

Research

In Vitro and In Vivo Effects of Seaweed Extract on Carbohydrate Digestion and Availability

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Abstract

We investigated *in vitro* and *in vivo* effects of seaweed extract on carbohydrate digestion and availability. In the *in vitro* study inhibition α -glucosidase and α -amylase activity was measured. In the *in vivo* study, postprandial blood glucose after a breakfast containing 50g of digestible 25 carbohydrates was measured in participants (N=10, >40 years, obese) who were given 0 mg, 800 mg and 1600 mg seaweed extract before breakfast in a randomized order during three study days. The extract inhibited α -amylase and α -glucosidase activity and IC50 concentrations were 0.88 ± 0.09 and 0.81 ± 0.26 $\mu\text{g/mL}$ for α -amylase and α -glucosidase, respectively. Of the participants, 60% had increased fasting blood glucose. Postprandially, the highest blood sugar 30 concentrations were reached between 30 and 45 minutes (9.5 ± 2.1 mmol/L) and reached again baseline concentrations (5.5 ± 0.7 mmol/L) after two hours. However, fasting blood glucose, Cmax, AUC and tmax were not significantly different between study days.

Keywords: *in vivo*, *in vitro*, seaweed, carbohydrates

Introduction

The prevalence of type 2 diabetes has been increasing in Western countries and is strongly related to obesity and physical inactivity [1]. Prospective observational studies and clinical trials have shown that individual nutrients, foods and dietary patterns are of importance in the prevention and management of type 2 diabetes [2]. Seaweed is one of the largest producers of biomass in marine environment and is a rich arsenal of functional ingredients which may possess the potential to prevent type 2 diabetes [3].

As reviewed by Holdt and Kraan [4], seaweeds contain, e.g., sulphated polysaccharides, carotenoid pigments and phlorotannins, a large group of polyphenols, displaying *in vitro* antioxidant activity, anti-diabetic- and anti-inflammatory properties. Several studies have shown, e.g., that seaweed extract inhibits α -amylase and α -glucosidase which are major carbohydrate hydrolysing enzymes in the small intestine [5,6,7]. This is of importance, because one of the therapeutic approaches in treating or preventing type 2 diabetes is to reduce postprandial hyperglycaemia by inhibiting major carbohydrate hydrolysing enzymes [8]. Anti-diabetic effects were further confirmed in animal models of diabetes, where seaweed as part of animal food or extracts were successfully given *per os* or intraperitoneally to improve glucose metabolism in mice, rats and rabbits [9,10,11,12].

In agreement with this experimental evidence, a cross-sectional study found a negative relationship between dietary algae (seaweed) consumption and the risk of type 2 diabetes mellitus in the Korean population [13].

However, when it comes to human trials, only little information is available. Kim et al [14]. could show that dry powdered sea tangle and sea mustard (equivalent to 48 g fresh weight) taken for 4 weeks improved glucose metabolism in patients with type 2 diabetes. A similar study which used a lesser amount of seaweed showed only some minor effects on diabetes related outcomes [15].

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In order to gain more knowledge on seaweed and carbohydrate metabolism, the aim of the present study was to investigate 1) the *in vitro* effects of seaweed extract on carbohydrate digestion due to inhibition of α -glucosidase and α -amylase; and 2) the acute effects of three different doses of seaweed extract on post-prandial blood glucose in overweight volunteers. The seaweed extract used in the present study derived from bladder wrack.

Materials and Methods

Seaweed extract

Extract from the brown seaweed bladder wrack (*Fucus vesiculosus*) was provided by Marinox Ltd., Reykjavik, Iceland (www.marinox.is). Microbiological and chemical quality of the extract was confirmed and the composition of the extract is shown in Table 1.

Dry matter, ash and salt

The samples were analysed for dry matter (g/100g), calculated as the loss in weight during drying at 103°C (ISO 6496: 1999), ash g/100g where the sample is ashed at 550°C for 3 hours and the residue weighed (ISO 5984: 2002) and salt content (g/100g) was determined using a potentiometric method (AOAC 976.18). The analyses were accredited by Swedish Board for Accreditation and Conformity Assessment (SWEDAC). The measurement uncertainty was $\pm 4\%$, $\pm 6\%$ and $\pm 4\%$, for water, ash and NaCl, respectively.

Proteins

Protein content (g/100g) was determined using the Dumas method and calculated using total nitrogen (N) x 6.25 (ISO 16634-1 2008) with slight modifications for Rapid N instrument from Elementar, accredited by Swedish Board for Accreditation and Conformity Assessment (SWEDAC), with a measurement uncertainty of $\pm 1\%$.

Lipids

The content of extractable fat was determined using the Soxhlet method (AOCS 1997).

Minerals and inorganic trace elements

The mineral analysis of sodium (Na), potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) were determined by ICP-MS (inductively coupled plasma mass spectrometry) after sample were diluted 50 times after analyses of inorganic trace elements.

Inorganic trace elements

The iodine (I) analysis was performed by LUFAGrolab GmbH, Bruckberg, Germany and determined by inductively coupled plasma mass spectrometry (ICP-MS), method DIN EN 15111. The analysis of iron (Fe), cadmium (Cd), lead (Pb), mercury (Hg), and total arsenic (As) was done by ICP-

MS (inductively coupled plasma mass spectrometry) after mineralization of the samples with closed vessel acid digestion. The analysis is accredited according to DIN EN ISO/IEC 17025. Portions (up to 200 mg weighed with 0.1 mg accuracy) of dried samples together with 3 ml HNO₃ and 1.5 ml H₂O₂ were transferred to 50 ml digestion bombs. Samples were digested in a Mars5 microwave oven (CEM, North Carolina, USA), according to in-house method SV-25-02-SN of the Icelandic Food and Biotech R&D Institute conducted according to the validated NMKL method no. 186. The digested sample solutions were quantitatively transferred to 50 ml polypropylene tubes and diluted to 30 ml with Milli-Q water. The concentration of the different elements (Cd, Cu, Zn, As, Se, Hg, Pb) in these digests was determined by ICP-MS (Agilent 7500ce, Waldbronn, Germany). ¹¹⁵In was used as internal standard.

Inorganic arsenic was analysed by Eurofins WEJ Contaminants laboratory, Hamburg, Germany with hydride generation atomic absorption.

Microbiological analysis

Aerobic microorganisms were analysed at 30°C according NMKL method no. 86. Moulds and yeasts were determined according to NMKL method no: 98.

Thermotolerant coliform bacteria and Escherichia coli were determined according to NMKL method no: 125.

Polyphenol content (TPC)

Total polyphenol content was determined according to the method by Singleton and Rossi [16], adapted to microplate format and some modifications. In short, 20 μ l of sample was mixed with 100 μ l of 0.2N Folin-Ciocalteu and allowed to stand at room temperature for 5 min. Then 80 μ l of 7.5% Na₂CO₃ was added, heated for 10 sec at 800 W in a microwave and 30 minutes incubated at room temperature under constant agitation. Absorbance was read at 730 nm with a microplate reader (POLARstar Optima BMG labtech, Offenburg, Germany).

Seven concentrations of phloroglucinol were used to create standard curves. Total phenol content was calculated as gram of phloroglucinol equivalents (PGE) per 100 g sample using interpolation from regression analysis.

***In vitro* study** **α -Glucosidase**

α -Glucosidase activity was assayed using the substrate p-nitrophenyl- α -D-glucopyranoside, which is hydrolysed by α -glucosidase to release the product p-nitrophenol, a colour agent that can be monitored at 405 nm [17]. Inhibition of enzyme activity was measured at different sample concentrations and concentration needed to reduce enzyme activity 50% was calculated (IC50).

 α -Amylase

The α -amylase inhibitory activity was determined using maltose from potato starch. The reaction was stopped with dinitrosalicylic acid colour reagent and absorbance was measured at 540 nm [18]. Inhibition of enzyme activity was measured at different sample concentrations and concentration needed to reduce enzyme activity 50% was calculated (IC50).

Human study**Subjects**

Participants (N = 10) were recruited through advertisements on the internal web of the University of Iceland. Inclusion criteria were age > 40 years and obesity (defined as BMI > 30 kg/m²), exclusion criteria were cardiovascular disease, diabetes, pregnancy, lactation or drug treatment for high blood sugar. The study was approved by the Icelandic National Bioethics Committee (VSN15-139-S1) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

Study design

This was a 3-way cross-over study in which participants were given 0, 800 and 1600 mg seaweed extract in a randomized order. The participants attended our clinical center for 3 times during a two weeks period between eight and nine o'clock in the morning. Each time they were in fasting condition. The length of each stay was around 3 hours. 0 mg dose: The participants swallowed 4 placebo capsules (containing lactose) and then ate breakfast containing 50 g of digestible carbohydrates in form of breakfast cereals (both sugar and starch) and 250 ml of water. 800 mg dose: The participants swallowed 2 placebo capsules and 2 seaweed extract capsules (each capsule contained 400 mg of seaweed extract) and consequently ate breakfast. 1600 mg dose: The participants swallowed 4 seaweed extract capsules and consequently ate breakfast.

Measurement of blood glucose

The measurement of blood sugar followed the recently updated standard GI testing protocol (ISO26642:2010) [19].

Blood glucose in fresh capillary blood will be measured at baseline and then 15, 30, 45, 60, 75, 90 and 120 minutes after breakfast using a finger prick test (Accu-Check, Roche Diagnostics GmbH, Mannheim, Germany). Accu-Chek® Aviva from Roche Diagnostics, Mannheim, Germany measures reasonably well with a mean bias of 5.3% and a mean absolute relative difference of 6.8% compared to the reference method YSI (Yellow Springs, OH) 2300 Stat Plus glucose analyser [20].

From the blood glucose measurements area under the curve (AUC_{0-2h}) was calculated using the linear trapezoidal rule. The highest blood glucose concentration (C_{max}) and the time reaching the maximum concentration (t_{max}) were determined by straight forward selection of the highest plasma concentration during the sampling period.

Anthropometric measurements

Body weight, height and body composition were measured using bioelectrical impedance analysis (InBody230, InBody, Seoul, Korea). Body mass index (BMI) will be calculated from the recorded height and weight (kg/m²). Waist circumference was measured halfway between the top of the lateral iliac crest and the lowest rib.

Estimated intake of iodine and heavy metals

The total intake of iodine and heavy metals on the study day with the high dose extract was estimated using data from the latest national dietary survey in Iceland [21], and the chemical composition of the seaweed extract. The estimated intakes were then compared to upper intake levels derived from NNR 2012 (iodine) [22] and WHO/FAO joint committee (2010) for heavy metals [23].

Statistical analyses

The data were analysed using statistical software (SPSS, version 24.0, SPSS, Chicago, IL, USA). Data were checked for normality using the Kolmogorov

Smirnov test. Data are presented as mean \pm standard deviation (SD) and percentage. Differences between the three time points were calculated using *General Linear Model - Repeated Measures*. The significance level was set at $P \leq 0.05$.

Results**Extract production and in vitro study**

The production resulted into 8.7 kg dry extract from 360 L liquid extract. Therefore the yield (w/v) was 3.3%. The microbiological analysis showed that the

extract was negative for E.coli contamination, below the threshold (<20 CFU/g) for mould and yeast contamination and the total count of microbial contamination at 30°C was 5700 CFU/g (Table 1).

The chemical analysis showed that only small amounts of water were left in the extract and that the main components of the dry matter were ash, salt and proteins. The extract contained the essential trace element iodine as well as detectable traces of the heavy metals mercury, arsenic, cadmium and lead (Table 1).

The extract inhibited *in vitro* α -amylase and α -glucosidase activity. The IC₅₀ concentrations were 0.88 ± 0.09 and 0.81 ± 0.26 μ g/mL for α -amylase and α -glucosidase, respectively.

Microbial status			
Total count (CFU/g)	5700		
Yeast and mold (CFU/g)	<20		
E.coli (CFU/g)	negative		

Chemical composition	100 g extract	800 mg extract	1600 mg extract
Water (g)	7.1	0.057	0.114
Dry material (g)	92.9	0.743	1.486
Ash (g)	32.6	0.261	0.522
Protein (g)	6.8	0.054	0.109
Fat (g)	0.3	0.002	0.005
Carbohydrates (g)	53.2	0.426	0.851
Hg (μ g)	6.0	0.048	0.096
Cd (μ g)	232.0	1.856	3.712
Pb (μ g)	16.0	0.128	0.256
As (mg)	5.7	0.045	0.091
Iodine (mg)	21.0	0.168	0.336
Polyphenol content*	26.7	0.214	0.427

Table 1: Microbial contamination and chemical composition of the spray-dried seaweed extract *phloroglucinol equivalents/100g dry extract

Human study

The characteristics of the participants can be seen in Table 2. All of the participants were obese and 60% had increased fasting blood glucose at least at one of the three visits. The majority of the participants were female.

A comparison in fasting blood glucose, C_{max}, AUC and t_{max} between the three study days can be seen in Table 3. None of the outcome measurements differed between the days.

Post-prandial changes in blood glucose can be seen in Figure 1. The blood glucose curves at the three study days were similar. The highest blood sugar concentrations were reached between 30 and 45 minutes and reached again baseline concentrations after two hours.

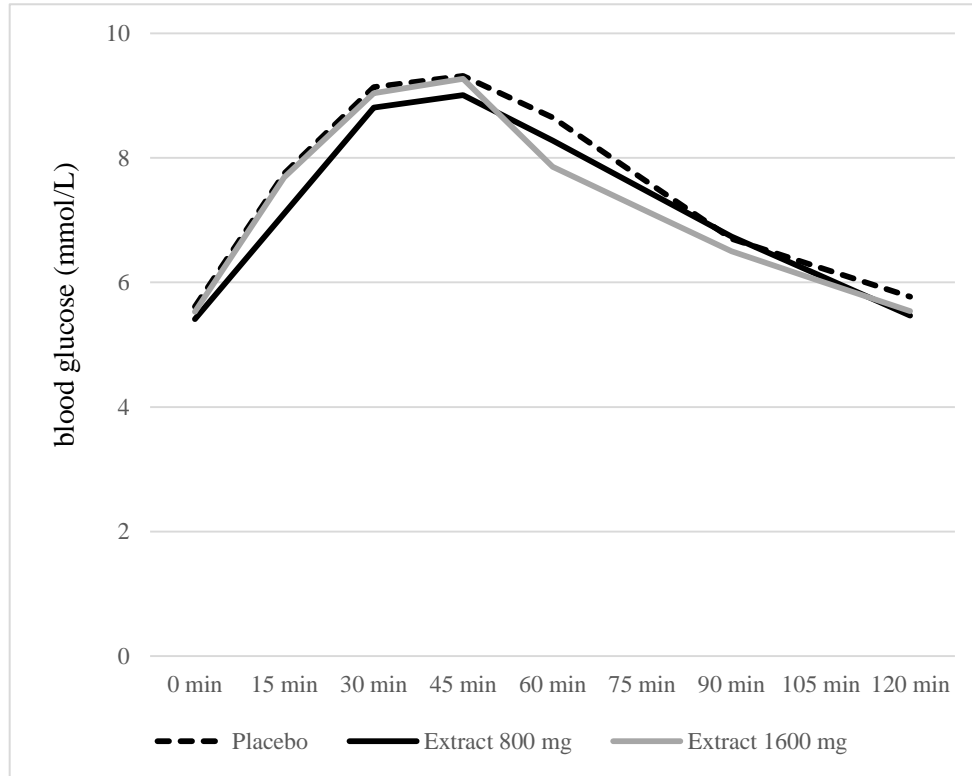


Figure 1: Post-prandial blood glucose after ingestion of 0, 800 and 1600 mg of seaweed extract.

	Mean	±	SD
Age (years)	55.1	±	10.7
Height (cm)	169.1	±	8.0
Body weight (kg)	98.7	±	14.3
Waist (cm)	109.3	±	10.3
BMI (kg/m ²)	34.4	±	3.3
Body fat (%)	41.0	±	5.3
Fasting glucose (nmol/L)*	5.52	±	0.67
Female (%)		70	
Obesity (%)		100	
Smokers (%)		10	
Increased fasting blood glucose (%)**		60	

Table 2: Baseline characteristics of the participants (N = 10).

*Mean fasting blood glucose from the three visits

**Fasting blood glucose above 5.6 nmol/L at least at one of the three visits

	Intake by food	intake by 1600 mg extract	total intake	upper level of intake*
Iodine (μg)	168	368	536	600
Cadmium (μg)	8.1	3.5	11.6	56.5
Lead (μg)	18.4	0.06	18	250
Mercury (μg)	5.6	0.1	6	70
Arsenic (μg)	126	77	203	**
Arsenic - inorganic (μg)	**	0.2		210

Table 3: Calculated intake of heavy metals and iodine (in μg) from food and seaweed extract in comparison to upper intake levels derived from NNR 2012 (iodine) and WHO/FAO joint committee (2010) for heavy metals.

*Iodine: upper intake level 600 $\mu\text{g}/\text{d}$

Cadmium: provisional tolerable monthly intake = 25 $\mu\text{g}/\text{kg}$ bw = 56.5 $\mu\text{g}/\text{d}$ for a 70 kg person

Lead: provisional tolerable weekly intake of 25 $\mu\text{g}/\text{kg}$ bw⁴ = 250 $\mu\text{g}/\text{d}$ for a 70 kg person

Mercury: provisional tolerable weekly intake of 4 $\mu\text{g}/\text{kg}$ bw⁴ for inorganic mercury = 70 $\mu\text{g}/\text{d}$ for a 70 kg person

Arsenic: 3 $\mu\text{g}/\text{kg}$ BW per day for inorganic arsenic = 210 $\mu\text{g}/\text{d}$ for a 70 kg person

**no estimate available for arsenic intake in a Icelandic population.

	Placebo			extract 800 mg			extract 1600 mg			P- value
	Mean	\pm	SD	mean	\pm	SD	mean	\pm	SD	
baseline glucose (mmol/L)	5.6	\pm	0.8	5.4	\pm	0.7	5.5	\pm	0.7	n.s.
C _{max} (mmol/L)	9.6	\pm	2.4	9.3	\pm	2.0	9.5	\pm	2.0	n.s.
C _{max} incremental (mmol/L)	4.0	\pm	2.0	3.9	\pm	1.7	4.0	\pm	1.5	n.s.
AUC _{0-2h} (mmol/L/2h)	15.3	\pm	3.1	14.8	\pm	2.5	14.8	\pm	2.5	n.s.
AUC _{0-2h} incremental (mmol/L/2h)	4.1	\pm	1.9	3.9	\pm	1.7	3.7	\pm	1.4	n.s.
t _{max} (min)	39.8	\pm	10.0	41.3	\pm	9.5	38.3	\pm	10.3	n.s.

Table 4: Post-prandial blood glucose after ingestion of 0, 800 and 1600 mg of seaweed extract.

Discussion

The present study investigated the *in vitro* and *in vivo* effects of seaweed extract on carbohydrate digestion and -availability. We used a hot water extract from the brown seaweed bladderwrack (*Fucus vesiculosus*) harvested in Iceland and found that the extract inhibited α -glucosidase and α -amylase *in vitro*, however, these promising effects did not translate into reduced post-prandial blood glucose after consumption of a carbohydrate rich breakfast in the human study.

Inhibitors of the carbohydrate digesting enzymes α -glucosidase and α -amylase are currently prescribed as treatment for diabetes mellitus [24]. Acarbose is sold as Precose® / Glucobay® and is a pseudotetrasaccharide derived from fungi (*Actinoplanes*) and has high affinity

(factor 104 to 105 higher than regular oligosaccharides) for the binding site of amylase and glucosidase enzymes which cannot cleave a C-N linkage in acarbose. It is very effective inhibitor of α -amylase and to a lesser extent of α -glucosidase [25,26], but its gastrointestinal side effects (flatulence, abdominal pain and/or diarrhoea), costs and frequency of administration cause the search for alternatives that could be used for diet therapy of diabetes [27].

Our extract showed good *in vivo* inhibition of carbohydrate digesting enzymes and the IC₅₀ concentrations were lower than what have been found for Acarbose or extracts from, e.g., fruits, in other studies [28,29,30].

Although it is difficult to compare to results from other *in vivo* studies, because inhibition might depend on species where the enzymes are derived from, we expected our extract to reduce post-prandial blood glucose in the human study similar to Acarbose. It has been reported, that even the smallest dosage of Acarbose reduces the post-prandial increase in blood glucose by around 20 - 30% [31].

However, in the human study, administration of different doses of seaweed extract did not affect post-prandial blood glucose in our participants. After ingestion of a standardized carbohydrate rich breakfast, blood glucose increased by around 80% on all three study days and neither C_{max} , t_{max} nor AUC were significantly different between placebo and seaweed administration. As 100% of our participants were obese and the majority had disturbed glucose metabolism to some degree, we think this group was appropriate to detect potential effects of seaweed extract on post-prandial blood glucose.

One possible reason for this discrepancy in results between our *in-vitro* and *in-vivo* study is that the amount of fucoidan in the extract might have been too low to yield any meaningful changes in blood glucose.

In vitro inhibition of carbohydrate digesting enzymes α -glucosidase and α -amylase could have been caused by unspecific protein binding of polyphenols rather than from specific binding from fucoidan [32]. The interaction of polyphenols with proteins has emerged as a relevant mechanism to explain several of polyphenol biological effects. For the nonspecific type of mechanisms, the chemical features shared by most of the polyphenols, i.e., the phenol group, are key to their biological actions. Further, it has been reported that different polyphenols are efficient inhibitors of the activity of a broad number of enzymes. However, it is possible that in the human study polyphenols got diluted with food and digestive secretions and thus did not affect carbohydrate digestion in the small intestine.

Since both animal studies and cross-sectional studies have indicated anti-diabetic effects of seaweed [9,10,11,12,13], we do not exclude the possibility that seaweed extract has long term effects on bloodglucose control by mechanisms that work independently from carbohydrate digestion. When investigating new compounds for human consumption, it is critical to consider safety issues. Although bladderwrack does not have GRAS status, it is freely available as dietary supplement and has been used as food (-ingredient), mainly in Asian cuisine. The non clinical toxicology and clinical toxicology and safety of fucoidan derived from bladderwrack and other sources has been recently reviewed by Fitton [33] and in humans doses up to 3 g/day seem to be well tolerated.

In our human study the participants did not report any adverse effects. The intake of the highest strength of seaweed extract adds to the total trace element intakes. Still, estimated total intakes stay far below upper levels of intake for lead, mercury and cadmium. However, intakes of iodine and arsenic deserve more consideration.

When ingesting the high dose of seaweed extract, the estimated total dietary iodine intake approaches upper levels of intake which is 600 μ g a day [20]. This estimated intake is based on mean intakes of the Icelandic population [19]. Considering the large standard deviation of iodine intake according national dietary survey 2012, it is clear that some individuals will actually achieve higher intakes than 600 μ g/d. The upper level of intake can be defined as the highest level of chronic nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals [20]. As in this study participants received the highest dose only once, we conclude that intake of iodine higher than 600 μ g/d will not be harmful for the participants. However, this can be a matter of concern for a long term intervention study or for chronic consumption of a high dose of seaweed extract.

The most toxic forms of arsenic are the inorganic arsenic compounds; the inorganic arsenic trioxide is well known as a rat poison. Methylated forms of arsenic have a low acute toxicity; arsenobetaine which is the principal arsenic form in fish and crustaceans is considered non-toxic. In shellfish, molluscs and seaweed dimethylarsinylriboside derivatives occur ("arsenosugars"), the possible toxicity of which is not known in detail [34]. In agreement with that, upper intake levels are 210 μ g/d for a 70 kg person [21]. Although the arsenic intakes are not known for the Icelandic population, the highest dose of seaweed extract adds only around 0.2 μ g of inorganic arsenic which is unlikely to play a significant role in risk for the participants.

Strengths and limitations

It is a strength of this study that it used both an *in vitro* study design as well as an *in vivo* study design in humans. However, as the human intervention tested only the acute effects on blood glucose, longer intervention trials have to investigate potential long-term effects on glucose metabolism and safety.

Conclusion

Our study shows that extract from the brown seaweed bladderwrack inhibits α -glucosidase and α -amylase *in vitro*, however, ingestion of 800 or 1600 mg extract does not result into reduced post-prandial blood glucose after consumption of a carbohydrate rich breakfast in obese, adult volunteers. Future studies have to determine whether seaweed extract has long term effects on blood glucose control by mechanisms that work independently from carbohydrate digestion.

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Conflict of interest

The authors declare no conflict of interests.

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