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## Fermentability characteristics of different *Saccharomyces cerevisiae* cell wall using cat faeces as *inoculum*

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### ABSTRACT

Yeast cell wall are a source of prebiotics in animal nutrition, able to improve microbial function in the large intestine. The purpose of this paper was to compare the fermentability of six *Saccharomyces cerevisiae* (SC) cell wall obtained from three production processes (alcoholic, brewers and bakers). For each production process the two aliquots characterised by the highest the lowest carbohydrate levels were selected. In order to perform the *in vitro* trial, each SC substrate was incubated at 39 °C under anaerobic condition for 72 h, using cat faecal *inoculum*. The results showed that the production processes did not affect organic matter disappearance, while significantly ( $p < .01$ ) influenced cumulative volume of gas which appeared the lowest for alcoholic substrates. The alcoholic SC substrate showed significantly highest ( $p < .01$ ) fermentation rate. All SCFA were affected by the production processes ( $p < .01$ ). In particular, brewers substrates showed the highest acetate and butyrate production, while the fermentation of SC cell wall obtained by bakers showed the highest production of valerate, iso-butyrate and iso-valerate. Considering the high fermentability and acetate and butyrate production, the SC cell wall obtained by brewers seem more suitable to be used as prebiotic in cat diet. The bakers extract seems less advisable, due to its less fermentability at which corresponded the highest production of gas and iso-butyrate and iso-valerate, which are considered the less desirable end-products. The study underlines that it is necessary to evaluate the fermentation characteristics of these ingredients before their use in diet formulation as source of prebiotics.

### HIGHLIGHTS

- The production process affects the *in vitro* fermentation parameters differently from the carbohydrate's concentration.
- The production processes significantly influenced cumulative volume of gas and all short chain fatty acids production.
- The SC cell walls obtained by brewers seem more suitable to be used as prebiotic in cat diet and the bakers extract seems the less advisable.

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Gas production; prebiotic; oligosaccharides; short chain fatty acids; ammonia; fermentation kinetics

## Introduction

Yeasts and their cell extracts have been recognised as safe supplements for human and animal diets by the US Food and Drug Administration (Rodriguez et al. 2015) and EU Reg 2017/1017. These products contain high concentration of mannan-oligosaccharides (MOS, about 14% on dry matter basis),  $\beta$ -glucans (about 24% on dry matter basis), lipids, chitin, vitamins (White et al. 2002; Jaehrig et al. 2008) and proteins which are often linked to the mannans and referred as the mannan-protein complex (Lipke and Ovalle, 1998).

$\beta$ -glucans and MOS are considered beneficial for gut microbiota, may be due to their fermentability in the large intestine or, as reported by Ringø et al. (2016), by their ability to limit the colonisation of pathogen bacteria. Several *in vivo* and *in vitro* studies have highlighted the cell wall function as prebiotic in human and animal nutrition (Chen and Seviour, 2007; Laroche and Michaud, 2007; Hai and Fotedar, 2009) by reducing the intestinal pathogen colonisation (Johnson et al. 2012; Ringø et al. 2016; Fomenky et al. 2017), decreasing the immunosuppression caused by stress

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(Magnani et al. 2009; Liu et al. 2011; Borchani et al. 2016) and supporting the immune system (Newman and Newman 2001; O'Quinn et al. 2001). Furthermore,  $\beta$ -glucans protect the body from infections (e.g. viral, fungal and bacterial) (Liu et al. 2011; Borchani et al. 2016).

As pointed out by Shurson (2018), animals have been fed with products containing yeasts from more than 100 years. However, in recent years, due to the need to limit the use of antibiotics, increasing interest has been addressed to the use of yeasts and their products in animal nutrition, in particular in pet-food industry, which produce a wide range of diets and supplements containing yeast, as well as yeast cell walls, both for dogs and cats. Different species of *Saccharomyces*, in particular *S. cerevisiae*, are largely adopted as prebiotics in animal nutrition (Desnoyers et al. 2009; Mackenthun et al. 2013), as well as supplements (Liu et al. 2008; Kafilzadeh et al., 2019).

Due to their ability to ferment carbohydrates into carbon dioxide and alcohols, *Saccharomyces cerevisiae* (SC) have been widely used in the food industry for millennia.

Many different processes are developed in order to obtain a better quality of yeast extracts. Yeast extracts may be obtained mechanically after autolysis by acid, alkali hydrolysis or by enzymatic treatment (Freimund et al. 2003; Borchani et al. 2014). The inclusion of SC cell wall in the diet could have different effects, in function of SC source and yield extraction, which affect the amount of nutrients and their purity (Freimund et al. 2003). It is assumed that the production methods can affect the chemical characteristics of yeast cell wall and fermentability in the hindgut and, consequently, their applications in cat nutrition to prevent or treat specific pathologies. The aim of the present study was to use the *in vitro* gas production technique (IVGPT) with feline faeces as *inoculum* in order to compare the fermentation characteristics of six yeast cell wall samples obtained by three production processes (alcoholic, brewers and bakers) with two carbohydrates (glucans + mannans) levels (high and low) and to evaluate their possible utilisation in cat-foods' production. Several authors consider the IVGPT, utilising faecal *inocula*, useful to evaluate the fermentability of specific ingredients for diets of monogastric species. In literature are present studies conducted with faecal *inocula* from dogs (Bosch et al. 2016), cats (Musco et al. 2016), pigs (Musco et al. 2015; Mercurio et al. 2016; Lee et al. 2018) and horses (Murray et al. 2014).

## Materials and methods

### Substrates sampling

At the processing plant the *Saccharomyces cerevisiae* collected from different food industries: alcoholic (A), brewers (BR) and bakers (BA) were individually hydrolysed and centrifugated in order to separate the cell wall. Then, the obtained pellets were dried with air insufflation (mean temperature 38–40 °C). Three aliquots of daily production were randomly analysed for carbohydrates (mannans + glucans) concentration; samples representative from those with higher and lower carbohydrates concentration of the annual production were chosen and divided in two groups (high and low). Hence, considering the production process and the carbohydrates concentration, the substrates were named as follows: SC-A high, SC-A low, SC-BR high, SC-BR low, SC-BA high and SC-BA low.

### Laboratory analyses

Mannans + glucans content was determined on all the aliquots at the laboratory of Biorigin (São Paulo, Brazil) in accordance with Freimund et al. (2005).

The proximate analysis of the substrates was performed (in quadruplicate) at the Feed Evaluation Laboratory of the Department of Veterinary Medicine and Animal Production (Napoli, Italy), according to AOAC (2005) procedures (dry matter ID 934.01, crude protein ID 954.01; ether extract ID 920.39 C; crude fibre ID 978.10; ash ID 942.05).

Each substrate was weighed ( $0.5033 \pm 0.002$  g) in quadruplicates into serum flasks with a medium (0.75 mL) containing nitrogen source, fatty acids, vitamins and minerals; a reducing agent solution (4 mL) was added to guarantee the bacterial growth, as well as the anaerobiosis (Calabrò et al. 2013). For this last purpose, flasks were continuously blowing with CO<sub>2</sub> until they were sealed with butyl rubber stoppers and aluminium crimps. To prepare the *inoculum*, faecal samples were collected from four healthy adult European cats (mean age:  $3.41 \pm 0.7$  years; mean live weight  $3.53 \pm 0.500$  kg). The cats were progressively adapted (over 10 days) to a commercial standard diet (% as fed: crude protein 36, crude fibre 2.0, ether extract 20) administered daily according to their energy requirements using the equation:  $100 \text{ kcal/kg}^{0.67}$  (NRC 2006). After collection the faecal samples were immediately transported to the laboratory and processed within 40 min. To reduce the variability (Bosch et al. 2017a, 2017b), the individual faecal samples were pooled, and successively diluted (1:10 v:v)

with 0.9% NaCl sterile solution, homogenised, filtered through six layers of gauze and added (5 mL) to the pre-warmed serum flasks. The flasks were placed in an incubator (PI400 Carbolite, Sheffield, UK) at 39 °C for 72 h (Bosch et al. 2017a). In addition, four flasks were incubated without substrate (negative control).

The fermenting gas production was recorded 16 times, at 3–4 h intervals, using a manual pressure transducer (Cole and Parmer Instrument, Vernon Hills, IL, USA). The cumulative volume of gas (OMCV, mL/g) obtained for each sample at 72 h was related to the quantity of incubated organic matter. The fermentation was stopped by cooling the flasks to 4 °C, and the fermenting liquor pH was measured (pH metre model 3030; Alessandrini Instrument SpA glass electrode Jenway, Dunmow, UK). Flask residues were filtered through pre-weighed sintered glass crucibles (porosity #2, DURAN Group GmbH Mainz, Germany), and residual organic matter was determined by burning the sample at 550 °C for 5 h. Organic matter disappearance (OMD, %) was calculated as the difference between incubated and residual OM.

Two aliquots (5 mL) of fermenting liquor were collected and frozen at –15 °C to determine the fermentation end-products. Short chain fatty acids (SCFA) were measured using gas chromatography technique, whereas ammonia (N-NH<sub>3</sub>) was determined using spectrophotometry technique (Musco et al. 2016).

All procedures involving animals were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Prot. 2019/0013729 of 08/02/2019).

### Data processing

The gas profile of each flask was fitted to the sigmoid model described by Groot et al. (1996) as follows:

$$G = \frac{A}{1 + \frac{C^B}{t^B}}$$

where G is the total gas produced (mL/g of incubated OM) at t (h) time; A is the asymptotic gas production (mL/g of incubated OM); B is the time at which half of the asymptote is reached (h); C is the curve switching characteristic. The model parameters were utilised (Bauer et al., 2001) to calculate the maximum fermentation rate ( $R_{max}$ , mL/h) and the time at which it occurs ( $T_{max}$ , h) according to the following formula:

$$R_{max} = \frac{A \cdot B^C \cdot B \cdot T_{max}^{(B-1)}}{(1 + C^B \cdot T_{max}^{-B})^2}$$

$$T_{max} = C \cdot [(B-1)/(B+1)]^{\frac{1}{B}}$$

The chemical composition of the SC cell walls and all the fermentation characteristics obtained (OMCV, OMD, SCFA, pH and N-NH<sub>3</sub>,  $T_{max}$ ,  $R_{max}$ ) were subjected to analysis of variance using the JMP software, version 14 (SAS Institute, Cary, NC, USA, 2014) to detect the differences between the production processes and carbohydrates concentration according to the following equation:

$$y_{ijk} = \mu + PP_i + CARB_j + PP * CARB_{ij} + \varepsilon_{ijk}$$

where y is the experimental data;  $\mu$  is the general mean; PP is the production process (i = alcoholic, brewers, bakers); CARB is the carbohydrates concentration (j = high, low); PP \* CARB is the first level of interaction in which the effect of one causal variable on an outcome depends on the state of a second causal variable;  $\varepsilon$  is the error term.

When significant differences among substrates were found by the analysis of variance, means were compared using the t test employing the JMP software (SAS Institute, Cary, NC, USA, 2014, version 14.0).

**Table 1.** Proximate analysis (% a.f.) of the *Saccharomyces cerevisiae* cell wall obtained from three production processes.

Substrate	DM	CP	EE	CF	Ash	Glucans + mannans
	Production process effect (PP)					
SC-A	93.52b	28.99A	2.92C	1.10	2.02B	60.60AB
SC-BR	95.95a	21.92C	3.21B	1.30B	6.29A	63.18A
SC-BA	93.07b	23.87B	11.60A	1.60A	2.46B	56.21B
	Carbohydrates concentration effect (CARB)					
High	94.52	23.40B	6.03A	1.21B	3.44	65.25A
Low	93.85	26.46A	5.80B	1.46A	3.74	54.75B
Interaction (P)	0.87	<0.00	0.76	0.00	0.00	0.07
SEM	1.03	0.61	0.08	0.10	0.51	2.29

SC-A: *Saccharomyces cerevisiae* alcoholic extract; SC-BR: *Saccharomyces cerevisiae* brewers extract; SC-BA: *Saccharomyces cerevisiae* bakers extract; High and Low: levels of carbohydrates concentration; DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; SEM: standard error of the mean.

Along the column for each effect: A, B, C =  $p < .01$ ; a, b =  $p < .05$ .

**Table 2.** *In vitro* fermentation parameters for *Saccharomyces cerevisiae* incubated with cat faeces.

Substrate	OMD, %	OMCV, mL/g	$T_{max}$ , h	$R_{max}$ , mL/h
Production process effect (PP)				
SC-A	96.65	147.50B	14.06C	8.30A
SC-BR	95.67	183.40A	16.74B	6.22B
SC-BA	88.35	173.30A	19.24A	6.44B
Carbohydrates concentration effect (CARB)				
High	91.26	166.30	16.36	7.17
Low	95.85	161.80	17.00	6.80
Interaction (P)				
SEM	12.42	10.87	1.38	1.10

SC-A: *Saccharomyces cerevisiae* alcoholic extract; SC-BR: *Saccharomyces cerevisiae* brewers extract; SC-BA: *Saccharomyces cerevisiae* bakers extract; High and Low: levels of carbohydrates concentration; OMD: organic matter disappearance (% of incubated OM); OMCV: cumulative volume of gas related to incubated OM (mL/g); Maximum fermentation rate ( $R_{max}$ ) and time at which it occurred ( $T_{max}$ ); SEM: standard error of the mean. Along the column for each effect: A, B, C =  $p < .01$ .

## Results

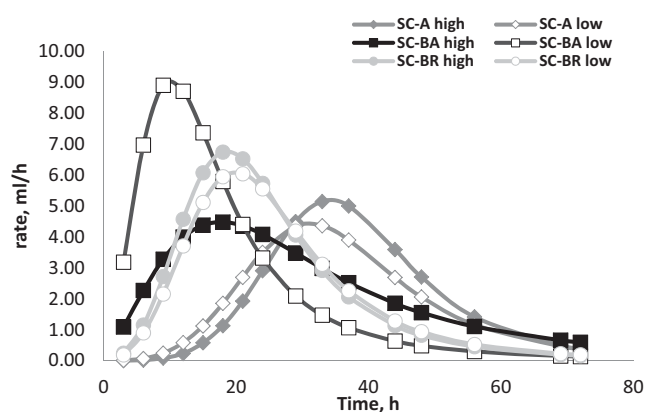
### Proximate analysis

The chemical composition characteristics of the tested substrates are depicted in Table 1.

All chemical parameters were affected by the production processes. In particular, bakers extract showed the highest ( $p < .01$ ) EE and CF compared to the alcoholic and brewer extracts. On the other hand, the alcoholic extract showed the highest ( $p < .01$ ) crude protein content, and brewers extract the highest ash and glucans + mannans levels, compared to the other samples. Considering the carbohydrates effect, the High SC showed highest ( $p < .01$ ) EE, while the Low SC showed the high content of CP and CF ( $p < .01$ ). The interaction was high significant for CP, CF and ash.

### In vitro fermentation characteristics

Usually a gas production trial with carnivores *inocula* is stopped at 48 h of incubation; however, in this study the incubation was prolonged (72 h) until gas production started to decrease clearly. The *in vitro* fermentation parameters are reported in Table 2. Neither the production process nor carbohydrates concentration affected the organic matter disappearance, which always resulted higher than 88%. On the contrary, the production processes significantly ( $p < .01$ ) affected all the other parameters: OMCV appeared lower for the yeast cell wall obtained by alcoholic process than that obtained from brewers and bakers ones, even if the interaction was significant for OMCV ( $p < .05$ ).  $T_{max}$  not linearly differs among the production processes, as indicated by the high significance of the interaction ( $p < .0001$ ). Compared to the other substrates, the



**Figure 1.** *In vitro* fermentation kinetics of *Saccharomyces cerevisiae* cell wall obtained from different extraction processes with different carbohydrates concentration. *Saccharomyces cerevisiae* obtained from alcoholic extract (SC-A), brewers extract (SC-BR) and bakers extract (SC-BA) with two levels of carbohydrates concentration (high and low).

fastest fermentation kinetics ( $p < .01$ ) was observed in SC-A, which reached the highest  $R_{max}$  values in the shortest time (14 h of incubation), whereas the slowest ( $p < .01$ ) was observed in SC-BA, which needed more than 19 h to achieve the maximum fermentation rate.

The fermentation rate profiles for the three production processes with the two levels of carbohydrates are illustrated in Figure 1. Considering the carbohydrates levels within the same production process, different trend appear: for A and BR the curves show similar shape between low and high (for both the processes is more rapid and consistent when high carbohydrates content is present), otherwise for BA large differences emerged between low and high carbohydrates level. In particular, the fermentation trend shows a bell-shaped curve when the carbohydrates content is low and a flatter shape when the carbohydrates content is high. The differences between the BA substrates are evidenced also by  $R_{max}$  value, which was more than double for low (9.13 mL/h) than high carbohydrates ones (4.49 mL/h).

### Fermentation end-products

In Table 3, the concentration of the fermentation end-products registered after 72 h of incubation are reported. The production processes significantly ( $p < .01$ ) influenced all short chain fatty acids, whereas the carbohydrates levels only few. In particular, cell wall obtained by brewers showed the highest acetate and butyrate production ( $p < .01$ ), while the fermentation of the cell wall obtained by bakers allowed the highest production of valerate, iso-butyrate and iso-valerate ( $p < .01$ ). The fermentation of yeast cell wall

**Table 3.** Fermentation end-products (mmol/g of incubated organic matter) and pH at 72 h of *Saccharomyces cerevisiae* cell wall.

Substrate	Acetate	Propionate	Iso-butyrate	Butyrate	Iso-valerate	Valerate	N-NH <sub>3</sub>	pH
			Production process effect (PP)					
SC-A	14.12B	6.98A	0.34B	1.57B	0.52B	0.58B	69.29	6.28
SC-BR	15.65A	5.92B	0.33B	2.10A	0.50B	0.58B	42.61	6.37
SC-BA	14.62B	5.18C	0.41A	1.76B	0.61A	0.91A	45.78	6.31
			Carbohydrates concentration effect (CARB)					
High	14.71	6.41A	0.35	1.94	0.51A	0.63B	53.40	6.30
Low	14.88	5.65B	0.36	1.68	0.57B	0.74A	51.72	6.34
Interaction (P)	0.00	0.00	0.58	0.00	0.71	0.00	<0.00	0.44
SEM	0.85	0.39	0.03	0.18	0.06	0.10	2.31	0.03

SC-A: *Saccharomyces cerevisiae* alcoholic extract; SC-BR: *Saccharomyces cerevisiae* brewers extract; SC-BA: *Saccharomyces cerevisiae* bakers extracts; High and Low: levels of carbohydrates concentration; N-NH<sub>3</sub>: ammonia; SEM: standard error of the mean.

Along the column for each effect: A, B, C =  $p < .01$ .

characterised by high mannans + glucans concentration produced a significantly ( $p < .01$ ) higher concentration of propionate and iso-valerate and a lower amount of valerate. No statistical differences were detected for pH and N-NH<sub>3</sub> production due to both factors. As regards to N-NH<sub>3</sub> content, only the interaction between the production system and carbohydrates concentration was statistically significant ( $p < .01$ ).

## Discussion

Fermentable carbohydrates are important components of pet diets due to their widely known properties. These polysaccharides remain almost intact in the first part of the intestine until they reach the foregut, where they become a substrate available for the bacteria attack (Musco et al. 2017). They are a source of organic matter for the large intestine microbiota and stimulate the microorganism growth throughout the production of short chain fatty acids. Instead, butyric acid represents the most important source of energy for the colonocytes, as described by Vastolo et al. (2019). All yeast cell walls, and in particular that one from *Saccharomyces cerevisiae*, have been recognised to be a newsworthy source of fermentable carbohydrates. All fungal cell walls consist of polysaccharides; in particular, the chitin and various glucans homopolymers of D-glucose were most frequently found in building blocks used by nature to construct a barrier that separates the fungus from the external environment. In the yeast cell, the  $\alpha$ - and  $\beta$ -glucans and the  $\alpha$ -mannan are the major polysaccharides utilised in cell wall construction (only chitin represents 1–2%) (Kwiatkowski and Kwiatkowski, 2012). In addition some authors (Freimund et al. 2005; Pinto et al. 2015) reported that the chemical composition of the yeast cell walls, in terms of ether extract, crude protein and crude fibre, is highly affected by the starting materials but also by the production process. The differences in nutrient composition noticeably affect the functional

effect when cat diets are supplemented with yeast cell wall (Aquino et al. 2013; Santos et al. 2018). As a consequence, it is quite difficult to predict the real effects of a new functional product and to compare the results of different studies using supplementations based on yeast cell wall, because their availability and fermentability in the large intestine could differ a lot. Furthermore, only a few studies have been performed using *in vitro* technique to evaluate the fermentation characteristics of carbohydrate-rich products using cat faces as *inoculum* (Musco et al. 2016; Bosch et al. 2017a, 2017b).

In this study, some significant differences in chemical composition among the tested substrates were observed, but the trend in fermentation parameters was not always easy explainable by these differences. These last could be due to the yeast strains and/or to the temperature of processing used in the food industries. For example, the significantly highest amount of mannans + glucans in *Saccharomyces cerevisiae* brewers extracts surely contributed to the high volume of gas produced, whereas the low gas production of the alcoholic extracts could be due to their high protein content. Probably, the significantly highest lipid concentration of bakers extract could explain the lower OMD of these substrates. However, it is difficult to explain the high gas production, even if produced slowly, recorded in these substrates.

The short chain fatty acids are the main end-products of the bacterial fermentation in the mammal's colon, and they are considered as indicators of nutritional evaluation of ingredients rich in carbohydrates used in pet-food (Cutrignelli et al. 2009). The main SCFA (acetate, propionate and butyrate) are rapidly absorbed and then metabolised by the gut epithelium, liver and muscle and have a trophic effect on the intestinal epithelium, maintaining the mucosal defence barrier against pathogens organisms (Mroz, 2005).

All the tested substrates ranged SCFA as follows: acetate > propionate > butyrate. This observation, together

with the pH values registered at the end of fermentation, suggests that the fermentation falls in the physiological ranges for cats (Younes et al. 2001). Notwithstanding, for all the samples the SCFA production and the proportion of each fatty acid were significantly affected by the production process, while the concentration of carbohydrates (high vs. low) significantly affected only the propionate, valerate and iso-valerate production.

It is interesting to compare these results with those obtained in our previous study (Musco et al. 2018), incubating the same substrates, in the same experimental conditions, but using dog faecal *inoculum*. All tested substrates were fermented by both *inocula*, but the faecal microorganism activities seem different in terms of fermentation pattern and obtained final products. In particular, the cat intestinal microbiota seems more influenced by the variations due to the chemical composition of substrates compared to dog ones, in fact several differences in the proportion of SCFA were observed in cat in function of carbohydrates concentration unlike dog. These differences could be due to the specific microbial populations and/or activities in the two animal species. Our observation is in accordance with that reported by Garcia-Mazcorro et al. (2015), which evaluating *in vivo* the effect of different prebiotic supplementation in dog and cat microbiota, concluded that the two species respond differently to the same treatment.

## Conclusions

From the findings obtained, it seems evident that the *Saccharomyces cerevisiae* cell wall production process affects most of the *in vitro* fermentation parameters differently from the carbohydrates (mannans + glucans) concentration. In particular, brewers extracts seem more useful supplement for cat diets. The high butyrate production obtained incubating these substrates suggest a better trophic effect on gut epithelium. Whereas the high gas production and fermentation rate registered suggest the importance of the dosage. In fact, the use of wrong quantity may cause undesirable effects, such as meteorism and abdominal pains. The bakers extracts seem a less advisable substrate because of its lower fermentability at which correspond the highest production of gas and iso-butyrate and iso-valerate, which are considered the less desirable end-products. These last, in fact, derived from the fermentation of branched-chain amino acids (valine, leucine and iso-leucine).

Therefore, it is suggested to test ingredients with functional characteristics, such as yeast, not only for

chemical composition (e.g. protein/carbohydrates ratio) but also for their *in vitro* fermentation patterns, before introducing them in cat diets.

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## Author contributions

M.I.C. and S.C. contributed to the project administration, conceptualisation and writing-original draft; N.M. and A.V. performed the analyses; F.R. contributed to the collection of samples; M.I.C., M.C., L.E. supported the acquisition and interpretation of data and contributed in writing the article; N.M. editing the paper. All the authors gave final approval to the manuscript and any revised version submitted.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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