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Received: 11 May 2014 Revised: 9 August 2014 Accepted article published: 15 August 2014 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6873

Prebiotic potential of Jerusalem artichoke (*Helianthus tuberosus* L.) in Wistar rats: effects of levels of supplementation on hindgut fermentation, intestinal morphology, blood metabolites and immune response

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Abstract

BACKGROUND: Many studies have been conducted using purified prebiotics such as inulin or fructooligosaccharides (FOS) as nutraceuticals, but there is very little information available on the prebiotic potential of raw products rich in inulin and FOS, such as Jerusalem artichoke (JA; *Helianthus tuberosus* L.). The present experiment aimed to evaluate the prebiotic effects of JA tubers in rats.

RESULTS: Seventy-two Wistar weanling rats divided into four groups were fed for 12 weeks on a basal diet fortified with pulverized JA tubers at 0 (control), 20, 40 and 60 g kg^{-1} levels. Enhanced cell-mediated immunity in terms of skin indurations ($P = 0.082$) and CD4+ T-lymphocyte population ($P = 0.002$) was observed in the JA-supplemented groups compared with the control group. Blood haemoglobin ($P = 0.017$), glucose ($P = 0.001$), urea ($P = 0.004$) and calcium ($P = 0.048$) varied favourably upon inclusion of JA. An increasing trend ($P = 0.059$) in the length of large intestine was apparent in the JA-fed groups. The tissue mass of caecum ($P = 0.069$) and colon ($P = 0.003$) was increased in the JA-supplemented groups, accompanied by higher ($P = 0.007$) caecal crypt depth. The pH and ammonia concentrations of intestinal digesta decreased and those of lactate and total volatile fatty acids increased in the JA-fed groups.

CONCLUSION: The results suggest that JA had beneficial effects on immunity, blood metabolites, intestinal morphometry and hindgut fermentation of rats.

Keywords: Jerusalem artichoke; *Helianthus tuberosus*; hindgut fermentation; intestinal morphometry; metabolic profile; immunity

INTRODUCTION

A prebiotic is a non-digestible food ingredient that beneficially affects the host by targeting indigenous components in the gut, such as gastrointestinal microflora.[1] The use of prebiotics is a promising approach for inducing desirable hindgut fermentation by the stimulation of benign or potentially health-promoting indigenous bacteria. Inulin and fructooligosaccharides (FOS) are plant-derived prebiotics containing soluble dietary fibres that are not digested or absorbed in the small intestine owing to the absence of β-fructosidase but are fermented in the large intestine by beneficial bacteria, resulting in the production of organic acids (lactic acid and volatile fatty acids such as acetate, propionate, butyrate, etc.) and gases (CO_2, CH_4, H_2, etc.). The former are extensively absorbed and help the host to recover a part of the chemical energy from these non-viable carbohydrates.[2]

Many other beneficial health effects claimed to accrue from the use of these fermentable carbohydrates have been reported relating to glucose homeostasis[3–6] and lipid metabolism.[7–9] Moreover, about 80% of the body's immune system is localized in the gastrointestinal tract; hence prebiotics with their known positive influence on gut metabolism are thought to have potential to modulate the body's immune system.[10–12] Further, prebiotics are found to improve the intestinal morphometry via a combined effect of hypertrophy and hyperplasia, resulting in a greater exchange surface area for better nutrient absorption and utilization. These different aspects are closely linked to the type of fermentable carbohydrates (dose, structure and relative molecular mass) and the duration of the experiment.[13]

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Many studies have been conducted using inulin or FOS as dietary supplements, but there is very little information on the prebiotic efficacy of inulin and FOS present in raw products consumed as part of the daily diet. Root vegetables including Jerusalem artichoke (JA; *Helianthus tuberosus* L.), burdock, chicory, leek and onion are especially rich sources of inulin and/or FOS. Also known as wild sunflower, *H. tuberosus* is a tuberous perennial plant of the Asteraceae family and contains 160–200 g kg^{-1} inulin and 120–150 g kg^{-1} FOS on a fresh weight basis.[14] However, there is a need to confirm its prebiotic effectiveness using reliable methodologies in different formulations.[15] Therefore, the objective of this research was to evaluate the prebiotic potential of JA through the assessment of targeted blood metabolites, cell-mediated immunity and morphometric and fermentative attributes of the hindgut in rats.

MATERIALS AND METHODS

Plant material

Helianthus tuberosus tubers were washed with cold water, skinned and sliced individually to a thickness of about 5 mm. The sliced material was sun dried, followed by drying at 70 °C in a forced air oven, and ground in a laboratory mill (SM 100, Retsch GmbH, Haan, Germany) through a 1 mm mesh screen.

Rats and management

Seventy-two albino rats of Wistar strain (90–130 g and 4–6 weeks old) were procured from the Laboratory Animal Research Section, Indian Veterinary Research Institute, Izatnagar, India. For the purpose of acclimatization to the new environment, all rats were maintained for 2 weeks on a standard diet. The rats were housed (three animals per cage, six replicates per treatment) in solid bottom polypropylene cages with wire tops in a small-animal holding room under environmentally controlled conditions of 22 ± 2 °C temperature, 80 ± 10% humidity and a 12 h light/12 h dark cycle with the dark period from 20:00 to 08:00. The rats were provided with fresh food (20 g kg^{-1} live body weight (BW) day^{-1}). All rats had free access to potable water at all times. All procedures complied with the Institute's ethical guidelines on the care and use of laboratory animals.

Experimental protocol, feeds and feeding

The experiment was conducted on 72 rats allocated into four experimental groups (CON, JA-20, JA-40 and JA-60) in a randomized block design and fed over 12 weeks. The CON group (control) was fed the basal diet (Table 1) without supplementation, while JA powder was supplemented at rates of 20, 40 and 60 g kg^{-1} in the JA-20, JA-40 and JA-60 groups respectively. The quantity of diet offered was calculated so as to meet the protein and energy requirements of rats in compliance with NRC recommendations.[16] Food consumption was monitored daily and body weight was recorded weekly. Immune response was assessed after 11 weeks of the study. At the termination of the experiment, rats were anaesthetized with chloroform according to the recommendation for euthanasia of experimental animals.[17]

Sampling and processing

Cell-mediated immunity was assessed by measurement of skin indurations as type-IV delayed-type hypersensitivity (DTH) reaction to phytohaemagglutinin-P (PHA-P; Sigma, St Louis, MO, USA) as a mitogen.[18] Four rats from each group were given a DTH challenge of 25 µL of PHA-P (4 mg mL^{-1}) on one side and 25 µL of saline solution (8.5 g L^{-1} NaCl) as a negative control on the other side via intradermal injection in the flank region. The difference in skin indurations assessed at 0, 12, 24, 48 and 72 h post-injection by digital Vernier's callipers represented the DTH response.

Table 1. Ingredient composition (g kg^{-1}) of basal diet[a]

Ingredient	Concentration
Rice	300
Sorghum	190
Bengal gram	250
Soybean meal	180
Soya oil	40
Skimmed milk powder	40

[a] Pressure cooked at 15 psi for 10 min.

After 12 weeks of the feeding trial, rats were sacrificed and blood samples were collected by cardiac puncture with sterile disposable syringes and placed into two chilled heparinized microcentrifuge tubes. Fresh whole blood samples were utilized for assessment of immune phenotyping of T-lymphocyte subtypes by flow cytometry as well as for haemoglobin and haematocrit estimation. Plasma was separated from the second blood sample within 1 h of collection by centrifugation at 2000 × *g* for 10 min at 4 °C and frozen at −20 °C until analysis.

The small intestine, caecum and colon were excised and their respective lengths were measured. The caecum and colon, complete with digesta, were removed and weighed. Total caecal digesta were drained from the colonic ligature into a 20 mL vial. The caecum was then flushed with clean ice-cold saline, blotted on filter paper and weighed (caecal tissue weight). Likewise, colonic tissue weight was determined. Digesta from the caecum, colon and rectum were pooled separately in duplicate. The pH was determined *in situ* using a digital pH meter (pH Spear, Eutech Instruments, Klang Selangor D.E., Malaysia). Digesta samples were processed for estimation of lactate, ammonia and volatile fatty acids (VFA) as described elsewhere.[19]

To examine the histomorphometry, samples of duodenum, caecum and colon were excised and fixed in 100 g L^{-1} buffered formalin solution at room temperature. After Gill's haematoxylin and eosin (H&E) staining, the villus height and crypt depth of intestinal segments were measured by Image Analysis Software (Microsoft Inc., Redmond, WA, USA) using a stereozoom microscope (Olympus, Tokyo, Japan).

Laboratory analyses

Immunophenotyping of peripheral blood lymphocyte T-cell subsets (CD3, general T-cell marker; CD4+, helper T-cells; CD8+, cytotoxic/suppressor T-cells) was performed with a dual-laser benchtop FACScan flow cytometer (FACSCalibur, Becton Dickinson, San Diego, CA, USA) as described previously.[20] Analysis was performed with CellQuest Pro-software (BD Biosciences, San Jose, CA, USA).

Lactate and ammonia concentrations were determined by standard procedures.[21,22] VFA concentrations were measured by gas–liquid chromatography (Neuon, Ashco Pvt. Ltd, Mumbai, India).[23]

Haemoglobin was determined by the cyanmethaemoglobin method immediately after collection and haematocrit was determined by the standard method using a capillary centrifuge.

Table 2. Chemical composition (g kg^{-1} dry matter) of experimental diets

Attribute	Dietary group[a]			
	CON	JA-20	JA-40	JA-60
Dry matter	960.6	962.7	953.8	964.0
Organic matter	930.8	923.9	926.1	927.9
Crude protein	202.7	200.9	199.2	197.5
Ether extract	49.3	49.3	47.7	47.4
Crude fibre	16.9	17.7	18.2	18.7
Nitrogen-free extract	661.8	656.1	660.9	664.4
Total carbohydrates	678.7	673.8	679.2	683.1
Calcium	10.8	11.1	10.5	10.3
Phosphorus	7.6	7.6	7.5	7.2

[a] Basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} Jerusalem artichoke powder.

Plasma concentrations of glucose, total protein, albumin, total cholesterol, triglycerides, high-density lipoprotein (HDL), urea, uric acid, creatinine and minerals (calcium, inorganic phosphorus, sodium and potassium) were estimated using diagnostic reagent kits (Span Diagnostics Ltd, Surat, India) in a UV-2601 double-beam UV–visible spectrophotometer (Beijing Rayleigh Analytical Instrument Co. Ltd, Beijing, China). The globulin value was calculated by subtracting the albumin from the total proteins. Very-low-density lipoprotein (VLDL) was calculated as one-fifth of the triglyceride concentration,[24] while low-density lipoprotein (LDL) was calculated as LDL = total cholesterol – HDL – VLDL. Atherogenic indices were determined as cholesterol/HDL and LDL/HDL.

Statistical analyses

Elementary statistical analysis was carried out with IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA). Data are presented as arithmetic mean and standard error of mean (SEM). Procedures used included one-way analysis of variance (ANOVA) with Tukey's *post hoc* test, except for DTH data. Differences among treatment level least squares means with a probability of $P \leq 0.05$ were accepted as statistically significant, while mean differences with P ranging from 0.06 to 0.15 were accepted as trends. Least squares means of JA-supplemented groups were compared with the control group for linear and quadratic contrasts. DTH data were analyzed using generalized linear model procedures to differentiate the effects of treatments and periods.

RESULTS

Diets

The chemical composition of the experimental diets is shown in Table 2. The composition of all nutrients matches the nutrient requirement profile for laboratory rats.[16] There was no appreciable variation in the nutritional profile among the four diets.

DTH response to PHA-P

All animals showed a positive DTH response to PHA-P, with the maximum increase in skin indurations attained at 12 h post-inoculation. The net increase in skin indurations tended to be higher ($P = 0.082$) in the JA-20 group (0.41 cm) than in the CON (0.37 cm) and JA-60 (0.37 cm) groups, while that in the JA-40 group (0.39 cm) was comparable to both the JA-20 and JA-60 groups (Fig. 1).

Peripheral lymphocyte sub-populations

The CD3 population decreased (linear, $P = 0.048$) while that of CD4+ T-lymphocytes increased ($P = 0.001$) with increasing levels of JA. When compared with the CON group (8.14%), the population of CD4+ T-cells was increased in the JA-40 (12.14%) and JA-60 (11.56%) groups; the corresponding value in the JA-20 group (10.09%) was comparable to the other three groups. The population of CD8+ T-lymphocytes, however, remained similar among the four groups (Table 3). The CD4+/CD8+ ratio was enhanced (linear, $P = 0.018$) with increase in dietary JA.

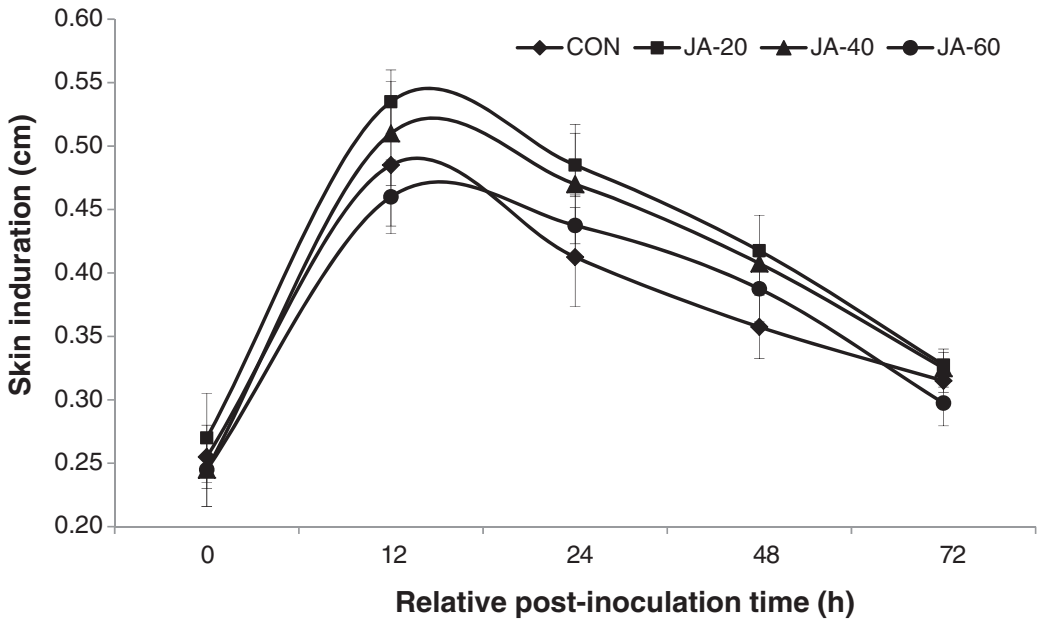

Figure 1. Effects of dietary Jerusalem artichoke (JA) on cell-mediated immunity of rats measured through DTH response to PHA-P (basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} JA powder). Values are mean of four rats per treatment.

Table 3. Effects of dietary Jerusalem artichoke on peripheral lymphocyte sub-populations (%) of rats after 12 weeks of feeding

Attribute	Dietary group[a]				SEM	*P* value (JA)	
	CON	JA-20	JA-40	JA-60		Linear	Quadratic
CD3	13.61	10.36	10.40	10.21	1.076	0.048	0.171
CD4+	8.14a	10.09ab	12.14b	11.56b	0.683	0.001	0.079
CD8+	8.21	6.14	7.07	7.20	0.952	0.625	0.262
CD4+/CD8+	0.99	1.67	2.00	1.95	0.279	0.018	0.205

Values are mean of six rats per treatment. Means with different letters in a row differ significantly. SEM, standard error of mean.
[a] Basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} Jerusalem artichoke powder.

Table 4. Effects of dietary Jerusalem artichoke on blood metabolites of rats after 12 weeks of feeding

Attribute[b]	Dietary group[a]				SEM	*P* value (JA)	
	CON	JA-20	JA-40	JA-60		Linear	Quadratic
General metabolic indices							
Haemoglobin (mmol L^{-1})	7.88a	8.09ab	8.81c	8.57bc	0.223	0.007	0.322
Haematocrit (%)	36.75	38.00	40.44	38.81	1.213	0.117	0.241
Glucose (mmol L^{-1})	7.95c	7.03bc	6.75ab	5.91a	0.324	<0.001	0.914
Lipid profile indices							
Cholesterol (mmol L^{-1})	3.53	3.06	3.31	3.34	0.145	0.588	0.091
Triglycerides (mmol L^{-1})	1.15	0.97	1.02	1.21	0.111	0.647	0.097
HDL (mmol L^{-1})	1.53	1.74	1.81	1.72	0.130	0.267	0.253
LDL (mmol L^{-1})	1.48b	0.89a	1.03a	1.07ab	0.149	0.100	0.039
VLDL (mmol L^{-1})	0.23	0.19	0.20	0.24	0.111	0.647	0.097
Cholesterol/HDL	2.50	1.95	2.04	2.03	0.172	0.090	0.129
LDL/HDL	1.12	0.67	0.75	0.68	0.149	0.067	0.211
Liver function indices							
Total protein (g L^{-1})	66.03	71.52	68.68	71.91	2.233	0.143	0.615
Albumin (g L^{-1})	37.43	38.42	37.74	39.81	1.583	0.363	0.735
Globulin (g L^{-1})	28.59	33.11	30.93	32.11	2.447	0.447	0.499
Renal function indices							
Urea (mmol L^{-1})	5.22bc	4.19a	4.60ab	5.45c	0.258	0.336	0.001
Uric acid (μmol L^{-1})	141.64	139.70	152.12	158.07	9.099	0.135	0.667
Creatinine (μmol L^{-1})	80.83	80.06	80.06	75.47	6.711	0.594	0.777
Minerals							
Calcium (mmol L^{-1})	2.28a	2.30ab	2.52b	2.51ab	0.112	0.012	0.836
Phosphorus (mmol L^{-1})	1.94	1.96	2.06	2.13	0.069	0.034	0.680
Sodium (mmol L^{-1})	149.38	144.19	148.51	147.71	7.046	0.982	0.756
Potassium (mmol L^{-1})	4.97	4.42	4.15	4.47	0.254	0.124	0.089

Values are mean of 16 rats per treatment. Means with different letters in a row differ significantly. SEM, standard error of mean.
[a] Basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} Jerusalem artichoke powder.
[b] HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

Blood metabolites

Blood haemoglobin increased (linear, $P = 0.007$) whereas haematocrit tended to increase (linear, $P = 0.117$) with increase in dietary JA. However, the glucose concentration was reduced (linear, $P < 0.001$) with increasing JA addition to the diet (Table 4). Total cholesterol, triglyceride and VLDL concentrations in plasma tended to decrease (quadratic, $P = 0.091 – 0.097$) with increased dietary JA levels. LDL levels also tended to reduce (linear, $P = 0.100$; quadratic, $P = 0.039$), so atherogenic indices also tended to decline in JA-supplemented groups. There was a trend of improvement (linear, $P = 0.143$) in total protein concentrations upon JA supplementation, whereas albumin and globulin concentrations were not affected. The plasma urea value showed a significant (quadratic, $P = 0.001$) variation resulting from JA inclusion. The calcium level varied ($P = 0.012$) among the groups, being highest in the JA-40 group. Inorganic phosphorus was also enhanced (linear, $P = 0.034$) with JA inclusion in the diet. Dietary treatments had no effects ($P > 0.05$) on blood sodium concentration, whereas potassium showed an increasing trend (linear, $P = 0.124$; quadratic; $P = 0.089$) in the treatment groups.

Intestinal morphometry

Pre-sacrifice BW of the four groups of rats did not show any apparent variation. The length of small intestine increased (linear, $P = 0.046$) in response to dietary JA inclusion. Caecum length showed no influence of JA, whereas there was an increase (linear,

Table 5. Effects of dietary Jerusalem artichoke on morphometric indices of intestinal segments of rats after 12 weeks of feeding

Attribute	Dietary group[a]				SEM	*P* value (JA)	
	CON	JA-20	JA-40	JA-60		Linear	Quadratic
Body weight (g)	305.03	325.07	336.01	333.95	18.864	0.251	0.560
Length (cm)							
Small intestine	114.50	115.06	117.94	122.17	2.848	0.046	0.522
Caecum	3.75	3.68	3.85	3.94	0.167	0.319	0.631
Colon	15.84	17.06	17.56	17.97	0.566	0.008	0.481
Weight (g)							
Caecum with digesta	4.07	4.13	4.86	4.93	0.312	0.019	0.986
Caecal tissue	1.31	1.42	1.58	1.61	0.092	0.010	0.651
Caecal digesta	2.76	2.71	3.28	3.32	0.259	0.055	0.856
Colon with digesta	2.97a	4.47b	4.40b	3.92b	0.256	0.019	<0.001
Colonic tissue	1.71a	2.19b	2.17b	2.18b	0.103	0.004	0.026
Colonic digesta	1.26a	2.28b	2.23b	1.74ab	0.215	0.155	0.001
Relative tissue mass (g kg^{-1} body weight)							
Caecum	4.42	4.48	4.78	4.88	0.251	0.140	0.941
Colon	5.85a	6.94b	6.52ab	6.64ab	0.281	0.122	0.089
Relative digesta (g kg^{-1} body weight)							
Caecum	8.95	8.47	9.87	10.02	0.612	0.098	0.613
Colon	3.97a	7.30b	6.90b	5.51ab	0.697	0.182	0.001

Values are mean of 18 rats per treatment. Means with different letters in a row differ significantly. SEM, standard error of mean.
[a] Basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} Jerusalem artichoke powder.

Table 6. Effects of dietary Jerusalem artichoke on histological attributes of various intestinal segments of rats after 12 weeks of feeding

Attribute	Dietary group[a]				SEM	*P* value (JA)	
	CON	JA-20	JA-40	JA-60		Linear	Quadratic
Duodenum (μm)							
Villus height	395.35	535.28	643.04	598.50	89.510	0.082	0.310
Crypt depth	266.40	363.22	385.38	324.37	81.089	0.465	0.192
Caecum (μm)							
Crypt depth	87.44a	86.09a	88.39a	124.86b	8.655	0.005	0.035
Colon (μm)							
Crypt depth	103.45	105.11	111.16	104.92	10.149	0.819	0.699

Values are mean of ten rats per treatment. Means with different letters in a row differ significantly. SEM, standard error of mean.
[a] Basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} Jerusalem artichoke powder.

$P = 0.008$) in colon length with JA supplementation. The total length of caecum and colon also increased (linear, $P = 0.008$) in the treatment groups.

The weight of caecal (linear, $P = 0.01$) and colonic (linear, $P = 0.004$; quadratic, $P = 0.026$) tissues increased with JA supplementation, and the same trend was apparent when the total weight was compared (Table 5). The weight of caecal (linear, $P = 0.055$) and colonic (quadratic, $P = 0.001$) digesta increased following JA supplementation. Moreover, the relative tissue mass and relative digesta (g kg^{-1} BW) of colon increased significantly in all treatment groups.

The histomorphometry of intestinal segments revealed that there was a trend of increase (linear, $P = 0.082$) in the villus height of duodenum with JA supplementation. The crypt depth of caecum was also increased (linear, $P = 0.005$; quadratic, $P = 0.035$), with the highest depth observed in the JA-60 group (Table 6).

Biochemical analyses of intestinal digesta

The pH of caecal digesta was lower in the JA-40 group than in the CON group, whereas that in the JA-60 group was comparable to both the CON and JA-20 groups. A similar trend also persisted with colonic ($P = 0.031$) and rectal ($P = 0.029$) digesta. The ammonia concentration in all intestinal segments was reduced in the treatment groups, particularly in JA-40 and JA-20, compared with the CON group (Table 7). The lactate concentration in caecal digesta (251.65–348.34 μmol g^{-1} dry matter) increased (quadratic, $P = 0.02$) for rat groups consuming JA. Total short-chain fatty acids (SCFA) as well as its fractions, i.e. acetate, propionate and butyrate, increased (linear, $P = 0.001–0.01$) in caecal digesta with increasing JA addition to the diet (Table 8). There was also an increase in total SCFA and butyrate (linear, $P = 0.024$ and 0.034 respectively) at colon level. At rectum level, all volatile fatty acids (VFA) except valerate increased (linear,

Table 7. Effects of dietary Jerusalem artichoke on biochemical characteristics of pooled intestinal digesta of rats after 12 weeks of feeding

	Dietary group[a]					*P* value (JA)	
Attribute	CON	JA-20	JA-40	JA-60	SEM	Linear	Quadratic
pH							
Caecum	6.51c	6.15b	5.74a	5.96ab	0.104	0.001	0.017
Colon	6.27	6.08	5.85	5.89	0.124	0.031	0.377
Rectum	6.67	6.32	6.15	6.21	0.141	0.029	0.171
Ammonia (μmol g^{-1} dry matter)							
Caecum	16.34b	11.16a	10.64a	14.17ab	1.442	0.297	0.011
Colon	8.95c	4.52a	8.93c	6.95b	0.565	0.541	0.051
Rectum	4.57a	3.51a	3.50a	6.20b	0.388	0.015	0.000
Lactate (μmol g^{-1} dry matter)							
Caecum	251.65a	348.34b	327.04b	311.82ab	20.831	0.113	0.020
Colon	299.81	345.74	382.70	337.41	33.362	0.335	0.197
Rectum	171.03	202.38	224.23	204.37	19.487	0.187	0.213

Values are mean of 16 rats (four pooled) per treatment. Means with different letters in a row differ significantly. SEM, standard error of mean.
[a] Basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} Jerusalem artichoke powder.

Table 8. Effects of dietary Jerusalem artichoke on volatile fatty acid concentration (μmol g^{-1} dry matter) in hindgut digesta of rats after 12 weeks of feeding

	Dietary group[a]					*P* value (JA)	
Attribute[b]	CON	JA-20	JA-40	JA-60	SEM	Linear	Quadratic
Caecal digesta							
Total VFA	279.80a	329.12ab	348.59bc	392.67c	15.230	0.001	0.868
Acetate	172.24a	197.09b	198.90b	213.58b	5.291	0.001	0.364
Propionate	59.79a	67.72a	81.69ab	96.93b	7.168	0.004	0.624
Butyrate	37.37a	52.60ab	56.01ab	69.27b	6.560	0.010	0.884
Total SCFA	269.40a	317.41ab	336.60bc	379.79c	14.744	0.001	0.874
Isobutyrate	3.48	4.12	4.17	4.47	0.349	0.089	0.642
Isovalerate	4.06	4.50	4.62	4.98	0.340	0.094	0.917
Valerate	2.86	3.10	3.20	3.43	0.282	0.187	0.995
Total BCFA	10.40	11.71	11.99	12.89	0.889	0.087	0.823
Colon digesta							
Total VFA	257.01	301.97	312.89	319.97	15.679	0.022	0.262
Acetate	156.30	179.36	181.73	185.33	9.534	0.069	0.337
Propionate	56.31	61.35	63.21	63.38	4.771	0.311	0.623
Butyrate	34.38	50.07	56.57	60.36	7.382	0.034	0.443
Total SCFA	246.98	290.78	301.51	309.06	15.849	0.024	0.286
Isobutyrate	3.46	3.94	3.96	3.78	0.322	0.505	0.337
Isovalerate	3.92	4.33	4.43	4.18	0.160	0.251	0.071
Valerate	2.66	2.92	3.00	2.94	0.224	0.382	0.504
Total BCFA	10.03	11.19	11.38	10.91	0.621	0.341	0.225
Rectal digesta							
Total VFA	134.83	164.51	178.49	181.32	11.870	0.020	0.291
Acetate	82.24	99.63	106.26	107.91	7.843	0.044	0.345
Propionate	29.65	34.79	38.38	39.16	2.945	0.041	0.481
Butyrate	18.22a	24.43b	27.69b	27.88b	1.376	0.001	0.060
Total SCFA	130.11	158.84	172.34	174.95	11.804	0.023	0.301
Isobutyrate	1.70	2.06	2.27	2.27	0.145	0.019	0.259
Isovalerate	1.79	2.12	2.28	2.44	0.174	0.028	0.626
Valerate	1.23	1.50	1.61	1.67	0.187	0.128	0.596
Total BCFA	4.73a	5.67ab	6.15b	6.37b	0.307	0.004	0.268

Values are mean of 16 rats (four pooled) per treatment. Means with different letters in a row differ significantly. SEM, standard error of mean.
[a] Basal diet supplemented with 0 (CON), 20 (JA-20), 40 (JA-40) and 60 (JA-60) g kg^{-1} Jerusalem artichoke powder.
[b] VFA, volatile fatty acids; SCFA, short-chain fatty acids; BCFA, branched-chain fatty acids.

$P = 0.001 - 0.044$) with increasing JA supplementation. Digesta total SCFA ($P = 0.023$) and total branched-chain fatty acids (BCFA; isobutyrate + isovalerate + valerate) ($P = 0.004$) also increased in JA-supplemented groups. Total VFA concentration increased linearly in all three segments for groups consuming JA.

DISCUSSION

The purpose of this work was to establish the functional attributes of *H. tuberosus* as a prebiotic *per se* rather than as a source of isolated inulin/FOS. The DTH response to the T-cell mitogen PHA-P tended to improve upon JA supplementation, indicating that JA enhances parameters of Th1-dependent immunity. In another study, dietary short-chain glucooligosaccharides and long-chain FOS were shown to have a similar effect on DTH responses besides modulating biochemical parameters (SCFA, lactate and pH).[10] The increased DTH response is suggestive of qualitative changes in the T-cell population, which was confirmed by the observed increase in CD4+/CD8+ ratio. This finding is supported by previous studies with different types of dietary fibres, suggesting that these non-digestible constituents exert distinct immunological effects in the peripheral blood.[11,12]

Improvements in the haemoglobin status of rats fed JA are indicative of enhanced iron bioavailability at intestine level.[25] The increased hindgut fermentation by prebiotics may decrease the pH of the luminal content, promote reduction of Fe(III) to Fe(II) and stimulate proliferation of epithelial cells to expand the absorptive surface area of caecum and colon mucosa for mineral absorption.[25,26] Lowered pH of intestinal digesta favours passive diffusion through mucosa; prebiotics therefore facilitate transfer of water into the large intestine, thus allowing minerals to become more soluble. The observed linear improvement in plasma calcium and phosphorus levels could also be explained on a similar basis. The results agree with previous reports on human[27] and animal[25,28] models. A reduction in plasma glucose with increasing levels of JA is consistent with the perceived influence of prebiotics in controlling glucose homeostasis. There are various reports that have highlighted the role of prebiotics in improved glucose tolerance and reduced glycaemia in diabetic rats[3] and stabilized blood sugar levels in normal rats[4] and human subjects.[5] This effect could be partly explained by the satietogenic role of prebiotics,[6] reduced hepatic gluconeogenesis, delayed gastric emptying and/or shortened transit time through the small intestine. The observed decline in total cholesterol, LDL, VLDL and triglyceride levels is in agreement with the work of several other research groups.[3,7] In an experiment with rats, Varlamova *et al.*[9] reported that the total cholesterol level was reduced with growing proportions of artichoke flour supplement in the diet. It has been proposed that hemicelluloses and pectins present in JA tubers might bind bile acids and thus make difficult the process of emulsification, digestion and absorption of triglycerides.[8] The reduction in cholesterol, therefore, could have been due to the precipitation and excretion of bile acids into the intestine.[29] Similar to the present findings, no influence was observed on plasma total protein and albumin concentrations when buckwheat was used as a prebiotic.[30] Feeding rats a diet supplemented with inulin and oligofructose was reported to reduce uraemia.[25,31] In the present study, similar uric acid content was found in groups of rats fed JA-supplemented and control diets, which is in agreement with other reports.[3,30] In contrast, a significant increase in serum urea accompanied by a decrease in serum creatinine was observed when JA was supplemented in diabetic rats.[3]

Feeding JA resulted in a linear increase in colon length; this is consistent with previous work on prebiotics in poultry[32] and pigs.[33,34] When the total length of caecum and colon was compared, there was a linear increase in the large intestine length, the increase being 5.87, 9.29 and 11.84% in the three JA-supplemented groups compared with the control group. It was previously reported that, as the dietary level of FOS increased, the caecum size appeared to increase.[7,26,35,36] There are other reports showing increased caecal digesta and tissue weight induced by inulin and FOS, resulting in a greater surface area being available for nutrient absorption.[13,37] In the present study, JA inclusion increased the colonic digesta besides increasing the tissue weight of both caecum and colon. These results, in turn, highlight the role of JA as a potential prebiotic.

The intestinal morphometrics showed an improving trend as a result of JA supplementation. The observed trophic effects are similar to those reported in previous studies using inulin and/or FOS.[35,36] In rats, SCFA stimulate large and small intestinal cell proliferation and lead to increased brush border digestion and nutrient absorption through increased villus height and crypt depth.

There was a linear decrease in the pH of intestinal digesta with increasing levels of JA inclusion. This is in agreement with other studies.[26,36] Low pH negatively impacts the growth of potential pathogens and improves nutrient absorption. The intestinal pH and lactic acid concentration are inversely related; a similar trend was observed with significant increase in the caecal lactate content. There was a commensurate decrease in ammonia concentration in all intestinal segments due to JA supplementation. These results are indicative of the beneficial effects of JA on intestinal health and agree well with previous studies.[26,27] Total VFA and its fractions increased significantly in JA-supplemented groups, further justifying the observed decrease in pH. It is well established that propionate enhances the absorptive capacity of the hindgut by stimulating epithelial proliferation, whereas butyrate is the preferred energy source for these cells. In agreement with our results, Le Blay *et al.*[37] also observed increased concentrations of butyrate and total SCFA in caecum and colon due to FOS supplementation.

Considering the data obtained and the subsequent discussion, it is concluded that *H. tuberosus* may constitute a prospective prebiotic additive because of its potential impacts on gut health attributes, blood metabolic variables and immune response.

ACKNOWLEDGEMENT

Financial assistance to the first author in the form of a Junior Research Fellowship from the Indian Council of Agricultural Research, New Delhi, India is gratefully acknowledged.

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