



## Effect of oat and soybean rich in distinct non-starch polysaccharides on fermentation, appetite regulation and fat accumulation in rat

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### ABSTRACT

Consumption of non-starch polysaccharides (NSP) is associated with reduced risk of obesity. This study aimed to compare the effects of cereals (oats) and legumes (soybean), rich in different classes of NSP, on appetite regulation and fat accumulation in rats. Soy pectin fermented more efficient than cereal arabinoxylan in rats. Soy pectin and oat  $\beta$ -glucan were utilized mainly in the caecum of rats. Only small amount of maltodextrin, cello-oligosaccharides and xylo-oligosaccharides were detected in the digesta. Caecal fermentation of soy pectin produced significantly higher concentration of short chain fatty acids (SCFAs) compared to the control. Retroperitoneal (RP) fat-pad weight was significantly lower for rats fed with soybean meal enriched diet than for controls. An inverse correlation between rat RP fat-pad weight and concentration (and proportion) of butyrate was observed. Consumption of soy pectin and oat  $\beta$ -glucan enriched foods to produce targeted SCFAs *in vivo* could be a potential strategy to lower fat mass accumulation and a potential tool to manage obesity.

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### 1. Introduction

Cereals (e.g. oats) and legumes (e.g. soybean) are important sources of both human food and animal feed products. Like other grains, oats are used as starch source (starch content  $\approx$  35% w/w) [1] in animal diets. Soybean (*Glycine max*) is attractive for its high protein content ( $\approx$ 48% w/w) [2]. In addition to starch and protein, oats and soybean are sources of non-starch polysaccharides (NSP). The content of NSP is 20% for oats [1] and 22% for defatted soybean flour [3]. NSP of oats mainly consist of mixed linked (1-3,1-4)- $\beta$ -D-glucan ( $\beta$ -glucan), arabinoxylans (AXs) and cellulose [3]. In soybean, pectic polysaccharides, xyloglucan and cellulose are the major NSP [4].

Human and animal studies consistently show that consumption of NSP can be associated with reduced risk of metabolic syndrome and obesity, and other health-promoting effects [5,6]. NSP cannot be digested by endogenous enzymes in the stomach and small intestine, and are potential substrates for fermentation by the gut microbiota. In pigs, the fermentability of NSP from oats and defatted soybean meal was found to be around 85% and 66%, respectively [7,8]. Microbial

fermentation of NSP in the large intestine leads to the production of short-chain fatty acids (SCFAs), including acetate, propionate and butyrate [9]. SCFA patterns and fermentability differs between NSP from various origins and depend on the solubility and structure of the NSP [10].

Numerous studies have demonstrated that SCFAs are associated with the enhanced release of anorectic gut hormones, like glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) from colonic L cells [11], and the regulation of body fat accumulation [12] and food intake [13]. Moreover, SCFAs may contribute to changes in energy balance in animals [13]. SCFAs are able to inhibit fat accumulation in adipose tissue of mice [14] and enhance fat oxidation in adipose and liver tissue of mice [15]. In most of these studies the SCFAs were orally administered [11,14]. However, SCFAs are unpalatable and are rapidly absorbed in the small intestine, where L cells are sparse. To overcome these disadvantages, isolated and specific NSP, with the purpose to be fermented in the large intestine to generate SCFAs, have been administered to potentially regulate the eating behaviour, body weight and body fat content [9,16]. However, the effects of oats and soybean diets rich in different populations of NSP on food intake behaviour and fat mass in mammals have not been reported so far.

The aim of the present study is to study the fermentation behaviour of NSP present in oats and soybean, and to compare the patterns of

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SCFAs produced during fermentation in the large intestine of rats. Food intake, body weight and visceral fat mass are compared between the groups to evaluate the potential effects of differences in NSP composition on SCFA patterns and energy homeostasis.

## 2. Materials and methods

### 2.1. Ingredients and diets

Oat (*Avena sativa*) grains and soybean meal (most fat has been removed) were supplied by Agrifirm (Apeldoorn, The Netherlands). Standard rat diet chow (RMH-B) [17] was purchased from AB Diets (Woerden, The Netherlands).

The fiber sources of RMH-B are whole wheat (42.3%), and small amounts of wheat middlings (8.3%), whole oat (8.3%) and maize (minor amount, info: AB diets). In terms of animal feeds, grains are often used without hull removal. Given oat hulls also contain certain amount of arabinoxylan which is our aiming carbohydrate polymer, the whole oat grains with hull were used. The soybean meal and oat samples were milled (<0.5 mm) and individually mixed with RMH-B to a weight percentage of 30% to reach approximately 6% (w/w) NSP. The mixtures were transformed into pellets and named oats enriched diet (OATS) and soybean meal enriched diet (SBM), respectively. Wheat based diet RMH-B was used as a control diet (CHOW). Although the created diets varied in macro- (and micro-) nutrient composition, the focus was on the differences in carbohydrate composition among the diets. The ingredients compositions of the diets are shown in Table 1. In addition, the flour of oats and soybean meal were also individually mixed with ground RMH-B to a weight percentage of 15% as Pre-Intervention diets, PI-OATS and PI-SBM, respectively.

### 2.2. Animals and sampling

Male Wistar rats, weighing  $323 \pm 3$  g, were purchased from Harlan Laboratories (Horst, The Netherlands). The rat was chosen as it is a suitable model, which has been widely used to study the *in vivo* fermentation of NSPs (e.g. pectin and  $\beta$ -glucan) and the subsequent effects on

regulation of eating behaviour [9,16]. Animals were housed solitary in the cages. Temperature was 21 °C with a light/dark cycle of 12 h/12 h. After habituation, animals were randomly subdivided into 3 experimental groups of 8 rats. Food and water were fed *ad libitum*. Animals in all groups were fed with diet CHOW for 3 weeks. Next, the diets of 2 groups were changed to PI-OATS or PI-SBM diets for adaptation for 2 weeks, followed by a change to the respective diets OATS or SBM for 5 weeks. The third group received CHOW throughout the complete experiment. Food intake and body weight development were measured daily throughout the whole experiment. All experiments were approved by the Animal Ethics Committee Groningen University (DEC 6375, Groningen, The Netherlands).

Animals were sacrificed after 7 weeks of diet treatment. Animals were anesthetized using isoflurane and subsequently decapitated. Caecal and colonic contents were immediately frozen at  $-80$  °C for preservation. Liver, spleen, kidneys, adrenals, epididymal (EPI) and retroperitoneal (RP) fat pads were removed and weighed.

### 2.3. Sample preparation

Pelleted diets were pre-milled passing a 2.0 mm sieve using a centrifugal mill (ZM200, Retsch, Haan, Germany), defatted by acetone and air-dried. Frozen digesta from rats ( $n = 8$ ) were thawed for SCFA analysis. The amount of digesta obtained from some of the rats was not sufficient for both SCFA and carbohydrate analysis. Those samples are not included in the SCFA analyses. For other analyses, the defrosted digesta ( $\approx 10$  g) of each rat ( $n = 8$ ) from the same group were pooled and freeze-dried. Dried pooled digesta and defatted diets were milled using ball-milling (MM2000, Retsch).

### 2.4. Preparation of destarched fractions from diets

In order to allow a proper NSP determination, fractions devoid of starch containing either all NSP or water-insoluble NSP were obtained as described elsewhere [18].

### 2.5. Isolation of water-solubles and water-insolubles from digesta

Freeze-dried digesta (2 g) were suspended in distilled water (40 mL) and the suspensions were boiled for 5 min. Next, the suspensions were stirred for 30 min at room temperature. After centrifugation (20 min, 30,000  $\times$ g, 20 °C), the supernatants were collected. The pellets were suspended in distilled water to repeat the solubilisation and centrifuging step. The final water insoluble solids and combined supernatants were freeze-dried.

### 2.6. Chemical analysis

#### 2.6.1. Moisture content and ash content

Moisture and ash contents were determined in duplicate by drying overnight at 103 °C in an oven and by incineration at 550 °C to a constant weight, respectively.

#### 2.6.2. Protein, fat, starch and $\beta$ -glucan

Protein contents were determined according to the Dumas method on a Thermo Quest NA 2100 Nitrogen and Protein Analyzer (Interscience, Breda, The Netherlands). The nitrogen to protein conversion factors of diets CHOW, OATS and SBM were 5.95, 5.82 and 5.87, respectively, based on the provided protein composition of diet (AB diets) and published factors of oats and soybean meal [19]. Fat content was determined by extraction with petroleum ether using the Soxhlet apparatus after hydrochloric acid hydrolysis (AOAC 920.39). Starch and  $\beta$ -glucan contents were analyzed using the total starch and  $\beta$ -glucan kits from Megazyme (Bray, Ireland), respectively.

**Table 1**  
Ingredient and chemical composition of the experimental diets.

	CHOW	OATS	SBM
Ingredient (% w/w)			
Chow	100	70	70
Oats	0	30	0
Soybean meal	0	0	30
Chemical composition (% w/w)			
Moisture	5.7	5.3	5.5
Protein	23.5	19.6	32.0
Fat	5.7	5.8	4.8
Ash	5.3	4.5	5.7
Carbohydrate	52.2	55.0	46.8
Starch	37.8	39.0	26.9
NSP	12.3	15.1	15.0
Insoluble	11.0	13.2	11.9
Soluble	1.3	1.9	3.1
Arabinoxylan	6.6	7.2	4.6
Pectin	0	0	4.7
$\beta$ -Glucan	0.6	1.3	0.5
Cellulose	3.4	4.9	4.0
ME (kJ/g)	13.7	13.4	13.4
GE (kJ/g)	18.0	17.9	17.5

Chow, a standard rat diet (RMH-B, AB diets, The Netherlands); starch and  $\beta$ -glucan contents were analyzed using the total starch and  $\beta$ -glucan kits from Megazyme (Bray, Ireland), respectively; arabinoxylan (AX) content was calculated as the sum of arabinose and xylose present in NSP from cereals (for SBM group, AX content is 70% of that in CHOW group); pectin content was calculated from the difference between non-glucan polysaccharides and AXs present in NSP; cellulose content was calculated from the difference between glucose present in NSP and glucose present in  $\beta$ -glucan; ME, metabolizable energy calculated from Atwater Fuel Energy of diet components; GE, gross energy determined using bomb calorimetry.

### 2.6.3. Short chain fatty acids

Short chain fatty acids were analyzed on the basis of a method described before [20]. Duplicates of 250–300 mg samples in 1.5 mL distilled water, or 1.5 mL standards (0.06 mg/mL to 0.6 mg/mL) were mixed with 1.5 mL of 0.3 mg/mL 2-ethylbutyric acid (internal standard) in 0.2 M HCl. After centrifugation (5 min, 30,000 ×g, 20 °C), 200 µL of the supernatant of either a sample or the standard was mixed with 50 µL of 0.15 M oxalic acid. After 30 min, the mixture was centrifuged again and supernatant was transferred into a vial for analysis. A TRACE GC Ultra system coupled with a FID detector (Interscience, Breda, The Netherlands) was used to quantify the SCFA levels [20].

### 2.6.4. Constituent monosaccharide composition

Neutral sugar composition of total NSP (t-NSP) and insoluble NSP was determined by gas chromatography (FOCUS-GC, Thermo Scientific) after pre-hydrolysis of samples in 72% (w/w) H<sub>2</sub>SO<sub>4</sub> at 30 °C for 1 h, followed by hydrolysis in 1 M H<sub>2</sub>SO<sub>4</sub> at 100 °C for 3 h, and derivatization of monosaccharides to their alditol acetates with inositol as an internal standard [21]. Uronic acid in the hydrolysate was determined using an automated colorimetric *m*-hydroxydiphenyl assay [22], including 0.3% (w/w) Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O in the concentrated H<sub>2</sub>SO<sub>4</sub>. Soluble NSP content and composition were calculated from the difference between t-NSP and insoluble NSP.

### 2.6.5. Oligosaccharide profiling

Oligosaccharide profiling was performed using high performance anion exchange chromatography (HPAEC) system with pulsed amperometric detection (PAD) as described before [1].

### 2.7. Statistical analysis

Normal distribution of the data was confirmed using the Kolmogorov-Smirnov test. Weekly food intakes and weekly body weight data were analyzed by repeated measures ANOVA (General Linear Model with time, diet and diet × time interaction as factors). Group comparisons for all other data were performed by ANOVA (one-way)

followed by Duncan's multiple-range tests. Pearson correlation coefficients were used to determine associations between RP fat-pad weight and SCFAs. These analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). A value of *p* < 0.05 was taken as the criterion of significance.

## 3. Results

### 3.1. Characteristics of NSP present in diet CHOW, OATS and SBM

A higher content of t-NSP was found for OATS (15.1 g/100 g diet) and SBM (15.0 g/100 g diet) compared to CHOW (12.3 g/100 g diet). The amount of soluble NSP was higher in SBM than in OATS and CHOW, respectively (Table 1). A higher content of AXs was found for OATS (7.2 g/100 g diet) compared to CHOW (6.6 g/100 g diet) and SBM (4.6 g/100 g diet). The pectin content in SBM was 4.7 g/100 g diet. The main constituent monosaccharides of NSP in CHOW and OATS were arabinose, xylose, glucose and uronic acid, which comprised >90% of the total carbohydrates (Table 2). The molar proportion of glucose in t-NSP from OATS was higher than that in the t-NSP from CHOW. Higher levels of galactose (13 mol%), uronic acid (10 mol%) and mannose (5 mol%) were observed for t-NSP of SBM compared to the other two diets (Table 2). Arabinose to xylose (A/X) ratios for t-NSP from the three groups can be ranked as: SBM > CHOW > OATS. The molar percentage of galactose in the soluble part of t-NSP from SBM was higher than those from CHOW and OATS. A significantly higher level of glucose was found in soluble part NSP from OATS (33 mol%) than in those of CHOW (7 mol%) and SBM (9 mol%).

### 3.2. Food intake, body weight and body composition

Rats were fed with CHOW, OATS and SBM containing different types and quantities of NSP and other components (Table 1). The food intake behaviour, body weight and body composition were measured to compare the influence of the different diets. There was no difference between weekly intake for CHOW, OATS and SBM groups at any time

**Table 2**

Constituent monosaccharide composition, carbohydrate and starch content of diets and digesta from different parts of rat intestine.

Group	Sample		Sugar composition (mol%)						A/X	Carbohydrate content (w/w%)	Starch content (w/w%)
			Ara	Xyl	Man	Gal	Glc	UA			
CHOW	Diet	<b>Total</b>	<b>20</b>	<b>38</b>	<b>3</b>	<b>3</b>	<b>29</b>	<b>6</b>	<b>0.52</b>	<b>31</b>	n.a.
		Insoluble	15	30	2	2	41	8	0.51	43	n.a.
		Soluble	26	38	3	7	7	19	0.67	25	n.a.
	Cae	<b>Total</b>	<b>20</b>	<b>31</b>	<b>2</b>	<b>3</b>	<b>36</b>	<b>7</b>	<b>0.64</b>	<b>24</b>	<b>0.42</b>
		Insoluble	21	34	2	2	34	7	0.61	29	0.12
		Soluble	7	16	4	8	55	10	0.43	8	1.11
	Col	<b>Total</b>	<b>20</b>	<b>31</b>	<b>3</b>	<b>3</b>	<b>37</b>	<b>6</b>	<b>0.67</b>	<b>25</b>	<b>0.31</b>
		Insoluble	21	33	2	2	35	7	0.65	32	0.06
		Soluble	10	21	5	10	41	12	0.46	9	1.13
OATS	Diet	<b>Total</b>	<b>15</b>	<b>38</b>	<b>2</b>	<b>3</b>	<b>38</b>	<b>5</b>	<b>0.37</b>	<b>37</b>	n.a.
		Insoluble	13	36	3	5	38	4	0.36	32	n.a.
		Soluble	18	41	2	0	33	5	0.44	47	n.a.
	Cae	<b>Total</b>	<b>15</b>	<b>31</b>	<b>3</b>	<b>2</b>	<b>42</b>	<b>6</b>	<b>0.47</b>	<b>27</b>	<b>0.58</b>
		Insoluble	15	35	3	2	39	6	0.44	34	0.15
		Soluble	7	12	5	8	57	11	0.57	9	1.57
	Col	<b>Total</b>	<b>15</b>	<b>32</b>	<b>4</b>	<b>2</b>	<b>40</b>	<b>6</b>	<b>0.47</b>	<b>31</b>	<b>0.35</b>
		Insoluble	16	35	3	2	37	6	0.45	34	0.09
		Soluble	9	16	5	9	49	11	0.55	8	1.19
SBM	Diet	<b>Total</b>	<b>18</b>	<b>26</b>	<b>5</b>	<b>13</b>	<b>28</b>	<b>10</b>	<b>0.68</b>	<b>24</b>	n.a.
		Insoluble	16	23	5	11	37	8	0.69	25	n.a.
		Soluble	22	34	3	17	9	14	0.67	19	n.a.
	Cae	<b>Total</b>	<b>17</b>	<b>27</b>	<b>3</b>	<b>3</b>	<b>42</b>	<b>7</b>	<b>0.65</b>	<b>23</b>	<b>0.73</b>
		Insoluble	17	30	2	2	41	8	0.56	29	0.21
		Soluble	9	13	4	8	55	10	0.69	9	2.25
	Col	<b>Total</b>	<b>18</b>	<b>27</b>	<b>3</b>	<b>3</b>	<b>40</b>	<b>7</b>	<b>0.65</b>	<b>23</b>	<b>0.48</b>
		Insoluble	18	30	2	2	40	8	0.59	27	0.08
		Soluble	9	15	6	12	46	11	0.64	9	1.50

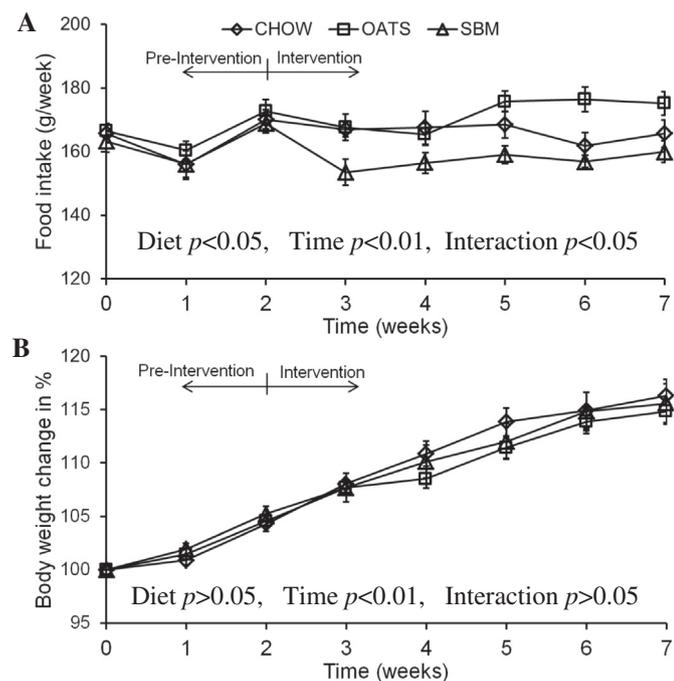
Diet, de-starched fraction containing all non-starch polysaccharides; Cae, pooled caecal contents from rats (n = 8); Col, pooled colonic contents from rats (n = 8); Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid; n.a., not analyzed. All values are the mean value of duplicate assays.

point during the Adaptation period with diet CHOW (data not shown) and the Pre-Intervention period with 15% oats/soy enriched diets (week 1–2,  $p > 0.05$ , Fig. 1A). The SBM group had a lower weekly food intake (153 g) than the CHOW group (167 g) during the first days of Intervention Period ( $p < 0.05$ , week 3). This difference between the SBM and CHOW groups became smaller at the end of this period ( $p > 0.05$ , Fig. 1A). In the last two weeks of the Intervention Period the weekly food intake was consistently higher in the OATS group than in the CHOW group. Significant effects of diet ( $p < 0.05$ ) and time ( $p < 0.01$ ) were observed (Fig. 1A). Despite the significant effect of diet  $\times$  time interaction ( $p < 0.05$ ) for weekly food intake, both the OATS and SBM groups do not show any significant difference of cumulative intake ( $p > 0.05$ ) compared to the CHOW group (Table 3). A significant ( $p < 0.05$ ) difference of weekly food intake between OATS and SBM groups was found. Consequently, the cumulative food intake of diet OATS was significantly higher than that of diet SBM ( $p < 0.05$ , Table 3).

With respect to weekly body weight gain, data analysis revealed a significant difference of time ( $p < 0.01$ ), but no difference of diets ( $p > 0.05$ ) and diet  $\times$  time interaction ( $p > 0.05$ ) between CHOW, OATS and SBM (Fig. 1B). There were no significant differences in body weight gain after 7 weeks between the three groups, neither in the weights of other markers that might indicate signs of metabolic syndrome, such as an empty gut, enlarged kidneys, liver, spleen and adrenals (Table 3). The EPI fat weight tended to be lower in SBM than in CHOW and OATS fed rats, although not significant. In contrast, the weight of RP fat was significantly lower in SBM ( $p < 0.05$ , Table 3) compared to CHOW, while that of OATS tended to be lower ( $p = 0.184$ ).

### 3.3. Characteristics of caecal and colonic digesta from rats

Just like for the diets, the main constituent monosaccharides of the NSP present in the digesta from all groups were arabinose, xylose, glucose and uronic acid (Table 2). The molar monosaccharide compositions of the corresponding NSP fractions were nearly the same in caecum and colon. In CHOW and OATS, the A/X ratio for all fractions of digesta, except for the soluble part from CHOW, were higher than that for the



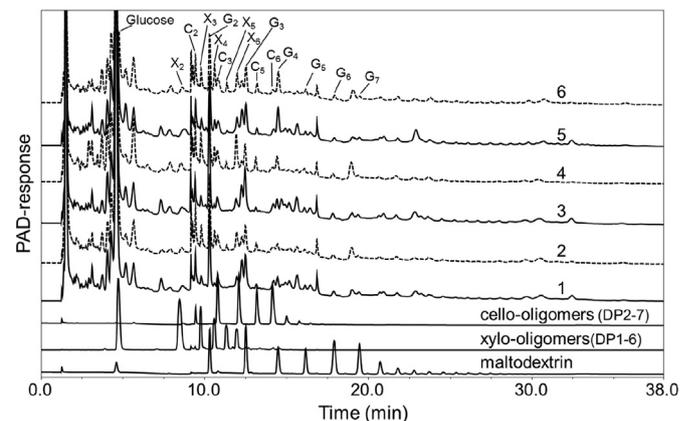
**Fig. 1.** Weekly food intake by rats (A) and body weight change of rats (B) fed with diet chow (CHOW), oats enriched chow (OATS) and soybean meal enriched chow (SBM). Values are means  $\pm$  SEM ( $n = 8$ /group). Data were analyzed by repeated measures ANOVA.

**Table 3**  
Cumulative food intake, body weight gain, organ weights, fat-pad weights and SCFA in rats fed with the experimental diets.

	CHOW	OATS	SBM
Cumulative food intake (kg)	1.11 $\pm$ 0.022 <sup>ab</sup>	1.14 $\pm$ 0.018 <sup>a</sup>	1.07 $\pm$ 0.015 <sup>b</sup>
Body weight (g)	458 $\pm$ 6.1	469 $\pm$ 10.0	456 $\pm$ 7.1
Empty gut (g)	16.4 $\pm$ 0.32	15.8 $\pm$ 0.36	16.3 $\pm$ 0.25
Kidneys (g)	3.1 $\pm$ 0.11	3.0 $\pm$ 0.07	3.2 $\pm$ 0.06
Liver (g)	16.1 $\pm$ 0.92	16.7 $\pm$ 0.65	16.1 $\pm$ 0.53
Spleen (g)	0.66 $\pm$ 0.038	0.68 $\pm$ 0.023	0.61 $\pm$ 0.029
Adrenals (mg)	50.3 $\pm$ 2.07	53.0 $\pm$ 2.64	55.5 $\pm$ 2.82
Epididymal fat (g)	9.3 $\pm$ 0.59	9.4 $\pm$ 0.48	9.0 $\pm$ 0.19
Retroperitoneal fat (g)	7.1 $\pm$ 0.65 <sup>a</sup>	6.2 $\pm$ 0.37 <sup>ab</sup>	5.6 $\pm$ 0.32 <sup>b</sup>
SCFA ( $\mu$ mol/g dry contents)		Caecum ( $n = 7$ )	
Acetate	257 $\pm$ 32 <sup>ab</sup> (55 $\pm$ 1.7)	211 $\pm$ 16 <sup>b</sup> (52 $\pm$ 1.8)	291 $\pm$ 25 <sup>a</sup> (52 $\pm$ 1.0)
Propionate	60 $\pm$ 8 <sup>b</sup> (13 $\pm$ 0.8 <sup>b</sup> )	51 $\pm$ 3 <sup>b</sup> (13 $\pm$ 0.6 <sup>b</sup> )	82 $\pm$ 6 <sup>a</sup> (15 $\pm$ 0.7 <sup>a</sup> )
Butyrate	130 $\pm$ 14 <sup>b</sup> (29 $\pm$ 2.0 <sup>ab</sup> )	130 $\pm$ 11 <sup>b</sup> (32 $\pm$ 2.3 <sup>a</sup> )	178 $\pm$ 25 <sup>a</sup> (31 $\pm$ 1.5 <sup>ab</sup> )
Branched SCFAs	12 $\pm$ 1 <sup>a</sup> (3 $\pm$ 0.1)	11 $\pm$ 1 <sup>a</sup> (3 $\pm$ 0.2)	12 $\pm$ 2 <sup>a</sup> (2 $\pm$ 0.4)
Total SCFAs	459 $\pm$ 49 <sup>b</sup>	403 $\pm$ 23 <sup>b</sup>	563 $\pm$ 53 <sup>a</sup>
SCFA ( $\mu$ mol/g dry contents)		Colon ( $n = 7$ )	
Acetate	132 $\pm$ 14 <sup>c</sup> (56 $\pm$ 1.9)	139 $\pm$ 12 <sup>c</sup> (53 $\pm$ 1.2)	140 $\pm$ 12 <sup>c</sup> (56 $\pm$ 0.9)
Propionate	33 $\pm$ 2 <sup>c</sup> (14 $\pm$ 0.4 <sup>ab</sup> )	34 $\pm$ 2 <sup>c</sup> (13 $\pm$ 0.6 <sup>ab</sup> )	35 $\pm$ 2 <sup>c</sup> (14 $\pm$ 0.8 <sup>ab</sup> )
Butyrate	65 $\pm$ 6 <sup>c</sup> (28 $\pm$ 2.0 <sup>ab</sup> )	82 $\pm$ 6 <sup>c</sup> (32 $\pm$ 1.0 <sup>a</sup> )	69 $\pm$ 7 <sup>c</sup> (28 $\pm$ 1.2 <sup>b</sup> )
Branched SCFAs	6 $\pm$ 1 <sup>b</sup> (2 $\pm$ 0.1)	6 $\pm$ 1 <sup>b</sup> (2 $\pm$ 0.2)	6 $\pm$ 1 <sup>b</sup> (2 $\pm$ 0.3)
Total SCFAs	236 $\pm$ 19 <sup>c</sup>	260 $\pm$ 19 <sup>c</sup>	249 $\pm$ 21 <sup>c</sup>

Values are means  $\pm$  SEM ( $n = 8$ /group, except the SCFA data). The molar proportions (%) of SCFA are present in the parentheses. Within rows, values without a common letter are significantly different ( $p < 0.05$ ), values with a same letter or without any letters are not significantly different ( $p > 0.05$ ).

corresponding fractions of the feed diets. In SBM, the A/X ratio for the insoluble fractions of digesta tended to be lower than that for the corresponding fractions from diet (Table 2). Interestingly, although the molar percentages of galactose and uronic acid are quite different in the diets, no difference was found between the molar percentages of these two monosaccharides present in the digesta of the different groups (Table 2). Small amounts of  $\beta$ -glucan (0.05–0.1%, w/w) were present in the caecal digesta from all the three groups (no further data shown). Next to the NSP, only minor amounts ( $< 0.73\%$ , w/w) of starch and its degradation products were detected in the digesta. To trace the utilization of oligosaccharides produced by microbiota in the digesta, HPAEC was used to detect oligomers present in the pooled sample per group (Fig. 2). Maltodextrin were the main oligosaccharides detected



**Fig. 2.** HPAEC elution patterns of water soluble fractions from pooled caecal digesta (solid lines) and pooled colonic digesta (dash lines) in CHOW (1 and 2), OATS (3 and 4) and SBM (5 and 6). X2 to X6: xylobiose to xylohexaose, respectively; C2 to C6: cellobiose to cellohexaose, respectively; G2 to G7: maltobiose to maltoheptaose, respectively.

in water soluble fraction. Besides maltodextrin, cello-oligosaccharides and xylo-oligosaccharides were present as well.

#### 3.4. SCFA contents in digesta

For all groups, the contents of total and individual SCFAs were significantly higher in caecum than in colon ( $p < 0.05$ , Table 3). The content of total SCFAs in caecal digesta was significantly higher ( $p < 0.05$ ) for the SBM group than for the OATS and CHOW groups (Table 3). Acetate was the major SCFA formed in the caecum and colon for all rats, followed by butyrate and propionate. The acetate content in the caecum of the SBM fed rats was significantly higher ( $p < 0.05$ ) than that in the OATS fed rats, but was not different from that in CHOW fed rats. The concentrations of propionate and butyrate in caecal digesta were significantly higher ( $p < 0.05$ ) for the SBM group than those for the OATS and CHOW groups. In contrast, the total and individual SCFA contents in the colon did not significantly differ between the three groups (Table 3). The SBM group had a significantly higher ( $p < 0.05$ ) proportion of propionate (15 mol%) in caecal digesta compared to the other two groups. The proportion of caecal butyrate tended to be increased by OATS and SBM diets, although the improvement was insignificant ( $p < 0.15$ ). The OATS group had a significantly ( $p < 0.05$ ) higher proportion of colonic butyrate (32 mol%) than that of the SBM group.

## 4. Discussion

NSP, abundantly present in cereals and legumes, have received widespread attention because of health promoting effects (e.g. preventing obesity and provoking antifungal immune responses) [2,23]. In the present study, oats and soybean meal were separately added to a wheat based diet to evaluate the effect of diets rich in different NSP composition on food intake and fat accumulation in rats. We found that pectin in soybean meal and of  $\beta$ -glucan in oats and wheat were extensively fermented as indicated by the disappearance of galactose and uronic acid for SBM, and  $\beta$ -glucan for OATS. Intake of soybean meal enriched diet correlated with an increase of butyrate and a decrease of the RP fat weight in rats.

NSP that escaped from pre-caecal digestion are fermented in the large intestine resulting in the formation of SCFAs. The microbial SCFA patterns depend on the type, structure, concentration and fermentability of the NSP [10,24], next to the composition of microbes present.

#### 4.1. Cereal $\beta$ -glucan and arabinoxylans (AXs)

Cereal  $\beta$ -glucan was highly fermentable in the caecum of rats as indicated by the low levels of  $\beta$ -glucan (0.05–0.1%, w/w) present in caecal digesta, which is in agreement with previous findings [25]. The branched-chain AXs represent the largest fraction of NSP in oats and wheat. These AXs are less preferable for microbiota than linear  $\beta$ -glucan, as described previously [18]. The results of the present study showed that the A/X ratio for cereal AXs in CHOW and OATS increased after fermentation in caecum and colon, which is consistent with previous results [26]. This indicates that, in rats, the low substituted oat AXs were preferably fermented over the high substituted AXs. Most of the studied xylanases preferably acted on unsubstituted regions of xylan backbone, and the presence of substituents hindered the accessibility of AXs for xylanases [27]. However, our results showed that xylo-oligosaccharides, which are degradable by the xylanases, were still present in the colonic digesta (Fig. 2). It is presumably that the observed oligosaccharides represent only those molecules which have been produced faster than they can be utilized by the resident microflora. Following the same reasoning, the presence of large relative amounts of maltodextrins (Fig. 2) presumably means that resistant starch was one of the contributors to caecal and colonic fermentation, with well-known impacts on butyrate production. This needs to be further

investigated by including ileal digesta for starch analysis in another batch of rat experiment.

Although the class of NSP in CHOW and OATS were the same, the abundances and structures of NSP ( $\beta$ -glucan and AXs) in the two diets were different as reported before [1], which resulted in different fermentation patterns. Less caecal SCFAs were found in the caecal sample of OATS group compared to that of CHOW group, despite the higher amounts of  $\beta$ -glucan present and fermented in OATS group compared to CHOW group. In the present study, the production of caecal SCFAs was mainly ascribed to the fermentation of both  $\beta$ -glucan and AXs. Because  $\beta$ -glucan is highly fermentable and CHOW is a wheat based diet, this suggests that the oat AXs were fermented less than wheat AXs in the caecum of rats. In contrast, previous study showed that the fermentability of oat AXs and oat cellulose are higher than that of wheat AXs and wheat cellulose, respectively, in the feces of rats [8]. Consequently, the fermentation of oat AXs in the present study might have shifted to the colon as also indicated by the trend of higher concentrations of colonic SCFAs in OATS group than in CHOW group (Table 3).

#### 4.2. Soy pectin

Arabinan side chains of soy pectin were fermented as indicated by the decreased A/X ratio for caecal digesta compared to that of the SBM diet (Table 2). Besides the fermentation of arabinan side chains, the galactan side chains and the uronic acid based backbone of soy pectin were also efficiently fermented in the rat caecum. This was indicated by the significantly lower molar percentages of galactose and uronic acid in caecal digesta compared to those in the SBM diet (Table 2). The higher concentration of caecal SCFAs and higher proportions of butyrate for SBM group compared to CHOW group is considered to be mainly due to the extensive fermentation of soy pectin. Consistent with our observation for soy pectin, a dietary fiber concentrate from okara has been found to enhance the total production of SCFAs, and in particular butyrate, in the caecum of rats [28]. Although some literatures show that pectic polysaccharides upon fermentation produce mainly acetate and/or propionate [10], the pectic polysaccharides used in their researches were mainly the water soluble fraction. The pectic fibers present in soybean meal are poorly soluble ( $\approx 25\%$  of the cell wall polysaccharide). In the present study, we used the whole soybean meal as a supplement. The polysaccharides present in our sample are more comparable to the ones present in okara which are mainly insoluble fiber fraction [28]. Nonetheless, in our current study lacking an indigestible marker in the diets to calculate the digestibility, the fermented NSP could not be quantified.

High contents of SCFAs have been reported to stimulate anorectic hormones secretion and inhibit orexigenic ghrelin secretion to reduce food intake and regulate energy homeostasis [11]. Hence, the significantly higher contents of caecal SCFAs ( $p < 0.05$ , Table 3) found in SBM group compared to those of CHOW and OAT groups might be associated with a higher circulating concentration of anorectic hormones. This may result in reduced food intake in rats in SBM group (Fig. 1A). In contrast to SBM and following the same reasoning, the higher food intake for rats in OATS group (Fig. 1A) might be explained by the lower contents of caecal SCFAs (Table 3). Beside the SCFA produced by fermentation of carbohydrate, protein was also reported to trigger the release of satiety hormones [29]. In SBM, the protein content was higher than the other two diets in this study (Table 1). Consequently, it seems likely that the high content of protein in SBM could also partly contribute to the inhibited food intake and fat accumulation, through the key underlying mechanism [29]. A more recent study in rats showed that rats fed high protein diet had consistently similar energy intake across all first 7 weeks, and had significantly higher energy intake at week 12 [30]. In contrast, our results showed lower energy intake for SBM-fed rats compared to controls. Hence, we infer that soy pectin rather than soy protein, in the present study, could be the major contributor to the inhibited food intake and fat accumulation. The soluble

fermentable fiber pectin appeared more effective than high protein for increasing satiety and decreasing energy intake and adiposity [31]. The soybean meal supplementation lowered weekly food intake for rats in the beginning of Intervention Period. The normalization of the lower food intake later on might indicate gut endocrine adaptation to the pectic polysaccharides from SBM. Nonetheless, total energy intake for SBM group was still much lower than for CHOW group due to the lower energy content and food intake. Although the similar weight gain for SBM and CHOW may have been influenced by differential energy expenditure, the similar weight gain might be correlated with a more efficient food conversion for the SBM diet. The adaptive response in rats fed with SBM allowed them to consume nearly the same amount of total energy as rats fed with CHOW, despite a continued protein intake in excess of needs for growth. In contrast, food intake for rats in OATS group was not different from that in CHOW till week 5 in Intervention Period. This might be partly explained by a quick adaptation of rats to NSP in OATS. The type (linkage, monosaccharide) of NSP present in oats and wheat (CHOW) is similar, although the A/X ratio and distribution of the arabinose substituents over the xylan backbone are different [1]. The high food intake in the last two weeks of Intervention Period may be due to the maintenance of energy homeostasis after being accustomed to lower metabolisable energy (ME) diet [32]. The differences in eating behaviour and energy homeostasis may be partly associated with viscosity. Viscous fibers, such as pectin in SBM, may create gastric distention and delay gastric emptying, consequently showing satiating effects [33].

EPI and RP fat-pad weights, both markers of abdominal fat mass, tended to be lower for SBM group than for CHOW group, although only the difference in RP fat-pad weight was significant. The lowered fat-pad weights in SBM could be a consequence of inhibited fat accumulation rather than reduced energy intake as the cumulative food intake and body weight of rats in SBM group were not different from those found for CHOW group (Table 3). This indicates that consumption of SBM diet leads to a redistribution of fat and to a decrease in the RP fat-pad weight. There are two main ways to achieve this metabolic regulation in adipose tissue. One is to inhibit fat release from small intestine into the circulation and fat deposition in adipose tissue, which is mediated or directly induced by SCFAs [34] produced during fermentation of dietary fibers. The other way to reduce adipose tissue in human and animals is to improve fat oxidation. SCFAs have been reported to induce a peroxisome proliferator-activated receptor (PPAR)  $\gamma$ -dependent switch from lipid synthesis to lipid utilization [15]. However, these previous studies that focused on the mechanism of regulation in fat metabolism by SCFAs used either orally administered SCFAs or *in vitro* models [14,15]. In the present study, SCFAs were produced by fermentation of NSP *in vivo*, thereby omitting the low efficiency of oral administration [35]. Our results demonstrate that the RP fat weight inversely correlates with the butyrate concentration in caecal digesta ( $p = 0.030$ , Fig. 3A). No correlation was found between RP fat weight and the concentrations of acetate and propionate (data not shown). These findings indicate the potential effects of butyrate on preventing the accumulation of RP fat. Butyrate has been suggested to have an important role in metabolism by *in vitro* and *in vivo* studies [34,36]. A similar trend in regulating fat distribution in rats was found for OATS. Diets enriched in whole oats [37] or oat  $\beta$ -glucan [33] have been reported to correlate with fat deposition in rats. However, in these studies SCFAs that mediated the modulating activity of oats on fat metabolism, were not considered. Butyrate is the preferred energy substrate of colonocytes, propionate is a substrate for hepatic gluconeogenesis, whereas acetate is a substrate for lipogenesis [38]. The present study shows that the RP fat weight is negatively correlated with molar proportion of caecal butyrate ( $p = 0.005$ , Fig. 3B), but positively correlated with the molar proportion of acetate ( $p = 0.003$ , Fig. 3C). Acetate has been reported to induce an anorectic neuropeptide expression profile and consequently decrease

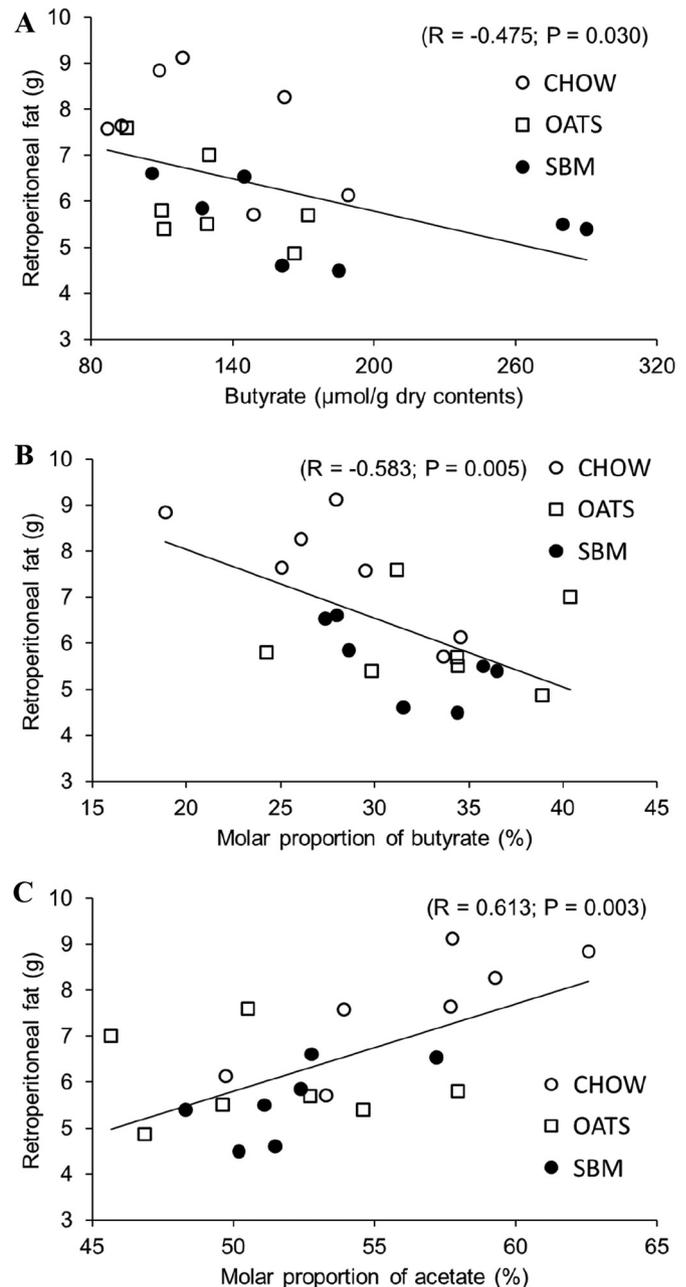


Fig. 3. Correlations between caecal SCFAs and retroperitoneal fat. Relationships with retroperitoneal fat for concentration of butyrate (A), proportion of butyrate (B) and proportion of acetate (C). The line is the regression line for all the data shown in each figure.

body weight [39]. Therefore, the effect of SCFAs on fat mass may be associated with not only the content of butyrate, but also with the molar proportions of both acetate and butyrate.

## 5. Conclusions

We studied the effects of oats and soybean on appetite regulation and fat accumulation in rats, and compared the fermentation patterns of different NSP from soybean meal and oats. A significant inverse correlation between rat RP fat-pad weight and concentration (and proportion) of butyrate, and a significant positive correlation between RP fat-pad weight and proportion of acetate were reported here for the first time. Easy-fermentable non-starch polysaccharides in oats ( $\beta$ -glucan) and soybean meal (soy pectin), which benefit the butyrate-producing bacteria are associated with low fat-pad weight in rats. Hence,

consumption of specific NSP (e.g. soy pectin) enriched food to produce SCFAs particularly butyrate *in vivo* could be a potential strategy for management of human obesity.

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### Author contributions

H.G., H.S., A.S., M.B., G.B., E.B. and P.V. conceived and designed the experiments; L.T. and J.S. performed the experiments; L.T., J.S., H.G., H.S. and A.S. analyzed the data; E.B., H.G., H.S. and A.S. contributed the reagents, materials and analysis tools; L.T., H.S. and H.G. drafted the manuscript. All authors read and approved the final manuscript.

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