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Feeding Jerusalem artichoke reduced skatole level and changed intestinal microbiota in the gut of entire male pigs

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*Different levels of dried Jerusalem artichoke were fed to entire male pigs 1 week before slaughter. The objective was to investigate the effect on skatole level in the hindgut and in adipose tissue, as well as the effect on microflora and short-chain fatty acids (SCFA) in the hindgut. Five experimental groups (*n *= 11) were given different dietary treatments 7 days before slaughtering: negative control (basal diet), positive control (basal diet + 9% chicory-inulin), basal diet + 4.1% Jerusalem artichoke, basal diet + 8.1% Jerusalem artichoke and basal diet + 12.2% Jerusalem artichoke. Samples from colon, rectum, faeces and adipose tissue were collected. Effect of dietary treatment on skatole, indole and androstenone levels in adipose tissue and on skatole, indole, pH, dry matter (DM), microbiota and SCFA in the hindgut was evaluated. Feeding increasing levels of Jerusalem artichoke to entire male pigs reduced skatole in digesta from colon and in faeces (linear,* $P < 0.01$*). There was also a tendency towards a decreased level of skatole in adipose tissue (linear,* $P = 0.06$*). Feeding Jerusalem artichoke decreased DM content in colon and faeces and pH in colon (linear,* $P < 0.01$*). Increasing levels of Jerusalem artichoke resulted in a reduced level of* Clostridium perfringens *in both colon and rectum (linear,* $P < 0.05$*) and a tendency towards decreased levels of enterobacteria in colon (linear,* $P = 0.05$*). Further, there was an increase in total amount of SCFA (linear,* $P < 0.05$*), acetic acid (linear,* $P < 0.05$*) and valerianic acid (linear,* $P < 0.01$*) in faeces. In conclusion, adding dried Jerusalem artichoke to diets for entire male pigs 1 week before slaughter resulted in a dose-dependent decrease in skatole levels in the hindgut and adipose tissue. The reduced skatole levels might be related to the decrease in* C. perfringens *and the increase in SCFA with subsequent reduction in pH.*

Keywords: jerusalem artichoke, inulin, pig, skatole, microbiota

Implications

In pig production, most entire male pigs are castrated due to the problem with boar taint. In many European countries, however, there is a trend towards a prohibition of surgical castration of male pigs for animal welfare reasons. This experiment showed that one of the main contributors to boar taint, skatole, decreased when entire male pigs were fed Jerusalem artichoke during a short period before slaughter. Feeding Jerusalem artichoke also had a positive influence on the microflora in the hindgut. Thus, adding Jerusalem artichoke to diets contributes to solving the boar taint problem in entire male pigs.

Introduction

In pig production, raising entire male pigs gives advantages due to better growth performance and leaner carcasses. In most European countries, however, surgical castration of male pigs is routine in meat production industry, mainly due to the problem with boar taint. This unpleasant odour is most obvious when meat is heated, and is primarily caused by the lipophilic skatole (3-methylindole) and androstenone accumulating in adipose tissue. The production of both substances increases during sexual maturity (Zamaratskaia, 2004). Skatole is produced in the hindgut by fermentation of the amino acid L-tryptophan (Yokoyama *et al.*, 1977; Zamaratskaia, 2004), whereas androstenone is produced in the testes (Bonneau, 1982). Skatole is thus produced in both sexes, but increased accumulation is observed in some entire male pigs with increasing age probably due to reduced metabolism in the liver (Babol *et al.*, 1999; Zamaratskaia *et al.*, 2004b). Owing to animal welfare reasons, surgical castration is controversial in many countries. In Norway, a law prohibiting surgical castration of all male pigs was supposed to pass in 2009, but is temporarily postponed due

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to lack of other feasible and efficient methods to reduce the problems with boar taint in entire male pig production (Norwegian Ministry of Agriculture and Food, 2008). Skatole production in the hindgut of pigs can be affected by the diet. Earlier studies report that feeding pigs diets supplemented with carbohydrates not enzymatically hydrolysed in the small intestine, but fermented in the hindgut, can reduce skatole levels in the hindgut (Jensen *et al*., 1995a; Jensen and Hansen, 2006; Øverland *et al*., 2011) and the accumulation in adipose tissue (Zamaratskaia *et al*., 2004a; Hansen *et al*., 2006; Pauly *et al*., 2008).

Inulin-type fructans are carbohydrates that include all β (2←1) linear fructans (Roberfroid, 2007). They are resistant to hydrolysis by enzymes in the small intestine, but are easily fermented by certain bacteria in the hindgut (primarily bifidobacteria; Flamm *et al*., 2001; Kleessen *et al*., 2007; Kolida and Gibson, 2007). Ingestion of inulin-type fructans has been given attention in recent years due to its beneficial health effects in humans (Kleessen *et al*., 2007; Kolida and Gibson, 2007; Ramnani *et al*., 2010) and animals (Apanavicius *et al*., 2007; Hansen *et al*., 2010; Jensen *et al*., 2011). Inulin-type fructans are found in several fruits and vegetables, but industrially, production is mainly based on chicory (*Compositae* family; Roberfroid, 2005). Experiments investigating Jerusalem artichoke (*Helianthus tuberosus*) as a source of inulin-type fructans are recently published (Kleessen *et al*., 2007; Ramnani *et al*., 2010). There is an increased interest among farmers to cultivate Jerusalem artichoke. It shows that good frost and drought tolerance, is resistant to diseases and can achieve a high yield of biomass (Slimestad *et al*., 2010). Thus, Jerusalem artichoke may become a new feed ingredient for pigs. To our knowledge, however, no studies have evaluated the effect of Jerusalem artichoke as a diet ingredient for entire male pigs on skatole levels. Øverland *et al*. (2011) concluded that inclusion of 3%, 6% and 9% chicory-inulin resulted in a dose-dependent reduction of skatole in the hindgut and backfat of entire male pigs; therefore, the present experiment was based on these results.

The main objectives of the present experiment were to investigate the effect of different levels of dried Jerusalem artichoke in diets for entire male pigs 1 week before slaughter on skatole in the hindgut and adipose tissue, and the effect on microflora and short-chain fatty acids (SCFA) in the hindgut.

Material and methods

One feeding experiment was conducted at the Experimental Farm of the Norwegian University of Life Sciences, Ås, Norway.

Dietary treatments

The dietary treatments were (i) negative control: basal diet on the basis of wheat and soyabean meal (low fibre content); (ii) positive control: basal diet + 9% chicory-inulin (6.3% pure inulin); (iii) basal diet + 4.1% dried Jerusalem artichoke (2.1% pure inulin); (iv) basal diet + 8.1% dried Jerusalem artichoke (4.2% pure inulin); and (v) basal diet + 12.2% dried Jerusalem artichoke (6.3% pure inulin). The calculated content of pure inulin in the diets was based on analyses of the Jerusalem artichoke product and on reported values from Orafti, Belgium. The basal diet was formulated to meet the requirements for all nutrients (National Research Council (NRC), 1998). The analysed chemical composition of the diets, the Jerusalem artichoke product and the chicory-inulin is given in Table 1.

Experimental animals

A total of 55 entire male pigs (Norwegian Landrace × Yorkshire) × (Norwegian Landrace × Duroc) from 11 litters were used. Average initial weight was 25.3 kg and average final weight was 111.7 kg. A randomized block design was used. The experimental animals were blocked by litter and by live weight and divided into five dietary treatments ($n = 11$).

Experimental procedure

The experimental period lasted for an average of 91 days. The experimental animals were fed a commercial diet (starting diet) for an average of 61 days, then the basal diet for 23 days, followed by a 7-day-period where the different dietary treatments were added to the basal diet as a top dressing. Pigs were kept in pens designed for individual feeding. Residual feed was collected after every feeding to determine average daily feed intake. The pigs were fed twice daily consistent with a restricted Norwegian feeding scale (Øverland *et al*., 2000), and had free access to drinking water at all times. Body weight was recorded once a week to determine average daily weight gain. The average room temperature was 16.1°C. Saw dust was used in the pens.

Sample collection

Uncontaminated faecal samples from each animal were collected from rectum 1 day before slaughter to determine content of skatole, indole and SCFA. The samples were frozen at −20°C pending analysis.

The pigs were slaughtered at a commercial slaughterhouse within 2 h after receiving the last meal. The entire digestive tract was taken out at the slaughter line, and the collection of uncontaminated samples from *colon ascendens* and rectum started immediately. Digesta from *colon ascendens* was collected to investigate skatole, indole, pH and selected groups of cultivable bacteria, and digesta from rectum was collected for bacterial examination. The samples from *colon ascendens* were taken 20 cm distal to the ileocaecal opening and from rectum approximately 5 to 10 cm proximal to the anus. All samples were kept on ice before either being frozen at −20°C pending analyses or incubated for bacterial examination. For the microbiological examination, 0.5 g samples were diluted in 4.5 ml 0.9% saline, and further serially diluted. From the dilution, 0.1 ml was placed on four selective media. All sampling were completed within 3 h after slaughtering, and all samples were processed for bacterial cultivation within 4 h after collection.

Samples of adipose tissue from the neck region were collected to determine levels of skatole, indole and androstenone.

The samples were kept frozen at −20°C until being analysed. Carcass weight and carcass lean percentage were measured at the slaughterhouse.

Bacterial counts from intestinal samples

Lactic acid-producing bacteria (LAB) were grown on MRS-lactobacillus agar (MERCK 10660, Darmstadt, Germany) aerobically at 37°C for 72 h. *Enterococcus* spp. were grown on Slanetz & Bartley agar (Oxoid CM377, Cambridge, UK) aerobically at 37°C for 72 h. Coliforms were grown on MacConkey agar (MERCK 5465, Darmstadt, Germany) aerobically at 37°C for 24 h. *Clostridium perfringens* was grown on TSC agar (Oxoid CM0587 Cambridge, UK) anaerobically at 37°C for 48 h with MERCK Anaerocult A (Darmstadt, Germany).

The figures from the bacterial counts were recorded as colony-forming units (CFU)/g digesta (Swanson *et al.*, 1992). In a few cases, the dilution ranges chosen for isolation (coliforms in *colon ascendens* and rectum 10^{-3}, 10^{-4} and 10^{-5}; enterococci in *colon ascendens* and rectum 10^{-2}, 10^{-3} and 10^{-5}; LAB in *colon ascendens* and rectum 10^{-5}, 10^{-6}, 10^{-7}) were either under- or overestimated. It was not practical to use undiluted or 10^{-1} diluted digesta for spreading on agar plates because of consistency of the material. When the bacterial concentrations were lower than our minimal detection level (10^{-2}), a $\log_{10}$ of 1.9 was registered. In cases of higher bacterial counts, compared with the dilutions permitted to count, a $\log_{10}$ value of 1 higher than the highest dilution was set as the count value.

Analytical methods

Dry matter (DM; EU Dir. 71/393), crude protein (Kjeldahl-N × 6.25; EU Dir. 93/28), crude fat (EU Dir. 98/64), crude fibre (EU Dir. 92/89) and ash (EU Dir. 71/250) contents were analysed in the starting diet, the basal diet, the dried Jerusalem artichoke product and the chicory-inulin product. In addition, the starting diet and the basal diet were analysed for Ca and P (NS-EN ISO 11885), and the basal diet for starch (AOAC 996.11), sucrose, glucose and fructose (LidVit.0A.26 Dionex), fructans calculated as inulin (AOAC 997.08) and the amino acids lysine, methionine, cysteine and threonine (SS-EN ISO 13903 : 2005). The Jerusalem artichoke product and the chicory-inulin product were analysed for fructans calculated as inulin (AOAC 997.08). Degree of polymerization (DP) was estimated on material hydrolysed in acid (200 mg in 10 ml pH 1.1 H_2SO_4, 100°C, 1 h). Fructose, glucose and rest sucrose were measured by using HPLC with evaporative light scattering detection (HPLC-ELSD), and intact inulin/fructo-oligosaccharides were extracted and analysed by using HPLC-ELSD as described by Asp (1993).

Skatole and indole in extracted fat were determined by using HPLC with fluorescence detection as described by Gibis (1994). The detection limit for both compounds was 0.005 μg/g. Androstenone in adipose tissue was analysed by using a time-resolved fluoroimmunoassay (Tuomola *et al.*, 1997), modified by using antiserum described by Andresen (1974). The digesta from intestine and faeces were analysed for skatole and indole by HPLC as outlined by Jensen and Jensen (1994), with chromatographic conditions described by Hansen-Møller (1994). The SCFA in faeces were analysed by using a modified method of capillary gas chromatography (Jensen *et al.*, 1995a).

Calculation and statistical analysis

Differences among the dietary treatments in skatole, indole and androstenone in adipose tissue and in pH, skatole, indole, DM, SCFA and microbiota in intestinal contents were tested using analysis of variance (ANOVA) by the GLM procedure of SAS Institute (1990). In addition, the Ryon–Einot–Gabriel–Welsch multiple range test was used to separate means. Polynomial orthogonal contrast was performed to compare differences between the positive control group and the dietary treatment with 12.2% Jerusalem artichoke. Orthogonal polynomials were used to test linear responses of increased levels of Jerusalem artichoke in the diets. Pearson correlation coefficient (r) was used to evaluate co-variance between skatole and androstenone and between skatole and indole in adipose tissue. In addition, the co-variance between digesta and adipose tissue for skatole and indole, respectively, was measured. $P < 0.05$ was set as level for significant difference among dietary treatments, and P-value between 0.05 and 0.10 was defined as a tendency.

Results

Analysed chemical composition of the starting diet, basal diet, dried Jerusalem artichoke product and chicory-inulin product revealed differences between the dried Jerusalem artichoke and the chicory-inulin in fructan content (50.6% *v.* 75.2%) and in average DP (5.1 *v.* 7.0: Table 1).

Jerusalem artichoke in diets for entire male pigs had in general no effect on growth performance and carcass traits (Table 2). A trend towards decreased feed conversion ratio was, however, noted (linear, $P = 0.09$).

Feeding increasing levels of Jerusalem artichoke to pigs significantly reduced skatole in digesta from *colon ascendens* and faeces (linear, $P < 0.01$; Table 3). ANOVA showed that pigs fed 8.1% and 12.2% Jerusalem artichoke had lower skatole levels in *colon ascendens* than those fed 4.1%. Indole content in digesta from *colon ascendens* was reduced when increasing amounts of Jerusalem artichoke was fed (linear, $P < 0.01$), whereas no such effect was observed in faeces. Feeding Jerusalem artichoke to pigs resulted in a decreased DM content in both *colon ascendens* and in faeces, as well as a decreased pH in *colon ascendens* (linear, $P < 0.01$). According to ANOVA, the DM content and pH in *colon ascendens* were significantly lower in pigs fed 8.1% and 12.2% Jerusalem artichoke compared with pigs fed 4.1%. The faecal DM content in the 12.2% Jerusalem artichoke group was lower than the positive control group (23.8% *v.* 26.6%). In adipose tissue, the variation in skatole between individuals within each experimental group was large. The mean values were in general low, ranging from 0.010 to 0.055 μg/g. There was, however, a tendency towards decreased levels of skatole with increasing levels of Jerusalem artichoke added ($P = 0.06$). In addition, there was a significant

Table 1 *Analysed chemical composition of the starting diet, basal diet, dried Jerusalem artichoke product and chicory-inulin product (g/100 g)*[1]

	Dietary treatment			
	Starting diet	Basal diet	Jerusalem artichoke	Chicory-inulin
Dry matter	85.8	86.7	93.1	97.3
Crude protein	16.2	17.1	8.0	6.6
Crude fat	4.2	3.1	<1	<1
Crude fibre	4.3	2.9	2.7	<1.0
Starch		43.2	<1.0	<1.0
Sucrose[2]		1.6	7.3	2.4
Glucose[2]		0.09	0.23	0.18
Fructose[2]		0.18	1.9	0.33
Fructans		0.9	50.6	75.2
Lysine[2]		0.94		
Methionine[2]		0.23		
Cysteine[2]		0.31		
Threonine[2]		0.64		
Ash	5.0	4.4	5.8	6.4
Ca	0.84	0.61		
P	0.49	0.41		
Average degree of polymerization [3]			5.1	7.0

[1]Eurofins Norsk matanalyse AS, PO Box 3055, 1506 Moss, Norway.
[2]Eurofins AB, PO Box 737, 53117 Lidköping, Sweden.
[3]Average degree of polymerization, PlantChem, PO Box 3082, Ganddal, Norway.

Table 2 *Effects of increasing levels of Jerusalem artichoke on growth performance and carcass traits of entire male pigs*

			Jerusalem artichoke[1]					
	Negative control	Positive control[2]	4.1%	8.1%	12.2%	s.e.m.[3]	*P*-value[4]	*P*-value[5]
Number of pigs	12	11	11	10	11			
Initial weight (kg)	25.4	24.9	25.1	25.3	24.6	0.50	ns	ns
Final weight (kg)	112.1	111.7	110.8	112.3	111.6	1.45	ns	ns
ADG (kg)	0.96	0.95	0.95	0.97	0.96	0.01	ns	ns
ADFI (kg)	2.16	2.12	2.14	2.16	2.09	0.02	ns	ns
FCR (kg/kg)	2.25	2.24	2.26	2.23	2.18	0.03	ns	0.09
Carcass weight (kg)	75.0	74.4	73.9	74.2	73.7	1.11	ns	ns
Lean meat (%)	62.6	62.4	61.5	61.8	62.1	0.46	ns	ns
Dressing (%)	66.9	66.6	66.7	66.1	66.1	0.40	ns	ns

ADG = average daily weight gain; ADFI = average daily feed intake; FCR = feed conversion ratio.
[1]Dried Jerusalem artichoke, containing 50.6% inulin-type fructans.
[2]9% chicory-inulin, Raftifeed® IPE (Orafti, Tienen, Belgium).
[3]Pooled s.e.m.
[4]*P*-value, GLM, all groups included.
[5]*P*-value, linear contrasts with increasing levels of Jerusalem artichoke (positive control omitted).
P-value: $^{**}P < 0.01$; $^{*}P < 0.05$; ns (non-significant) $P > 0.05$.

positive correlation between skatole and androstenone ($r = 0.499$, $P < 0.001$) and between skatole and indole ($r = 0.367$, $P < 0.01$) in adipose tissue. Significant correlations in skatole content between faeces and colon ($r = 0.451$, $P < 0.001$), between faeces and adipose tissue ($r = 0.458$, $P < 0.001$) and between colon and adipose tissue ($r = 0.523$, $P < 0.0001$) were observed. No such correlations were found for indole ($P > 0.05$).

Pigs fed increasing amounts of Jerusalem artichoke had a tendency to reduced levels of enterobacteria in *colon ascendens* (linear, $P = 0.05$), whereas no such relationship was observed in rectum (Table 4). The levels of *Enterococci* spp. were not influenced by the dietary treatments. For LAB, a difference among the dietary treatments was observed in *colon ascendens* contents, ranging from 9.18 in the 4.1% Jerusalem artichoke group to 9.89 in the 12.2% group (log CFU/g). This effect was not observed in the rectum contents. Levels of *C. perfringens* were reduced in both *colon ascendens* and rectum (linear, $P < 0.05$).

The effect of increasing levels of Jerusalem artichoke on SCFA content in faeces is reported in Table 5. There was an

Table 3 *Effects of increasing levels of Jerusalem artichoke on skatole (mg/kg DM), indole (mg/kg DM), DM (%) and pH in hindgut content and on skatole, indole and androstenone in adipose tissue (μg/g) of entire male pigs*

			Jerusalem artichoke[1]						
	Negative control	Positive control[2]	4.1%	8.1%	12.2%	s.e.m.[3]	*P*-value[4]	*P*-value[5]	*P*-value[6]
Number of pigs	12	11	11	10	11				
Colon ascendens (mg/kg DM)									
Skatole	4.6^{ab}	1.3^{b}	7.4^{a}	1.8^{b}	0.5^{b}	1.25	**	ns	**
Indole	43.6	31.5	32.6	27.4	24.5	5.43	ns	ns	**
DM (%)	17.3^{ab}	17.7^{ab}	19.6^{a}	15.6^{b}	14.3^{b}	0.92	**	*	**
pH	5.35^{ab}	5.31^{ab}	5.51^{a}	5.22^{b}	5.23^{b}	0.06	*	ns	**
Faeces (mg/kg DM)[7]									
Skatole	13.0^{ab}	9.7^{ab}	15.6^{a}	7.6^{ab}	4.7^{b}	2.24	*	ns	**
Indole	24.0	12.7	18.5	20.6	20.6	3.03	ns	0.07	ns
DM (%)	27.5^{a}	26.6^{ab}	25.3^{bc}	25.1^{bc}	23.8^{c}	0.53	**	**	**
Adipose tissue (μg/g)									
Skatole	0.037	0.017	0.055	0.015	0.010	0.01	0.08	ns	0.06
Indole	0.049	0.021	0.026	0.056	0.046	0.02	ns	ns	ns
Androstenone	1.8	1.3	2.7	2.0	1.6	0.34	0.08	ns	ns

DM = dry matter.
[a,b,c]Significant differences between dietary treatments using Ryon–Einot–Gabriel–Welsch multiple range test.
[1]Dried Jerusalem artichoke, containing 50.6% inulin-type fructans.
[2]9% chicory-inulin, Raftifeed® IPE (Orafti, Tienen, Belgium).
[3]Pooled s.e.m.
[4]*P*-value, GLM, all groups included.
[5]*P*-value, orthogonal contrast, positive control *v.* 12.2% Jerusalem artichoke.
[6]*P*-value, linear contrasts with increasing levels of Jerusalem artichoke (positive control omitted).
[7]$n = 11$ in negative control group.
P-value: $^{**}P < 0.01$; $^{*}P < 0.05$; ns (non-significant) $P > 0.05$.

Table 4 *Effects of increasing levels of Jerusalem artichoke on microbiota in colon ascendens and rectum of entire male pigs (log CFU/g digesta)*

			Jerusalem artichoke[1]						
	Negative control	Positive control[2]	4.1%	8.1%	12.2%	s.e.m.[3]	*P*-value[4]	*P*-value[5]	*P*-value[6]
Number of pigs	12	11	11	10	11				
Colon ascendens (log CFU/g digesta)									
Coliforms	6.68	6.45	6.50	6.10	6.21	0.221	ns	ns	0.05
Enterococci spp.	6.18	6.72	6.26	6.35	5.92	0.328	ns	0.09	ns
Lactic acid bacteria	9.82^{a}	9.66^{ab}	9.18^{b}	9.43^{ab}	9.89^{a}	0.144	**	ns	ns
Clostridium perfringens	6.09	5.42	5.92	5.08	4.98	0.319	0.07	ns	*
Rectum (log CFU/g digesta)									
Coliforms	5.96	6.25	5.82	5.99	5.70	0.310	ns	ns	ns
Enterococci spp.	6.15	6.32	6.09	5.96	5.93	0.283	ns	ns	ns
Lactic acid bacteria	9.57	9.31	9.29	9.40	9.52	0.162	ns	ns	ns
Clostridium perfringens	5.83	4.79	5.60	5.71	5.04	0.307	0.09	ns	*

CFU = colony-forming unit.
[a,b]Significant differences between dietary treatments using Ryon–Einot–Gabriel–Welsch multiple range test.
[1]Dried Jerusalem artichoke, containing 50.6% inulin-type fructans.
[2]9% chicory-inulin, Raftifeed® IPE (Orafti, Tienen, Belgium).
[3]Pooled s.e.m.
[4]*P*-value, GLM, all groups included.
[5]*P*-value, orthogonal contrast, positive control *v.* 12.2% Jerusalem artichoke.
[6]*P*-value, linear contrasts with increasing levels of Jerusalem artichoke (positive control omitted).
P-value: $^{**}P < 0.01$; $^{*}P < 0.05$; ns (non-significant) $P > 0.05$.

increase in total amount of SCFA (linear, $P < 0.05$), acetic acid (linear, $P < 0.05$) and valerianic acid (linear, $P < 0.01$) with increasing amounts of Jerusalem artichoke added. According to ANOVA, the 4.1% group was similar to the negative control but lower than the positive control for valerianic acid, whereas the 8.1% and 12.2% Jerusalem artichoke group were similar to both the positive and negative control ($P < 0.01$).

Table 5 *Effects of increasing levels of Jerusalem artichoke on SCFA (mmol/kg DM) in faeces of entire male pigs*

			Jerusalem artichoke[1]						
	Negative control	Positive control[2]	4.1%	8.1%	12.2%	s.e.m.[3]	*P*-value[4]	P-value[5]	P-value[6]
Number of pigs	11	11	11	10	11				
Organic acids (mmol/kg DM)									
Total amount	499.7	519.7	517.6	533.3	584.8	27.88	ns	ns	*
Formic acid	3.0	1.7	0.5	0.0	0.8	0.77	0.06	ns	0.06
Acetic acid	296.3	296.1	299.5	316.2	353.2	15.30	0.06	*	*
Propionic acid	112.6	117.9	115.3	116.9	121.9	7.21	ns	ns	ns
Isobutyric acid	11.3	10.1	11.1	11.8	12.7	1.05	ns	0.09	ns
Butyric acid	61.3	66.5	69.0	65.5	69.5	6.04	ns	ns	ns
Isovalerianic acid	7.2	7.4	7.7	8.3	8.2	1.02	ns	ns	ns
Valerianic acid	10.4[b]	18.9[a]	12.0[b]	13.3[ab]	15.4[ab]	1.57	**	ns	**
Caproinic acid	0.5	2.1	1.6	1.0	2.1	0.55	ns	ns	0.10
Succinic acid	0.0	0.4	1.1	0.2	1.6	0.47	ns	0.08	ns
Lactic acid	bdl	bdl	bdl	bdl	bdl	nd	nd	nd	nd

SCFA = short-chain fatty acids; DM = dry matter; bdl = below detection level.
[a,b]Significant differences between dietary treatments using Ryon–Einot–Gabriel–Welsch multiple range test.
[1]Dried Jerusalem artichoke, containing 50.6% inulin-type fructans.
[2]9% chicory-inulin, Raftifeed® IPE (Orafti, Tienen, Belgium).
[3]Pooled s.e.m.
[4]*P*-value, GLM, all groups included.
[5]*P*-value, orthogonal contrast, positive control *v.* 12.2% Jerusalem artichoke.
[6]*P*-value, linear contrasts with increasing levels of Jerusalem artichoke (positive control omitted).
P-value: $^{**}P < 0.01$; $^{*}P < 0.05$; ns (non-significant) $P > 0.05$.

Discussion

A major objective in the present experiment was to investigate the effect of adding the fructan-rich Jerusalem artichoke in diets for entire male pigs during a short period before slaughter on skatole formation in the hindgut and further deposition in adipose tissue. Our results showed that increasing levels of Jerusalem artichoke in the diet resulted in a dose-dependent decrease in skatole levels in the hindgut and a corresponding trend for skatole in adipose tissue. In agreement with the present experiment, several studies with pigs reported reduced skatole levels in intestinal content, plasma or adipose tissue when fermentable carbohydrates were fed (Knarreborg *et al.*, 2002; Jensen and Hansen, 2006; Øverland *et al.*, 2011). Hansen *et al.* (2006) added crude and dried chicory and chicory-inulin in diets for pigs (25% of total energy intake) during different periods before slaughter, ranging from 1 to 9 weeks. They concluded that adding dried chicory for as little as 3 days reduced skatole in plasma. The optimal feeding period and dietary level of inulin-type fructans are, however, not stated. Skatole is produced in the hindgut by specialized bacteria by fermentation of the amino acid L-tryptophan (Yokoyama *et al.*, 1977; Zamaratskaia, 2004). Carbohydrates that escape digestion in the small intestine serve as an energy substrate for bacteria in the large intestine (Gibson and Roberfroid, 1995; Kolida and Gibson, 2007). When carbohydrates are used as energy by bacteria, the demand for N from amino acids will increase due to higher microbial protein synthesis. Thus, if sufficient amounts of fermentable carbohydrates are available, L-tryptophan will probably be incorporated into the bacteria to a larger extent. On the other hand, if the amount of fermentable carbohydrates in the hindgut is insufficient, L-tryptophan will be used as energy source for proteolytic bacteria with skatole as a possible end product.

The significant correlations between skatole in hindgut and adipose tissue observed within individual animals in the present experiment indicate that the amount of skatole deposited in adipose tissue is, to some extent, determined by the amount produced in the hindgut. This is in accordance with an earlier study by Knarreborg *et al.* (2002), reporting highly correlated skatole concentrations between faeces and blood. Jensen *et al.* (1995a) also reported correlation between skatole in hindgut and adipose tissue, but large individual differences among animals were observed. The high correlation between skatole and androstenone in adipose tissue observed in the present experiment is in accordance with a study by Zamaratskaia *et al.* (2004b), reporting high correlations between skatole and androstenone in plasma in older pigs (20 weeks). This may be caused by the inhibitory effect of the increased levels of sex steroids on the enzymes involved in skatole metabolism at this age (Babol *et al.*, 1999; Doran *et al.*, 2002; Whittington *et al.*, 2004).

The decreased DM content in faeces of pigs fed Jerusalem artichoke is possibly due to an osmotic effect caused by non-absorbed sugars in the gut lumen. The lower DM content in faeces of pigs fed 12.2% Jerusalem artichoke compared with those fed the positive chicory inulin-based control, despite the similar dietary level of inulin-type fructans of 6.3%, may be caused by the shorter DP in inulin-type fructans from Jerusalem artichoke compared with those from chicory (5.1 *v.* 7.0). This could result in an increased osmosis into the

gut lumen due to elevated numbers of particles, explaining the lower DM content.

The reduced pH in the large intestine of pigs fed increasing amounts of Jerusalem artichoke may be explained by the increased SCFA levels. Addition of fermentable carbohydrates to diets for pigs has shown to raise SCFA production in the hindgut (Claus *et al*., 2003). Reduced protein fermentation due to increased microbial protein synthesis may also result in less NH_3, and thus contribute to the decline in pH. It is possible that pH in the hindgut is an environmental factor that can reduce the amount of skatole-producing bacteria present or alter the metabolic activity in the bacteria towards indol production at the expense of skatole. A study on 3-methylindole production in *Clostridium scatologenes* ATCC 25775 by Doerner *et al*. (2009) found that skatole production decreased if pH was reduced from 7 to 5, and they further suggested that skatole production can be downregulated because it is not an essential metabolic function. A study by Jensen *et al*. (1995b) measuring skatole and indole formation from L-tryptophan in pig faecal slurries *in vitro*, reported that the amount of L-tryptophan converted to skatole at the expense of indole was increased at pH 5 compared with pH 8.

The inhibitory effect of inulin-type fructans on *C. perfringens* observed in the present experiment is also reported in earlier studies evaluating *Clostridium* spp. (Gibson *et al*., 1995; Xu *et al*., 2002). Bacteria reported as skatole producers often belong to the genus *Clostridium* (Jensen *et al*., 1995b; Attwood *et al*., 2006; Doerner *et al*., 2009). The amount of *C. perfringens* in the digesta can potentially be used as an indicator on the amount of bacteria from the genus *Clostridium* present (H. Sørum, personal communication). Thus, it may be hypothesized that adding inulin-type fructans in feed results in reduced growth of the genus *Clostridium* in general, resulting in reduced skatole synthesis. Studies using methods that can identify the bacteria-producing skatole in the hindgut of pigs, however, as well as the magnitude of the skatole production in these bacteria will be necessary to further confirm this hypothesis.

In the present experiment, we observed a significant effect of diet on total SCFA, acetic acid and valerianic acid content in faeces. This increase can be explained by fermentation of inulin-type fructans to SCFA. The higher levels of valerianic acid when inulin-type fructans was added in the diet are in agreement with a study by Øverland *et al*. (2011), adding chicory-inulin in diets to entire male pigs. Earlier studies report that feeding easily fermentable carbohydrates as raw potato starch to pigs results in higher butyrate production in the hindgut (Claus *et al*., 2003; Mentschel and Claus, 2003). Increased butyrate in the hindgut has been suggested to inhibit apoptosis of colonic mucosal cells (Hass *et al*., 1997; Mentschel and Claus, 2003), and is implied as an explanation to lower skatole production in the hindgut due to less substrate for skatole production (Claus *et al*., 2003). In the present experiment, there was no effect of diet on butyrate concentration in the hindgut. The sampling method used in the present experiment, however, may be limited. Faecal samples should ideally be frozen immediately after sampling and with no exposure to O to preserve the authentic amount of SCFA. The faecal samples in the present experiment were exposed to O in the test containers that may have influenced the butyrate concentration. In addition, the faecal collection process took 1 h, and the samples were not frozen before sampling from all test animals was completed. Thus, it is possible that further metabolism of SCFA into CO_2 and H_2O in the bacteria might have occurred. In addition, SCFA are, to a large extent, absorbed in the hindgut in humans, as discussed by Kleessen *et al*., (2007). Faecal concentration of SCFA may thus not be representative for the true levels in the hindgut. The faecal level of butyric acid and other SCFA in the present experiment may thus have been underestimated and thereby masking the possible dietary effect.

In conclusion, adding dried Jerusalem artichoke to diets for entire male pigs 1 week before slaughter may be an effective method to reduce skatole levels in hindgut and adipose tissue. Inclusion of Jerusalem artichoke in the diet resulted in a dose-dependent decrease in *C. perfringens* and increase in SCFA with subsequent reduction in pH.

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