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Effects of dietary supplementation with Jerusalem artichoke (*Helianthus tuberosus* L.) tubers on growth performance, nutrient digestibility, activity and composition of large intestinal microbiota in rats

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ABSTRACT. In order to investigate the effects of dietary supplementation with Jerusalem artichoke (*Helianthus tuberosus* L.; JA) as prebiotic, 72 Wistar rats were allocated into 4 groups (JA-0, JA-2, JA-4 and JA-6) and fed for 12 weeks a basal diet supplemented with 0, 2, 4 and 6% of pulverized JA tubers. The JA addition did not affect the growth performance of animals and had no detrimental effect on feed intake, however fibre digestibility was improved (linear, $P = 0.003$) whereas dry matter and organic matter digestibility (linear, $P = 0.012$ and 0.001, respectively) were decreased. Apparent digestibility of calcium and phosphorus was increased (quadratic, $P = 0.002$ and 0.005, respectively) in rats fed diets with JA supplementation. The JA addition into diets significantly ($P \leq 0.05$) increased the populations of beneficial *Lactobacillus* spp. and *Bifidobacterium* spp. microbiota in the caecal, colonic and rectal digesta. Acetic acid (linear, $P = 0.019$), propionic acid (linear, $P = 0.008$) and total short-chain fatty acids (SCFA; linear, $P = 0.007$) concentrations were elevated as the level of JA in the diet increased. Lactic acid content was increased (quadratic, $P = 0.009$) whereas pH (linear, $P = 0.024$ and quadratic, $P = 0.047$) and ammonia concentration (linear, $P < 0.001$) were reduced in faeces due to JA supplementation. In conclusion, feeding diets supplemented with JA tuber powder beneficially augments fibre utilization along with better apparent absorption of calcium and phosphorus and positive shift in large intestinal microbiota populations and SCFA concentrations.

Introduction

Prebiotics are dietary components resistant to digestion in the small intestine which are selectively fermented by the microbiota, mainly in the large intestine. They are expected to provide health promoting properties (Roberfroid et al., 2010) being a substrate for potentially beneficial endogenous gut microbiota (e.g., *Bifidobacterium* spp. and *Lactobacillus* spp.) residing in the hindgut, but not to the potentially pathogenic bacteria such as toxin producing *Clostridium* spp., proteolytic *Bacteroides* and toxigenic *Escherichia coli* (Samal and Behura, 2015). Among wildly known prebiotics, inulin-type fructans are the most commonly used. These carbohydrates are β-(2→1) linear fructans with a terminal

glucose unit linked by an α-(1→2) bond. They are neither hydrolysed by the low pH of gastric fluid nor by the host endogenous digestive enzymes in the small intestine (Flickinger et al., 2003). So they reach the hindgut in the intact form and are available for fermentation by bacteria yielding a variety of products, such as short-chain fatty acids (SCFA) and gases (CO_2, CH_4, H_2, etc.). The SCFA can contribute up to 30% of the energy supplied to the host. Another positive side effect of the SCFA production is the reduction of the digesta pH and, in consequence, the proliferation of beneficial lactic acid-producing bacteria (Flickinger et al., 2003). Moreover, the undissociated SCFA, as anti-bacterial agent, can act against pH-sensitive pathogenic bacteria (den Besten et al., 2013).

Inulin-type fructans are either synthesized from sucrose or prepared commercially from inulin-rich plants such as chicory (*Cichorium intybus* L.) root and Jerusalem artichoke (*Helianthus tuberosus* L.; JA) tuber. The yield potential, both for biomass and sugars, is higher in JA than chicory. The JA plant shows good frost and drought tolerance, is resistant to diseases and can achieve a high yield of biomass (Slimestad et al., 2010). The inulin content in JA tuber ranges from 7 to 30% of fresh weight (Kays and Nottingham, 2007) or from 60 to 85.5% of dry weight (Aduldecha et al., 2016). Tubers of JA have high metabolizable energy content of 15 MJ · kg^{-1} dry matter. Kleessen et al. (2007) confirmed its prebiotic features and equivalence to the inulin from chicory in snack bars. However, most of the research has been limited to chicory-derived inulin and the JA tubers are being seldom investigated for its prebiotic effect on different formulations (Ramnani et al., 2010). Inulin obtained from different plant species may differ in its properties, i.e. degree of polymerization (DP), and thus in its biological activity (Mensink et al., 2015). Such factors as inulin content in raw material, dietary level, animal species, duration of feeding, etc. may also determine the effect of inulin. The inulin DP further depends on plant source, climate, growing conditions, harvesting maturity and storage time after harvest (Rubel et al., 2014). *In vivo* studies on prebiotics like inulin and fructooligosaccharides have demonstrated encouraging results, mainly attributed to the effect of these probiotics on fibre utilization, apparent absorption of calcium and phosphorus and modulation of hindgut fermentation (Delzenne et al., 1995; Wolf et al., 1998; Strickling et al., 2000; Hesta et al., 2001; Swanson et al., 2002; Propst et al., 2003; Rideout and Fan, 2004; Kleessen et al., 2007; Ramnani et al., 2010; Samal et al., 2012). In a recent study, the authors have demonstrated the beneficial effects of JA tubers powder addition on gut health (Barszcz et al., 2016). Unlike to the supplementation with purified inulin, the present experiment was undertaken to assess the influence of different levels of pulverized JA tuber addition on growth performance, nutrient utilization and hindgut fermentation in rats.

Material and methods

Preparation of JA powder

Fresh JA tubers were purchased from the local market, washed with cold water and sliced. The sliced material was sun-dried followed by drying at 70 °C in a forced-air oven, ground to pass through a 1-mm sieve in a laboratory mill (SM 100, Retsch GmbH, Haan, Germany) and stored in air-tight containers.

Experimental animals and management

Seventy two Crl:WI(Han) Wistar rats (4-week old; 36 males and 36 females weighting 100–150 g and 90–120 g, respectively) were obtained from the Laboratory Animal Research Section of the Indian Veterinary Research Institute (Izatnagar, India). The experimental procedures, housing facilities and routine care of the rats were in accordance with the ethical protocol, and were approved by the Staff Research Council and Institutional Animal Ethics Committee (IAEC) of the Indian Veterinary Research Institute (Izatnagar, India). The rats were housed in polypropylene cages with wire tops (cage outside dimensions: 38.74 × 40.13 × 24.13 cm^3) in a well-ventilated and climate-controlled room (temperature: 22–24 °C, relative humidity: 40–70%, ventilation rate: 15–20 air changes per hour) under strictly hygienic conditions. A 12-h light/12-h dark cycle was maintained. Chopped paddy straw was used in the cages as bedding material.

Diets and experimental design

The rats were fed for 2 weeks a standard diet. After this acclimation period, rats were randomly allocated (3 rats in each cage and 6 replicates per group) by live weight (LW) and by sex and divided into 4 experimental groups (JA-0, JA-2, JA-4 and JA-6). A randomized block design was used. All rats were fed conventional basal diet to meet the NRC (1995) requirements. The diet (Table 1) was pressure-cooked at 15 psi for 10 min and 20 g mineral-vitamin premix per kg diet was added before feeding.

Table 1. Composition of basal diet for rats

Indices	Content
Ingredient composition, g · kg^{-1} air-dry	
rice	300
sorghum	190
Bengal gram	250
soyabean meal	160
soya oil	40
skimmed milk powder	40
mineral-vitamin premix[1]	20
Chemical composition, g · kg^{-1} DM	
dry matter, g · kg^{-1}	960.6
organic matter (OM)	930.8
crude protein (CP)	202.7
ether extract (EE)	49.3
crude fibre (CF)	16.9
nitrogen-free extract[2]	661.8
total carbohydrates[3]	678.7
Ca	10.8
P	7.6

[1] mineral-vitamin premix was added in the amount of 20 g per kg diet and contained (per kg DM): g: Ca 113, P 95; mg: Mg 378, Na 2079, K 4.2, Cl 2740.5, Fe 83.2, Cu 10.4, Zn 94.5, Mn 5.3, vit. E (α tocopherol) 28.4, vit. K (menadione) 1.5, thiamine 1.3, riboflavin 5, pyridoxine 1.4, niacin 16.1, pantothenic acid 14.2, choline 1606.5; µg: Se 330.8, I 831.6, cholecalciferol 13.0, cobalamin 33.1, folic acid 255.2, vit A 1431.7; [2] calculated as: OM − (CP + EE + CF); [3] calculated as: OM − (CP + EE)

The amount of feed offered to rats was adjusted weekly (according to each rat LW changes). The animals from control group (JA-0) were fed basal diet with no supplementation whereas the animals from JA-2, JA-4 and JA-6 groups were fed basal diet supplemented with 2, 4 and 6% of JA tubers powder, respectively. Clean and fresh drinking water was offered *ad libitum*.

Growth trial

During 12 weeks of feeding experiment, the feed intake of rats, allocated in replicates, was monitored daily. Residual feed was collected after every feeding to determine average daily feed intake (ADFI). Individual LW was recorded on two consecutive days each week to determine average daily live weight gain (ADG). The feed conversion ratio (FCR) (feed-to-gain ratio) was calculated on the basis of unit feed consumed to unit ADG for each replicate separately. Feed conversion efficiency (FCE; gain-to-feed ratio) was calculated as the difference in LW between the final and initial weights divided by the amount of feed consumed between the days when the initial and final weights were measured. The protein conversion efficiency (PCE; crude protein intake in g per body mass gain in g) was calculated on the basis of unit protein consumed to unit ADG for each replicate separately.

Digestibility trial and sample collection

Following 7 weeks of feeding, a 6-day digestibility trial (2-day adaptation period and 4-day collection period) was performed on all rats to assess the intake and output of nutrients. Two weeks before the collections, the animals were shifted and acclimatized to well-aerated 18×24×18 cm^3 wire-bottom metabolic cages fitted with removable feeders for quantitative collection of feeds offered and residues left and tray for collection of faeces voided. A total collection method was employed. The regular routine adopted during the feeding trial was used. The LW of the individual rat was monitored before and after trial period. Faecal collection began at 09:00 on day 1 and ended at 09:00 on day 4. All faeces were stored in refrigerated (4 °C) conditions until the sampling time at 09:00 next day. A round-the-clock watch was kept to ensure immediate collection and storage of faeces so as to avoid loss of volatile compounds and to prevent microbial growth. The faecal samples collected for 24 h were pooled replicate-wise in a clean airtight low-density polyethylene (LDPE) bags and weighed for the quantity of daily faecal output. The pH of the fresh faecal samples was measured *in situ* using a digital pH meter (pH Spear, Eutech Instruments, Klang Selangor D.E., Malaysia). A suitable aliquot of fresh faeces was drawn for DM determination and another aliquot was acidified with 10 ml of 4.6 mol · l^{-1} sulphuric acid for subsequent estimation of nitrogen. Separate aliquots were also taken for estimation of ammonia, lactic acid and SCFA concentrations. Representative samples of feed, refusals and faeces were pooled replicate-wise over the period of 4 days, dried at 70 °C in a forced-air oven, ground to pass through a 1-mm mesh screen in a laboratory mill (SM100, Retsch GmbH, Haan, Germany) and stored in airtight LDPE bags until further analysis.

Microbial count from large intestinal samples

After 12 weeks of feeding trial, rats were anaesthetized with chloroform and the abdominal cavity was opened to take intestinal digesta samples. The caecum, colon and rectum contents were dispensed in separate sterile tubes and stored anaerobically for microbiological examination. From a suspension of 1 g of homogenized digesta and 9 ml of sterile 0.9% saline (pH 7.4), two sets of dilution series (10^{-1} to 10^{-9}) were prepared. One ml of each

dilution was inoculated onto petri dishes with selective media. *Lactobacillus* spp. were grown on Difco™ Rogosa SL agar (BBL-Difco Laboratories, Detroit, MI, USA) and incubated aerobically at 37 °C for 24 h. *Bifidobacterium* spp. were grown on Bifidobacterium agar (Himedia, Mumbai, India) and incubated anaerobically at 37 °C for 24–48 h in BD GasPak™ EZ incubation container (BD Diagnostics, Sparks, MD, USA). Coliforms were grown on Difco™ MacConkey agar (BBL-Difco Laboratories, Detroit, MI, USA) and incubated aerobically at 37 °C for 48 h. *Clostridium* spp. were grown on Reinforced Clostridial agar (Himedia, Mumbai, India) and incubated anaerobically at 37 °C for 24–48 h in BD GasPak™ EZ incubation container (BD Diagnostics, Sparks, MD, USA). Bacterial counts were recorded as colony-forming units (CFU) and were expressed as $\log_{10}$ CFU · g^{-1} digesta. All samples were processed for bacterial cultivation within 4 h after collection.

Chemical analyses

The ground feed, residues and faecal samples were analysed in triplicate for dry matter (DM; ID 934.01) by oven drying method at 105 °C, crude fat or ether extract (EE; ID 920.39) by Soxhlet extraction, crude fibre (CF; ID 978.10), crude protein (CP; Kjeldahl-N×6.25; ID 984.13) and ash (ID 942.05) according to AOAC (2005) procedures. Organic matter (OM) and nitrogen free extract (NFE) were calculated by difference. The calcium content in the samples was determined by titrametric method (Talapatra et al., 1940) and phosphorus content was estimated spectrophotometrically adopting the metavanadate method (ID 965.17; AOAC, 2005). Ammonia, lactic acid and SCFA concentrations were determined in the supernatant of faecal aliquots as described by Kore et al. (2012).

Calculations and statistical analyses

Statistical analysis was carried out with IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA). Microbial populations and SCFA data were analysed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test. All other experimental data were analysed by two-way ANOVA with JA inclusion level and sex as factors. Data are presented as arithmetic mean and standard error of mean (SEM). Significance was declared at $P \leq 0.05$, whereas a trend/tendency was set between $P > 0.05$ and $P \leq 0.1$. The data were further analysed for linear (L) and quadratic (Q) contrasts associated with graded levels of JA tuber powder in the diets.

Results

Voluntary dry matter intake and live weight changes

Supplementation of rat diets with JA tuber powder had no effect on growth performance (Table 2). Contrary to the treatment effect, sex of the animal had a significant impact on the weekly averages of LW with higher values observed for males ($P < 0.001$) as compared to females (reflection of higher ADFI by the males). The ADG of male rats was higher ($P < 0.001$) when compared to their female counterparts (2.9 vs 1.5 g). The FCR and PCE were significantly improved in males.

Table 2. Effects of dietary Jerusalem artichoke (JA) supplementation on growth performance and feed utilization efficiency in rats after 12 weeks of feeding

Group[1]	Total gain[2]	ADG[2]	ADFI[2]	FCR[2]	FCE[2]	PCE[2]
	g			g · g^{-1}		
Males						
JA-0	238.7	2.84	17.34	0.95	1.08	0.19
JA-2	251.3	2.99	18.45	0.89	1.14	0.18
JA-4	237.9	2.83	17.75	0.90	1.11	0.18
JA-6	253.8	3.02	19.00	0.91	1.11	0.18
Females						
JA-0	127.7	1.52	15.29	1.58	0.65	0.32
JA-2	133.4	1.59	17.22	1.55	0.65	0.31
JA-4	128.6	1.53	16.27	1.67	0.62	0.33
JA-6	127.8	1.52	15.70	1.50	0.66	0.30
SEM[3]	3.30	0.04	0.27	0.04	0.02	0.01
Two-way ANOVA[4]						
D	0.654	0.654	0.286	0.902	0.949	0.844
S	0.000	0.000	0.002	0.000	0.000	0.000
I	0.803	0.804	0.557	0.902	0.938	0.909
Contrasts[5]						
L	0.649	0.653	0.365	0.769	0.818	0.571
Q	0.903	0.905	0.295	0.842	0.899	0.861

ADG – average daily live weight gain; ADFI – average daily feed intake; FCR – feed conversion ratio; FCE – feed conversion efficiency; PCE – protein conversion efficiency; [1] basal diet supplemented with 0 (JA-0), 2 (JA-2), 4 (JA-4) and 6% (JA-6) Jerusalem artichoke tuber powder; [2] values are mean of 18 rats per treatment; [3] SEM – standard error of mean; [4] D – diet effect, S – sex effect, I – interaction of sex and diet; [5] L – linear effect of JA, Q – quadratic effect of JA

Nutrient intake and digestibility

The total tract apparent digestibility of DM, OM, NFE and total carbohydrates was reduced linearly by JA tuber powder addition ($P = 0.012$, $P = 0.001$, $P = 0.022$ and $P = 0.024$, respectively; Table 3). The apparent digestibility of CF was, however, improved (linear, $P = 0.003$) by dietary supplementation with JA tuber powder. When com-

Table 3. Effects of dietary Jerusalem artichoke (JA) supplementation on coefficient of total tract apparent digestibility (CTTAD) of nutrients in rats after 7 weeks of feeding, %

Group[1]	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extract	Total carbohydrates	Ca	P
Males									
JA-0	85.40	87.67	84.13	86.95	34.82	80.28	80.77	44.23	47.57
JA-2	83.94	86.11	80.26	88.51	35.80	78.50	79.06	51.66	56.68
JA-4	82.53	85.83	82.19	88.37	43.41	77.42	78.04	54.42	56.25
JA-6	83.03	85.64	84.39	89.49	44.12	76.74	77.36	44.34	49.84
Females									
JA-0	85.58	87.57	85.53	90.75	26.61	80.68	81.16	50.47	52.63
JA-2	84.63	86.59	87.44	90.65	43.59	79.02	79.57	49.69	53.26
JA-4	83.04	84.85	82.90	90.98	42.21	77.11	77.44	54.91	57.02
JA-6	82.89	84.68	82.25	91.10	41.44	77.91	78.50	47.88	52.92
SEM[2]	0.36	0.24	0.59	0.66	0.91	0.34	0.33	0.80	0.78
Two-way ANOVA[3]									
D	0.056	0.009	0.589	0.894	0.013	0.102	0.109	0.008	0.030
S	0.668	0.432	0.149	0.072	0.681	0.651	0.651	0.215	0.389
I	0.978	0.663	0.077	0.943	0.211	0.961	0.961	0.328	0.285
Contrasts[4]									
L	0.012	0.001	0.286	0.463	0.003	0.022	0.024	0.968	0.439
Q	0.350	0.275	0.468	0.966	0.097	0.372	0.384	0.002	0.005

[1–4] see Table 2; values are mean of 6 replicates, each consisting of 3 rats per treatment

paring two sexes, a trend towards increased EE digestibility was apparent in female rats ($P = 0.072$).

The mean daily Ca intake in all 4 groups was similar. However, the apparent Ca digestibility was improved (quadratic, $P = 0.002$) in JA-4 group in comparison to JA-0 group, whereas that in the JA-2 group was comparable to values observed in both the JA-4 and JA-6 groups. A comparison of the effect of sex on the Ca metabolism revealed that male rats consumed more ($P < 0.01$) Ca than females which was observed in increased Ca excretion in faeces as well. However, this trend apparently did not persist to facilitate an augmentation in the Ca digestibility in male rats. No impact of JA supplementation was evident on the mean daily intake and faecal excretion of P by the rats. There was, however, an increase (quadratic, $P = 0.006$) in the amount of P absorbed, which increased (quadratic, $P = 0.005$) the apparent P digestibility in JA-4 group in comparison to the JA-0 group, whereas that in the JA-2 group was comparable to values observed in JA-4 and JA-6 groups. Comparison of the P metabolism data on the basis of sex revealed a similar picture as that of Ca.

Microbial populations

The counts of *Lactobacillus* spp., *Bifidobacterium* spp. and *Clostridium* spp. averaged about 8.1, 8.4 and 8.3 $\log_{10}$ CFU · g^{-1} digesta DM across treatments, respectively (Table 4). Coliforms were present in low concentrations, usually ranging from 4.3 to 5.7 $\log_{10}$ CFU · g^{-1} digesta DM. The JA tuber powder addition increased the level of *Lactobacillus* spp. in the caecum (linear, $P = 0.023$ and quadratic, $P = 0.002$), colon (linear, $P = 0.018$ and quadratic, $P = 0.019$) and rectum (quadratic,

Table 4. Effects of dietary Jerusalem artichoke (JA) supplementation on microbial count in large intestine of rats after 12 weeks of feeding, $\log_{10}$ CFU · g^{-1} digesta

Indices	Dietary group[1]				SEM[2]	*P*-value[3]	
	JA-0	JA-2	JA-4	JA-6		L	Q
Caecum							
Lactobacillus spp.	7.18[b]	8.55[a]	8.62[a]	8.03[a]	0.11	0.023	0.002
Bifidobacterium spp.	7.75[b]	8.80[a]	8.93[a]	8.09[ab]	0.14	0.367	0.007
Coliforms	5.71	4.92	4.76	5.53	0.21	0.711	0.084
Clostridium spp.	8.63	8.41	8.37	8.35	0.19	0.606	0.795
Colon							
Lactobacillus spp.	6.99[b]	8.23[a]	8.51[a]	8.11[a]	0.15	0.018	0.019
Bifidobacterium spp.	7.55[b]	8.61[a]	8.87[a]	8.46[a]	0.10	0.010	0.006
Coliforms	5.72	4.77	4.81	5.30	0.22	0.533	0.123
Clostridium spp.	8.00	7.77	7.72	8.05	0.25	0.961	0.567
Rectum							
Lactobacillus spp.	7.72[b]	8.69[a]	8.45[a]	8.25[ab]	0.11	0.169	0.020
Bifidobacterium spp.	7.33[b]	8.87[a]	8.75[a]	8.42[a]	0.16	0.045	0.014
Coliforms	5.26	4.33	4.94	4.80	0.21	0.676	0.352
Clostridium spp.	8.79	8.66	8.62	8.56	0.20	0.674	0.929

[1] see Table 2; [2] SEM – standard error of mean; [3] L – linear effect of JA, Q – quadratic effect of JA; values are mean of 6 replicates, each consisting of 3 rats per treatment; [ab] – means with different superscripts in a row are significantly different

Table 5. Effects of dietary Jerusalem artichoke (JA) supplementation on faecal pH, short-chain fatty acid (SCFA) concentrations and molar ratios (MR), lactic acid and ammonia concentrations in rats after 7 weeks of feeding

Indices	Dietary group[1]								SEM[2]	*P*-value[3]	
	JA-0		JA-2		JA-4		JA-6				
	μmol · g^{-1}	MR	μmol · g^{-1}	MR	μmol · g^{-1}	MR	μmol · g^{-1}	MR		L	Q
pH	6.41^{a}	-	6.17^{b}	-	6.08^{b}	-	6.16^{b}	-	0.04	0.024	0.047
Concentration											
acetic acid	141.43^{b}	62.82	164.83^{ab}	61.27	173.16^{a}	60.33	176.73^{a}	60.13	10.51	0.019	0.350
propionic acid	51.32^{b}	22.74	66.56^{ab}	23.68	71.01^{a}	24.80	74.12^{a}	25.10	5.90	0.008	0.309
butyric acid	29.29	13.04	35.91	13.64	39.13	13.45	40.59	13.41	4.35	0.063	0.557
isobutyric acid	0.89	0.41	1.11	0.41	1.18	0.40	1.30	0.47	0.24	0.231	0.829
valeric acid	1.06	0.50	1.28	0.51	1.47	0.55	1.63	0.47	0.47	0.416	0.999
isovaleric acid	1.03	0.49	1.23	0.49	1.33	0.47	1.53	0.42	0.43	0.375	0.946
total SCFA	225.01^{b}	-	270.92^{ab}	-	287.28^{a}	-	295.90^{a}	-	18.05	0.007	0.307
lactic acid	197.94^{b}	-	225.23^{ab}	-	247.99^{a}	-	215.43^{b}	-	5.05	0.115	0.009
ammonia	4.19^{a}	-	3.31^{b}	-	3.11^{b}	-	2.77^{b}	-	0.09	0.000	0.157

[1,2] see Table 2; [3] see Table 4; values are mean of 6 replicates, each consisting of 3 rats per treatment; [ab] – means with different superscripts in a row are significantly different

P = 0.02). Increased level of *Bifidobacterium* spp. was also observed in the caecum (quadratic, P = 0.007), colon (linear, P = 0.01 and quadratic, P = 0.006) and rectum (linear, P = 0.045 and quadratic, P = 0.014). Feeding diet supplemented with JA tuber powder tended to decrease coliforms level in caecum (quadratic, P = 0.084). This effect was observed in neither colonic nor rectal digesta. The *Clostridium* spp. count was not affected by dietary treatment.

Faecal pH, SCFA, lactic acid and ammonia concentrations

The pH of the faecal samples ranged from 6.08 to 6.41 and was decreased (linear, P = 0.024 and quadratic, P = 0.047) by JA addition into diet. Feeding diets supplemented with JA resulted in a dose-dependent increase in total SCFA (P = 0.007), acetic acid (P = 0.019), propionic acid (P = 0.008) and butyric acid (P = 0.063) concentrations (Table 5). The isobutyric, valeric and isovaleric acid contents were not influenced by dietary treatment. Ammonia concentration in faeces decreased (linear, $P < 0.001$) with increasing level of JA tuber powder in the diets. Faecal lactic acid concentration increased due to inclusion of JA tuber powder (quadratic, P = 0.009).

Discussion

The aim of present experiment was to investigate the effect of dietary supplementation with fructan-rich JA tuber powder as prebiotic on growth performance, nutrient digestibility and hindgut fermentation in rats. All diets were accepted readily by the rats and incorporation of JA tuber to the basal diet had no undesirable impact on the palatability. Inclusion of JA tuber also did not affect growth pattern of rats over the 12-week period. Earlier studies also reported that supplementation of dietary fibres from potatoes (Lærke et al., 2007) and wheat bran (Al-Shagrawi et al., 1999) did not influence rat weight gain. However, Cieślik et al. (2005) observed that JA flour, incorporated at 5, 10 and 15%, decreased the rat growth rate. Wolf et al. (1998) did not observe any influence on the ADFI and ADG of rats by feeding fructooligosaccharides (FOS) up to 5% level and this is in agreement with our findings. However, it was observed that during the restricted-feeding phase, rats fed FOS-containing diets showed a linear decrease in intake, higher ADG and improved FCE as compared to controls with increasing FOS supplementation. The inulin supplementation had limited effect on the amount of feed intake and had no effect on body weight of mice (Kuo et al., 2013).

Reduced nutrient digestibility due to JA tuber powder supplementation is consistent with the earlier reports on inulin and FOS (Propst et al., 2003), FOS (Flickinger et al., 2003) and monooligosaccharides (MOS) (Zentek et al., 2002) supplementation. Wolf et al. (1998) also observed lower apparent DM digestibility in rats consuming FOS-containing diets. Nevertheless, no difference in DM and OM digestibility was reported when diets contained FOS or MOS (Strickling et al., 2000; Swanson et al., 2002) as well as JA (Samal et al., 2012; Pradhan et al., 2015). On the contrary, Pradhan et al. (2015) observed a reduction in protein digestibility upon dietary supplementation with 2% of JA in the diet. Increased CF digestibility in JA-fed rats is in agreement with the findings of Zentek et al. (2002),

Pawar (2007) and Kore et al. (2012) who used MOS as a prebiotic. Samal et al. (2012) also recorded increased CF digestibility when JA was included in the diet. The significant improvement in the fibre digestibility can be attributed to greater fermentability of the dietary fibre in the hindgut resulting from bacteria growth stimulation. Improvement in apparent Ca absorption, found in the present study, is in agreement with other studies (Coudray et al., 1997; Pawar, 2007; Samal et al., 2012). Likewise, inulin (Ohta et al., 1994), FOS (Delzenne et al., 1995) and a mixture of inulin and FOS (Zafar et al., 2004) had also a positive effect on Ca absorption in rats. Similarly to the present findings, higher digestibility of P was encountered upon JA supplementation (Samal et al., 2012) but Pawar (2007) did not find any effect of MOS on P absorption. Contrary to the present observation, Rideout and Fan (2004) reported that inulin had no effect on P absorption but reduced urinary P excretion in growing pigs. Wolf et al. (1998) studied the influence of FOS on mineral bioavailability using 1–5% prebiotic addition to the rat diet and observed no stimulatory effect. Lowered pH of intestinal digesta favours passive diffusion through mucosa. Therefore prebiotics facilitate transfer of water into the large intestine, thus allowing Ca and P to become more soluble and better absorbed. It may suggest that caecum fermentation is important for Ca absorption in rats.

The increase in *Lactobacillus* spp. and *Bifidobacterium* spp. populations by JA tuber powder addition represents a clear improvement in the status of eubiosis. This may be the result of selective fermentation of inulin and FOS by these beneficial bacteria in the hindgut. Several studies (Swanson et al., 2002; Samal et al., 2012; Utami et al., 2013) also showed that inulin-type fructans stimulate growth of *Lactobacillus* spp. and *Bifidobacterium* spp. Abhari et al. (2015) and Massot-Cladera et al. (2015) also observed increased faecal count of these bacteria in inulin-fed rats. The increase in *Lactobacillus* spp. populations is in agreement with greater lactic acid concentration in rat faeces. The present findings support those of Kleessen et al. (2007) who reported increased faecal counts of *Bifidobacterium* spp. with concomitant reduction in *Bacteroides/Prevotella* (in number) and *Clostridium histolyticum/C. lituseburense* group (in frequency) in human volunteers. In another study related to the bifidogenic potential of JA, Semjonovs et al. (2007) observed that supplementation of milk and oat hydrolysate medium with JA concentrate and subsequent fermentation with different probiotic dairy starters resulted in a substantial stimulation of beneficial bacterial growth (*Bifidobacterium lactis* and *Lactobacillus acidophilus* as well as *L. bulgaricus*) and acidification rate. In the present study, no discernible variation was noted in the *Clostridium* spp. count among the groups. However, there was a declining trend of coliform populations in the caecal digesta. In contrast, the inhibitory effect of inulin-type fructans on *Clostridium* spp. was reported in the earlier studies (Xu et al., 2002; Vhile et al., 2012). Strickling et al. (2000), Flickinger et al. (2003) and Samal et al. (2012) found no influence of MOS, FOS and JA on the coliforms count, respectively.

The decreased faecal pH in rats fed diet supplemented with JA tuber powder may be explained by the increased SCFA levels. Faecal pH is affected by the metabolic reactions occurring in the gastrointestinal tract, including the degradation of fibre (Al-Shagrawi et al., 1999). Feeding diets with JA addition resulted in a dose-dependent increase in total SCFA content in faeces. Lower ammonia level, observed due to JA supplementation, may be explained by an enhanced synthesis of bacterial protein and may also contribute to lower faecal pH. Low pH exerts a negative impact on the growth of potential pathogens and improves nutrient absorption. The faecal pH and lactic acid concentrations are inversely related (Brooks et al., 2001). However, prebiotics do not always efficiently decrease the faecal pH, as it was shown by Hesta et al. (2003) and Kore et al. (2012). Hesta et al. (2001) examining FOS or inulin usage as prebiotic observed decreased faecal pH and increased concentration of total SCFA but only when higher levels were used and not even with 3% level of supplementation. Zentek et al. (2002) and Flickinger et al. (2003) noted decreased ammonia concentration due to diet supplementation with MOS or FOS, respectively, in the agreement with the present results. Nevertheless, no influence of prebiotics on the faecal ammonia concentration was observed (Strickling et al., 2000; Swanson et al., 2002; Kore et al., 2012). In contrast, the faecal ammonia content was higher due to MOS (Pawar, 2007) and FOS or inulin (Propst et al., 2003) supplementation. Similarly to the present results, the faecal lactic acid concentration was significantly higher upon FOS (Swanson et al., 2002) or MOS (Pawar, 2007) supplementation. As it was presented in other studies (Propst et al., 2003), total SCFA content increased linearly with increasing concentrations of prebiotic, with the greatest value noted for the 0.3% FOS treatment. Addition of fermentable carbohydrates to pig

diets raised SCFA production in the hindgut (Claus et al., 2003; Vhile et al., 2012). As it was previously shown, the faecal concentrations of acetate and propionate were much higher than that of butyrate in rats (Rodríguez-Cabezas et al., 2003). Massot-Cladera et al. (2015) observed that the diet containing inulin tended to reduce acetic acid and increase propionic acid proportion with the respect to their initial values as observed in the present study. No effect of JA tuber powder addition into diet on butyric acid concentration in the hindgut was observed in a study by Vhile et al. (2012). Lærke et al. (2007) reported greater propionate production due to dietary supplementation of soluble fibres from potatoes in rats. The total SCFA and particular acids produced during fermentation of oligofructose from yacón (*Smallanthus sonchifolius*) tuber were greater than in the control diet for rats (Utami et al., 2013). In the present experiment, there was no effect of diet on valeric acid concentration in the hindgut. In contrast, higher level of valeric acid was observed when inulin-type fructans were added to the diet in the studies by Øverland et al. (2011) and Vhile et al. (2012). However, no changes in concentration of faecal SCFA were observed in human volunteers consuming snack bars supplemented with JA (Kleessen et al., 2007).

Conclusions

Diet supplementation with Jerusalem artichoke tuber powder exerts a positive influence on the microbiota (*Lactobacillus* spp. and *Bifidobacterium* spp.) in the hindgut. This is connected with increased production of short chain fatty acids and subsequent reduction in digesta pH. A discernible encouraging impact was also evident on the fibre utilization and apparent absorption of minerals.

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