaccharides in the filtrate were calculated using the equations described by Johnson *et al.* (2013).

Determination of dietary fibre (DF) content

The insoluble dietary fibre (IDF), soluble dietary fibre (SDF), and total dietary fibre contents were analysed by

enzymic gravimetric method. Pumpkin samples were gelatinised with termamyl and subjected to enzymatic digestion by protease and amyloglucosidase to remove protein and starch. The residue was filtrated and washed with water. The washed residue was used for the determination of IDF and the filtrate was used for the determination of SDF according to the method described by Prosky *et al*. (1992).

Faecal sample collection and processing

At the end of the feeding period, fresh faecal samples were collected from each and every animal just after defecation. Immediately following the mixing, approximately one gram (1 g) of faecal material was added into sterilised sampling bottles containing 9 mL of autoclaved peptone water (Oxoid, CM009). Then, 1 mL of homogenised sample was serially diluted in 10-fold increments in sterilised peptone water for microbial enumerations.

Enumeration of bacteria in swine faecal matter

Diluted faecal samples were homogenised using K-550-GE vortex machine (Scientific Industries Inc, Bohemia, NY, USA). From each dilution level, 0.1 mL was plated on the following agar media. Lactic acid bacteria were enumerated on *Lactobacillus* MRS Agar (HIMEDIA, M641-500G) after incubation at 37 ºC for 48 – 72 hrs in an incubator (ALP Co, Ltd., Tokyo, Japan) where anaerobic environment was generated using AnaeroGen sachets (Oxoid, AN0025A) in anaerobic jars (Oxoid Ltd., Hampshire, England). Bifidobacteria were enumerated on modified *Bifidobacterium* agar (HIMEDIA, M1858-500G) after anaerobic incubation at 37 ºC for 48 – 72 hrs in an incubator (ALP Co, Ltd., Tokyo, Japan). Coliform bacteria were counted on MacConky agar (HIMEDIA, MH081-500G) after aerobic incubation at 37 ºC for 24 hrs.

Statistical analysis

For the analysis of water soluble prebiotic carbohydrates and dietary fibres, completely randomised design (CRD) was conducted and for the feeding trial randomised complete block design (RCBD) was conducted. Bacterial counts were transformed to $\log_{10}$ values before statistical analysis. All data were analysed by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS software programme (SAS Institute, Cary, NC, USA). Significant differences among means were separated by the Duncan's multiple rang test (DMRT). Differences at $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Dietary fibre content in local varieties and imported hybrids of pumpkin

Dietary fibre, a group of plant carbohydrate polymers, includes both oligosaccharides and polysaccharides which may be associated with lignin and other non-carbohydrate components such as polyphenols, saponins and waxes (Elleuch *et al*., 2011). Dietary fibres have been categorised into two major groups called insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) based on their water solubility (Elleuch *et al*., 2011). In the current study both IDF and SDF contents were analysed separately. Table 2 shows the dietary fibre composition of seven pumpkin varieties grown in Sri Lanka. IDF content was different ($p < 0.05$) among seven varieties tested in the current study. The values ranged from 16.32 to 22.34 % DM where the highest IDF value was observed in 'Suprima' variety and the lowest in 'Villachchi' variety.

SDF recorded in the present study accounted for less than 13 % of dry matter (DM). In particular, local varieties had higher ($p < 0.05$) SDF than that of the hybrid varieties where the highest value was observed in local variety Villachchi (12.2 % DM). Hence, it could be argued that there may be higher prebiotic potential in local pumpkin varieties than hybrid varieties available in Sri Lanka. However, the total dietary fibre content of pumpkin pulp was not significantly different among varieties. Furthermore, SDF represented 30.4 % of the total dietary fibre in pumpkin and the rest was IDF.

Composition of water soluble carbohydrates in local varieties and imported hybrids of pumpkin

The concentrations of water soluble carbohydrates (WSC) detected by HPLC technique are presented in Table 3. The results showed that the WSC profile of pumpkin composed of glucose, fructose, sucrose, sorbitol, mannitol, starchyose, raffinose, verbascose, kestose, and nystose. Collectively, the reducing sugar concentration (glucose+fructose) of pumpkin pulp varied from 1492.7 to 8139.8 mg/100 g DM in Pragathi and Arjuna varieties, respectively where the average value was 5566.8 mg/100 g DM. The reducing sugar content of pumpkin pulp did not significantly depend on the variety.

Table 2: Dietary fibre content (DF) of local varieties and imported hybrids of pumpkin pulp (dry matter basis)

Variety/ hybrid	Insoluble DF (%)	Soluble DF (%)	Total DF (%)
Moragollagama	16.6 ± 0.1 [b]	11.8 ± 0.4 [a]	28.4 ± 0.3 [a]
Villachchi	16.3 ± 2.2 [b]	12.2 ± 1.9 [a]	28.5 ± 0.3 [a]
Abishek	22.1 ± 0.5 [a]	7.2 ± 0.7 [b]	29.3 ± 0.1 [a]
Pragathi	19.8 ± 0.1 [ab]	6.7 ± 1.6 [b]	26.5 ± 1.5 [a]
Suprima	22.3 ± 1.7 [a]	7.4 ± 2.2 [b]	29.7 ± 0.5 [a]
Lara	21.4 ± 2.3 [a]	7.2 ± 1.4 [b]	28.6 ± 3.7 [a]
Arjuna	19.7 ± 0.6 [ab]	7.4 ± 0.2 [b]	27.1 ± 0.4 [a]
Mean	19.7 ± 2.6	8.6 ± 2.5	28.3 ± 1.6

Values are means ± standard deviation. n = 2

Means with different superscript letters within a column are significantly different ($p < 0.05$)

In terms of non-reducing sugar (sucrose), Arjuna variety had a significantly higher content of sucrose (2326.5 mg/100 g DM) compared to that of the other varieties. According to the current study, fructose was the most abundant WSC in pumpkin pulp followed by glucose and fructose.

As shown in Table 3, sorbitol and mannitol were the sugar alcohols identified in pumpkin pulp. Mannitol tends to be more common in vegetables, whereas sorbitol is found more commonly in fruits (Muir *et al.*, 2009). The average sugar alcohol content of four pumpkin varieties analysed was 127.8 mg/100 g DM and the highest concentration was found in the variety Villachchi. Furthermore, this variety was characterised by the highest amount of mannitol (66.8 mg/100 g DM). However, analysis of sugar alcohols in pumpkin varieties did not reveal significant differences among varieties.

The average total starchyose + raffinose concentration of analysed varieties was 76.5 mg/100 g DM and values were not significantly different ($p > 0.05$) among varieties. Moragollagama variety showed the highest concentration of starchyose+raffinose and Villachchi variety showed the lowest concentration among all investigated varieties.

The mean verbascose + kestose concentration was 294.5 mg/100 g DM in pumpkin pulp where the highest concentration was found in Villachchi variety and the lowest concentration was in Pragathi variety. However, analysis of verbascose + kestose in pumpkin pulp did not reveal variation by variety. The nystose concentration of pumpkin was found in a range from 32.1 to 48.6 mg/100 g DM for Arjuna, Moragollagama, and Pragathi varieties. The mean nystose concentration of pumpkin varieties analysed was 37.5 mg/100 g DM and the values were not significantly different among varieties.

In previous studies, a number of food crops, including vegetables, roots and tuber crops have been reported as potential sources of prebiotic carbohydrates (Dwivedi *et al*., 2014). Prebiotic carbohydrate profile of pumpkin analysed in the present study includes sugar alcohols (sorbitol and mannitol), raffinose family sugars (raffinose, stachyose and verbascose) and fructooligosaccharides (kestose and nystose). Composition and content of prebiotic carbohydrates in plant tissues depend highly on genetic factors and environmental conditions (Johnson *et al*., 2013).

An understanding of the prebiotic concentrations in pumpkin provides a better tool to select a more nutritious pumpkin variety and opportunities to further improve overall pumpkin nutritional quality through breeding programmes.

Effect of pumpkin powder incorporated diets on the faecal bacterial population of pigs

Lactic acid bacteria, bifidobacteria, and coliform bacteria counts in faecal matter of pigs were affected by the diets. SD with probiotic, PD, and PD with probiotic fed groups had higher ($p < 0.05$) counts of faecal lactic acid bacteria and bifidobacteria in comparison to the SD fed group. The highest population of lactic acid bacteria was observed in faeces of pigs fed with PD with probiotic. Furthermore, the highest counts of bifidobacteria were observed in faeces of pigs fed with PD with probiotic. However, there was no difference ($p > 0.05$) in faecal bifidobacteria

Table 3: Prebiotic carbohydrates of four pumpkin varieties grown in Sri Lanka (mg/100g, dry matter basis)

Variety	Reducing sugar		Non reducing sugar	Sugar alcohols		Stachyose + Raffinose	Verbascose + Kestose	Nystose
	Glucose	Fructose	Sucrose	Sorbitol	Mannitol			
Villachchi	2643.4 ± 3325.3 [a]	3317.5 ± 3447.9 [a]	648.9 ± 582.6 [b]	229.9 ± 307.3 [a]	66.8 ± 106.0 [a]	19.3 ± 8.6 [a]	464.8 ± 782.8 [a]	37.3 ± 21.8 [a]
Moragollagama	2455.8 ± 1533.6 [a]	4217.6 ± 3060 [a]	1185.9 ± 603.8 [b]	87.1 ± 34.5 [a]	36.1 ± 16.2 [a]	176.5 ± 202.0 [a]	368.0 ± 152.2 [a]	32.1 ± 3.8 [a]
Pragathi	473.0 ± 537.3 [a]	1019.7 ± 1357.7 [a]	299.8 ± 322.1 [b]	43.6 ± 9.4 [a]	26.8 ± 11.5 [a]	42.0 ± 30.3 [a]	46.4 ± 44.6 [a]	48.6 ± 27.0 [a]
Arjuna	3440.8 ± 5216 [a]	4699.0 ± 6112.8 [a]	2326.5 ± 217.3 [a]	66.2 ± 6.6 [a]	36.0 ± 9.1 [a]	68.3 ± 50.1 [a]	298.9 ± 212.7 [a]	32.0 ± 37.4 [a]
Mean	2253.3 ±1261.3	3313.5 ± 1632.8	1115.3 ± 885.9	106.7 ± 84.0	41.4 ± 17.5	76. 5 ± 69.6	294.5 ± 178.8	37.5 ± 7.8

Values are means ± standard deviation. n = 3

Means with different superscript letters within a column are significantly different ($p < 0.05$).

counts in pigs fed with SD with probiotic and PD diets. The faecal coliform counts in SD with probiotic and PD with probiotic fed pigs were significantly higher than the other diets fed pigs. The lowest counts of faecal coliform counts were observed in pigs fed with PD with probiotic diet.

In the present study, an increase in the number of lactic acid bacteria and bifidobacteria in the faeces of pigs fed with pumpkin powder incorporated diets has been observed. This could be related to the chemical composition of pumpkin powder as it is characterised by a high content of dietary fibre including considerable amount of prebiotic carbohydrates. The Villachchi variety used for the feeding experiment had the highest amount of prebiotic carbohydrates and dietary fibre among all the varieties analysed. Dietary fibres of pumpkin reach the colon and is fermented by specific colonic bacteria and converted to short chain fatty acids (SCFAs). Lactobacilli and bifidobacteria are involved in the fermentation process and the fermented products provide energy for the growth and replication of these beneficial bacteria (Stephen & Cummings, 1980; Lattimer & Haub, 2010). Particularly, slower passaging rate in the colon facilitates the establishment of a dense and complex anaerobic microflora and prebiotic mechanism (Kidder & Manners, 1978). Similarly, several studies have shown that the caecal population of beneficial microorganisms increased through the dietary fibre rich diets in humans, pigs, and rats (Liu *et al.*, 2012; Gunasekarum *et al.*, 2013).

According to the present study, pumpkin contains considerable amounts of sugar alcohols (sorbitol, mannitol), raffinose family oligosaccharides (raffinose, verbascose, stachyose) and fructooligosaccharides (FOS) (nystose, kestose).

In the present study, supplementation of diet with pumpkin powder is associated with significant reduction in coliform bacteria. This could be associated with higher counts of beneficial bacteria in the digestive tract of pigs. Bifidobacteria and lactobacilli inhibit pathogens like *E. coli*, *Campylobacter* and *Salmonella* spp. and lowers the pH of the gut which makes an unfavourable condition for pathogens through metabolic products such as SCFAs, competition for nutrients and colonisation sites, as well as direct antagonism through natural antimicrobial excretion (Gibson & Wang, 1994).

In vitro study by Sreenivas and Lele (2013) has shown that *Cucurbita maxima* supports the growth of *Lactobacillus fermentum*, *Bifidobacterium breve* and *Clostridium acetobutilicum* species. As supported by the previous *in vitro* study and the present *in vivo* study, prebiotic ability of pumpkin powder extends the scope of their use in functional foods. Possibilities for incorporation of pumpkin into common foodstuffs have been investigated in several studies. For example, addition of *Cucurbita maxima* in wheat bread, reduced-fat set-type yoghurt and reduced-fat frankfurters has been reported, showing its industrial applications (Różyło *et al*., 2014; Kim *et al*., 2016; Bakirci, *et al*., 2017).

Despite the health protective value, addition of prebiotic rich food ingredients reduces the caloric value and improve sensory characteristics of a wide range of food applications such as dairy and bakery products (Al-sheraji *et al*., 2013).

Effect of pumpkin powder incorporated diets on growth of probiotics

The faecal counts of lactic acid bacteria and bifidobacteria in SD with probiotic fed group was significantly higher ($p < 0.05$) than the counts of SD fed group. Possibly, this reflects the ability of probiotics in colonisation of the gut. Moreover, the population of bifidobacteria in faeces was higher ($p < 0.05$) in pigs fed with PD with probiotic diet than in those fed with SD with probiotic diet. This observation indicated that survival and colonisation of supplemented *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus acidophilus* have been favoured in pigs fed with pumpkin powder. This could be due to the presence of dietary fibre including prebiotic carbohydrates in pumpkin powder. A previous study has shown that mice fed with different prebiotics increase the numbers of probiotic bacteria such as *Lactobacillus acidophilus* L10, *Bifidobacterium lactis* B94 and *Lactobacillus casei* L26 present in the faeces when compared with those fed with glucose (Su *et al*., 2007). A recent study also indicated that there might be a potential of using prebiotics alone, or in combination with probiotics, to immunomodulate diseases like rheumatoid arthritis and cancer in humans (Macfarlane *et al*., 2008).

CONCLUSION

This study showed that pumpkin (*Cucurbita maxima*) pulp contains nutritionally important prebiotic carbohydrates (mannitol, sorbitol, stachyose, raffinose, verbascose, nystose and kestose). Moreover, local pumpkin varieties are characterised by the highest concentration of prebiotic carbohydrates and soluble dietary fibres. The beneficial microorganism composition of pig faeces was increased by the pumpkin powder incorporated diet, indicating the prebiotic potential of pumpkin flour. However, quantitative estimation of the composition of microbial community of faecal matter using a molecular genetic method to confirm the prebiotic property of pumpkin is necessary. Further studies are also required to analyse the morphological changes in gut and SCFAs concentrations in the gut or faecal matter of the experimental animal with the incorporation of pumpkin powder into the diet.

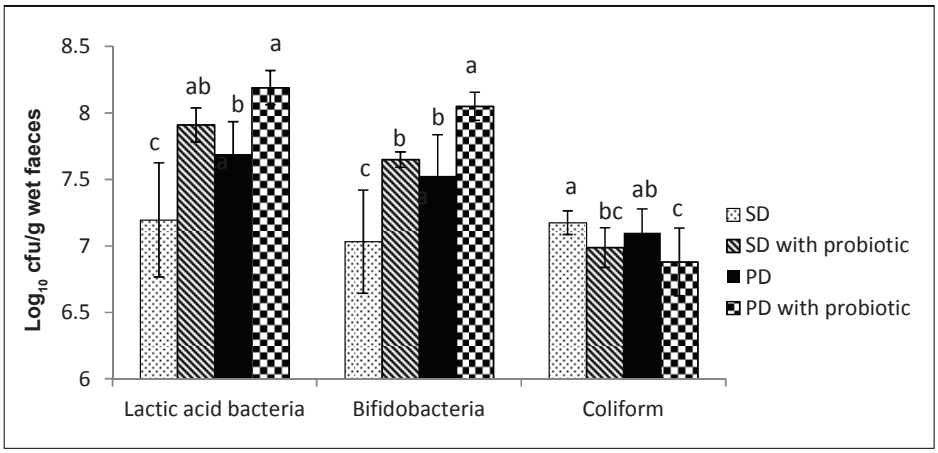

Figure 1: Faecal bacterial population of pigs fed with experimental diet
Standard diet (SD); SD which was partly replaced with pumpkin powder (100 gkg^{-1}) (PD). Values are given as means of four replicates ± SD. Means with different superscript letters within same bacterial group are significantly different ($p < 0.05$).

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