Supplementation of broiler's rations with *Saccharomyces Cerevisiae* prebiotic at different growth phases: effect growth performances and caecal microbiota

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SUMMARY

This study aims to investigate the effect of a dietary supplementation with *Saccharomyces cerevisiae*-derived prebiotic at different growth phases on growth performance and the caecal microbiota of broilers. A total of 192 male chicks Arbor Acres (n=8 chickens/cage) were divided into three groups: the first group was considered as a control and received a basal diet (T0). The second group was fed with a 2 g prebiotic /kg ration during the starter period (first two weeks) (T1). The third group received a basal diet supplemented with a 2 g prebiotic /kg ration until the fifth week of rearing (T2). Body weight, feed intake were recorded for three representative growth periods per weeks 0-3, 4-6, and 0-6 and bodyweight gains (DWG), and feed conversion ratios (FCR) were then calculated. At 7, 21, and 35 days of age, eight birds were selected from each group, and slaughtered after 12 h fasting. After dressing, the intestinal tract was directly eliminated. The caecum content was evacuated to perform microbiological analysis (lactic acid bacteria, total coliforms, and *Escherichia coli*). The results showed a significant decrease ($P = 0.04$) in FCR during the period from 0 to 3 weeks and the whole period was observed in the T2 group. The microbiological analysis showed an increase in *Lactobacillus*, and a decrease in *Escherichia coli* and total coliforms, in the group T2 that received prebiotic during the fifth week ($P < 0.001$). On day 21 of the experiment, the count of *Lactobacillus* was higher in the group T2 (6.40±0.01; $P < 0.001$). *Escherichia coli* and Coliforms counts were higher in broilers subjected to control diet and T1 ($P < 0.001$).
In conclusion, the addition of *Saccharomyces cerevisiae*-derived prebiotic during the fifth week of rearing can improve chickens' live performances through a selective effect on caecal microflora, leading to better protection against pathogens.

KEY WORDS

Saccharomyces cerevisiae-derived prebiotic, gut microbiota, broilers, breeding period, FCR.

INTRODUCTION

Considerable research has been conducted to assess the potential performance and health benefits of yeast-based products for animals (1). Many studies showed that supplementation with yeast culture (YC) enhances animal nutrition and health and that dietary supplementation of *Saccharomyces cerevisiae* could increase chicken growth and feed efficiency, and stimulate the immune system in animals (2). The gut microbiota plays fundamental roles in nutrient utilization, intestinal morphology, productive traits, immunity, and well-being by interacting with nutrients and the development of the gut system of the host (3, 4). Over the past decades, a common task to maintain host health is to optimize the gut microbiota of chickens using dietary supplementations. Although they have been banned by the European Union since 2006 due to the increasing concerns about resistance and its potential threat to consumers (5), antibiotics have been used at sub-therapeutic levels as growth promoters (AGPs) to boost the health and production of poultry (6). Finding alternatives to antibiotics has become an urgen need to maintain a balanced gut and satisfactory level of poultry production. Various feed additives have been proposed as natural growth promoters, including organic acids, cinnamon, enzymes, phytogenics, antimicrobial peptides, hyperimmune egg antibodies, probiotics, bacteriophages, nano-particles, and metals (7).
During the last years, prebiotics have gained considerable at-

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tention as potential alternatives to antibiotics and their beneficial effects on gut microbiota have been well demonstrated. Gibson and Roberfroid (8) defined prebiotics as indigestible food ingredients that promote one or more beneficial bacteria in the GIT, enhance GIT health, and potentially improve host health. Several types of indigestible oligosaccharides, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), mannan oligosaccharides (MOS), and isomaltose oligosaccharides (IMO), are considered to be prebiotic and have been studied as alternatives to AGPs (9,10). Previous studies reported that prebiotics can significantly modulate the intestinal microbial community by increasing the number of *Lactobacillus* and *Bifidobacterium* and reducing intestinal colonization of pathogenic bacteria by blocking their attachment sites on the intestinal mucosa through a process known as competitive exclusion (11,12). Therefore, prebiotics has the obvious ability to selectively enrich beneficial microorganisms associated with health and well-being. Moreover, refined functional carbohydrates (RFC) produced from enzymatic hydrolysis of the cell wall of *Saccharomyces cerevisiae*, including MOS, -glucans, and D-mannose, have also shown beneficial effects on broilers growth performance and gut pathogen colonization (13). Furthermore, the supplementation chicken's diet with prebiotics showed to decrease human foodborne pathogenic bacteria, such as *Salmonella* (9) and *Campylobacter* (13), consequently ensuring safe poultry products . However, a previous study conducted by Askri et al. (15) indicated that a short administration with *Saccharomyces cerevisiae*-derived prebiotic during only the starter phase did not improve growth performances. Consequently, theses results sowed that shorter periods of supplementation was not enough to elicit growth performances. This suggested that the beneficial prebiotics effects were dependent not only upon the individual constituent components of a prebiotic product but also in function of the growth phases.

The current study aimed to determine whether a *Saccharomyces cerevisiae*-derived prebiotic supplementation in broiler diet can affect performances and caecal microflora composition and if the growth phases play a significant role.

MATERIALS AND METHODS

Ethical considerations

The animal care protocol was approved by the Official Animal Care and Use Committee of the National Agronomic Institute of Tunisia (protocol N° 05/15) before the initiation of research and followed the Tunisian guidelines.

Birds and housing

This experiment was carried out in the poultry unit of the National Agronomic Institute of Tunisia. One hundred and ninety-two male day-old chicks from the 'Arbor Acres' strain were used in the current trial over 42 days. All birds were individually identified, weighed, divided into three groups, and were housed in cages. There were eight replicates for each group with 8 chicks per cage. All birds were vaccinated against Newcastle Disease, Infectious Bronchitis, and Gumboro (IBD). The climatic conditions and lighting program followed the commercial recommendations. Routine hygiene practices such as fumigation were recorded during the rearing period as recommended. The room temperature was gradually decreased from 33 °C, on day 3 to 24 °C until the end of the experiment. Chicks were kept under continuous light by the use of lamp lighting on a light regime consisting of 24 h light. Temperature, humidity, and ventilation were monitored recorded daily, and adjusted in response to bird comfort. The feeders and waterers were adjusted, according to the progressive growth of the chicks. Feed and water were supplied *ad libitum* throughout the experiment. All chicks were fed starter and grower-finisher diets from 1 to 14 d and 15 to 42 d, respectively. From day one chickens were fed either a corn and soybean meal basal diet formulated to meet the recommendations of the National Research Council. The nutrient composition of diets fed during the starter and finisher periods was 2900 Kcal/kg, and 2970 Kcal/kg of metabolized energy ; 20.5% and; 19.5% of crude protein, respectively (15).

Dietary Treatments

All diets were given in the mash form and did not contain antimicrobial growth promoters or coccidiostats. The prebiotic develeped using a revolutionary enzymatic hydrolysis process that breaks down the yeast cell wall into highly available Refined Functionnal Carbohydrates. The product is based on a yeast culture and products of the enzymatic hydrolysis of the *Saccharomyces cerevisiae* wall such as mannan oligosaccharides (MOS), mannose, beta-glucans, and galactosamine. The control group received a basal diet (T0). The second group received a basal diet supplemented with a dose of 2 g/kg of prebiotic during only the first two weeks (T1). The third group was fed a basal diet with a dose of 2 g of prebiotic for 5 weeks (T2). According to the recommendations of Askri et al (15), the prebiotic was removed one week before slaughter in order to preserve meat sensory quality. Feed and drinking water were provided *ad libitum*.

Growth Performance

Birds were weekly weighed and feed intake was calculated as the difference between the amount of feed supplied to the birds and the amount of feed refused. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to body weight gained. The body weight gains (BWG/g), and feed conversion ratios (FCR) were also calculated as per standard methods for each growing phase as follows: from weeks 0 to 3, weeks 4 to 6, and weeks 0 to 6 for the entire six weeks of the experimental study. The mortality rate was checked over the production trial period and no additional adjustment was performed because no mortality occurred.

Microbiological analysis

Sampling

At 7, 21, and 35 days of age, eight birds were slaughtered from each group after feed deprivation for 12 h, and directly after dressing the intestinal tract was eliminated. Intestinal content from the two caecum was evacuated and mixed in sterile glass bottles. The sealed bottles were saved in the laboratory at -20 °C till the enumeration of the microbial population.

Incubation Conditions

Escherichia coli and total coliform were chosen as markers of opportunistic bacteria and *Lactobacillus* was chosen as a marker of beneficial microflora. About 1 g of fresh samples was diluted 1:10 with sterile 0.1% peptone water in sterile test tubes (PW, Oxoid CM9) and a serial dilutions were performed in 1% peptone solution. Aliquots of 0.1 mL of each dilution were then spread on petri dishes containing the appropriate agar medi-

um: MRS (Gélose de Man, Rogosa, Sharpe), VRBL (Violet Red Bile Lactose Agar), and MacConkey agar plates to isolate the lactic acid bacteria, total coliforms, and *Escherichia coli*, respectively. The cultures were then incubated at 37°C for 48 h for *Lacotobacillus* and for 24 h fortotal coliforms, and *Escherichia coli*. The microflora colonies were counted manually after removal from the incubator. The concentration of microflora was finally expressed as log10 colony-forming units per gram of digest content.

Statistical Analysis

The data were subject to one-way ANOVA analysis with the GLM procedure of the statistical software package SAS version 9.4 (SAS Institute Inc., Cary, NC). The statistical assumption of residual normality was evaluated using the Shapiro-Wilk, while Levene's test was used for homogeneity of variances. Means difference was determined using the Student-Newman-Keuls test. Significance was considered at $P < 0.05$. The data were expressed as a means ± standard error. The statistical model was

$$Y_{ijk} = \mu + \alpha_i + e_{ij}$$

Where: Y_{ijk} = response variable. = overall mean value for Y. αi = fixed effects (j=1-3), and e_{ij} = error term.

RESULTS AND DISCUSSION

Effect of prebiotic administration Time on broiler performances

The effect of the basal diet supplemented with *Saccharomyces cerevisiae*-derived prebiotic on the performance of broilers was presented in Table 1. There was a significant difference in the average body weights (BW) of broilers among groups ($P < 0.05$). On day 21, the diets supplemented with *Saccharomyces cerevisiae* (T2) increased the BW of broilers compared to control group. The highest BW was recorded in T2 groups compared to T0 and T1 at (P=0.04). At day 42 too, BW and DWG showed an increasing trend reaching the highest value in T2 (P=0.02). The *Saccharomyces cerevisiae* did not affect BW and DWG in the early phase of growth. The body weights were not affected by the prebiotic administration during the breeding starter period on day 21 as well as day 42. Supplementation of broiler diets with *Saccharomyces cerevisiae*-derived prebiotic during the starter period (T1) did not affect FI and FCR. Moreover, FIs were significantly ($P < 0.05$) affected by the supplementation of *Saccharomyces cerevisiae*-derived prebiotic, indeed, decreases in FI were observed with T2 during the period 0-3 weeks, as well as from 4-6 weeks. Moreover, the FCR was improved by the prebiotic administration during the whole growing period from 0 to 3 weeks ($P = 0.04$), suggesting that the effect of prebiotics would be conditioned to its presence. Our results confirmed those found by Askri et al. (15) who showed that the prebiotic incorporation during the starter period did not improve growth performance. This study indicated that the presence of prebiotic in a broiler diet for a longer period was recommended to reach optimum live performance, particularly for FCR. These results could be explained by the fact that the duration for adaptation and the exposure of gastrointestinal tract (GIT) microbes to the supplemented prebiotic play a role in enhancing growth performance. Harmoniously, Hanning et al. (16) found a better result as they recorded enhancements in the villi height and crypt depth of the intestine when FOS was provided for a longer duration. Consistent with this study, the effects of *Saccharomyces cerevisiae* on performance were also reported for broilers and showed that yeast products did not affect their performance (17).

Effect of Prebiotic Administration Time on Caecal Microflora Population of Broilers

The results of the microbiological analysis were shown in Fig-

Table 1 - Effect prebiotic supplementation at different breeding stages on the growth performance of broiler chickens.

Item	Control	T1	T2	CV	P-value
			Bodyweights (g)		
14 day	297^{b} ±8.98	287^{c}±8.31	301^{a} ±8.98	7.50	0.03
21 day	569^{b}± 28.82	541^{c}±26.68	584^{a} ±28.82	12.52	0.04
42 day	1928^{b}±95.74	1803^{c}± 88.64	2026^{a}±95.74	12.25	0.02
			Weight gains (g/b/d)		
0-3 weeks	24.9^{b}±1.35	23.6^{c}±1.25	25.7^{a}± 1.35	13.48	0.03
4-6 weeks	69.7^{b} ±4.08	64.0^{c} ±3.78	74.3^{a} ±4.08	14.5	0.02
0-6 weeks	45.9^{b}±2.33	42.8^{c}±2.15	48.3^{a} ±2.33	12.53	0.02
			Feed intakes (g/b)		
0-3 weeks	41.8^{b}±1.68	43.8^{a}±1.56	40.5^{c}±1.59	9.17	0.03
4-6 weeks	106.9^{b} ±4.29	116.4^{a}±3.98	105.1^{b}±4.32	9.67	0.01
0-6 weeks	58.9^{b}±2.83	63.6^{a}±2.62	59.0^{ab}±2.83	11.43	0.04
			FCR (g/g)		
0-3 weeks	1.90^{ab}±0.17	2.20^{a}±0.16	1.53^{b}±0.17	7.10	0.04
4-6 weeks	1.61±0.16	1.81±0.15	1.62±0.16	11.71	0.50
0-6 weeks	1.73^{ab}±0.07	1.89^{a}±0.07	1.60^{b}±0.07	15.69	0.04

Data presented as (means ± SE); $^{a, b, c}$ Different letters in the same row denote significant ($P<0.05$) differences among treatments.
T:0 Control; T1: Group fed 2 g/kg of prebiotic during the starter period; T2: Group fed 2 g/kg of prebiotic until the fifth week of rearing.

ure 1. On day 7, results demonstrated the positive effect of prebiotic on the caecal microflora. *Lactobacillus* counts were significantly higher in the experimental group T1 and T2, on day 7. However, the removal of prebiotic in the T1 group has significantly reduced *Lactobacillus* loads at days 21 and 35, when compared to the T2 group still receiving the prebiotic. Our results suggested a direct effect of the prebiotic on *Lactobacillus* growth.

These findings are consistent with those of Jung et al. (18) demonstrating that prebiotics promoted the growth of beneficial bacteria such as *Lactobacilli* in the gut. Interestingly, our results indicated that prebiotic supplementation induced a significant increase in the caecal population of *Lactobacillus* in treated groups ($P < 0.05$), when the prebiotic was given. This confirmed that prebiotics can serve as a substrate for one or several beneficial bacteria present in the intestine. It was demonstrated that prebiotics selectively stimulates indigenous beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* (19). These outcomes are consistent with the findings of Jung et al. (18) who reported that dietary inclusion of prebiotic-based oligosaccharides in broiler chicks promoted the growth of beneficial bacteria such as *Lactobacilli.* Similarly, Baurhoo et al. (20) demonstrated that the incorporation of MOS in the broiler diet increased the *Lactobacilli* and *Bifidobacteria* population in comparison with the group fed antibiotic growth promotors (Virginiamycine). In another study, *in-ovo* injection of prebiotic as a new mode of administration which consists of early stimulation of intestinal microbiota development in the chicken gut had a positive effect on growth and performance due to the ability of prebiotics to increase intestinal *Lactobacilli* and *Bifidobacteria* populations, and these beneficial bacteria compete with harmful bacteria for colonization (21).

On the other hand, prebiotic supplementation led to a significant decrease in the caecal population of *Escherichia coli* and total coliforms in the T2 group received prebiotic until the fifth week of rearing ($P < 0001$). Our results are in agreement with those of Vieira et al. (22) who showed the positive effect of prebiotics supplementation in broiler diets in the protection against enteric pathogens, decrease of *E. coli* count. Likewise, Kleessen et al. (23) reported that using Jerusalem artichokes as prebiotic in-water supplementation reduced *Clostridium perfringens* number and endotoxin levels in broilers. Li et al. (24) showed that yeast cell wall (YCW) powder reduced significantly the *E. coli* population in the caecal content at 35 days ($P < 0.05$) but didn't have any significant effect on caecal *Salmonella* ($P > 0.05$). Furthermore, Askri et al. (12) revealed that the incorporation of *Saccharomyces cerevisiae* derived prebiotic in broiler diet had a significant improvement on caecal microbiota balance with a reduction of *E. coli* associated with an increase in the *Lactobacillus* population. Xu et al. (9) reported that using FOS as prebiotic in a broiler diet not only increased significantly to the growth of the *Bifidobacterium* and *Lactobacillus* population but also inhibited undesirable bacteria such as *E.coli*. Moreover, prebiotics FOS supplement on laying hens diet showed a positive effect on intestinal microbiota with a significant increase in *Lactobacilli* and a significant decline in *Campylobacter* and *Salmonella* (25). In contrast, Salehimanesh et al. (4) reported that prebiotic inclusion in broiler diet had no significant effect on

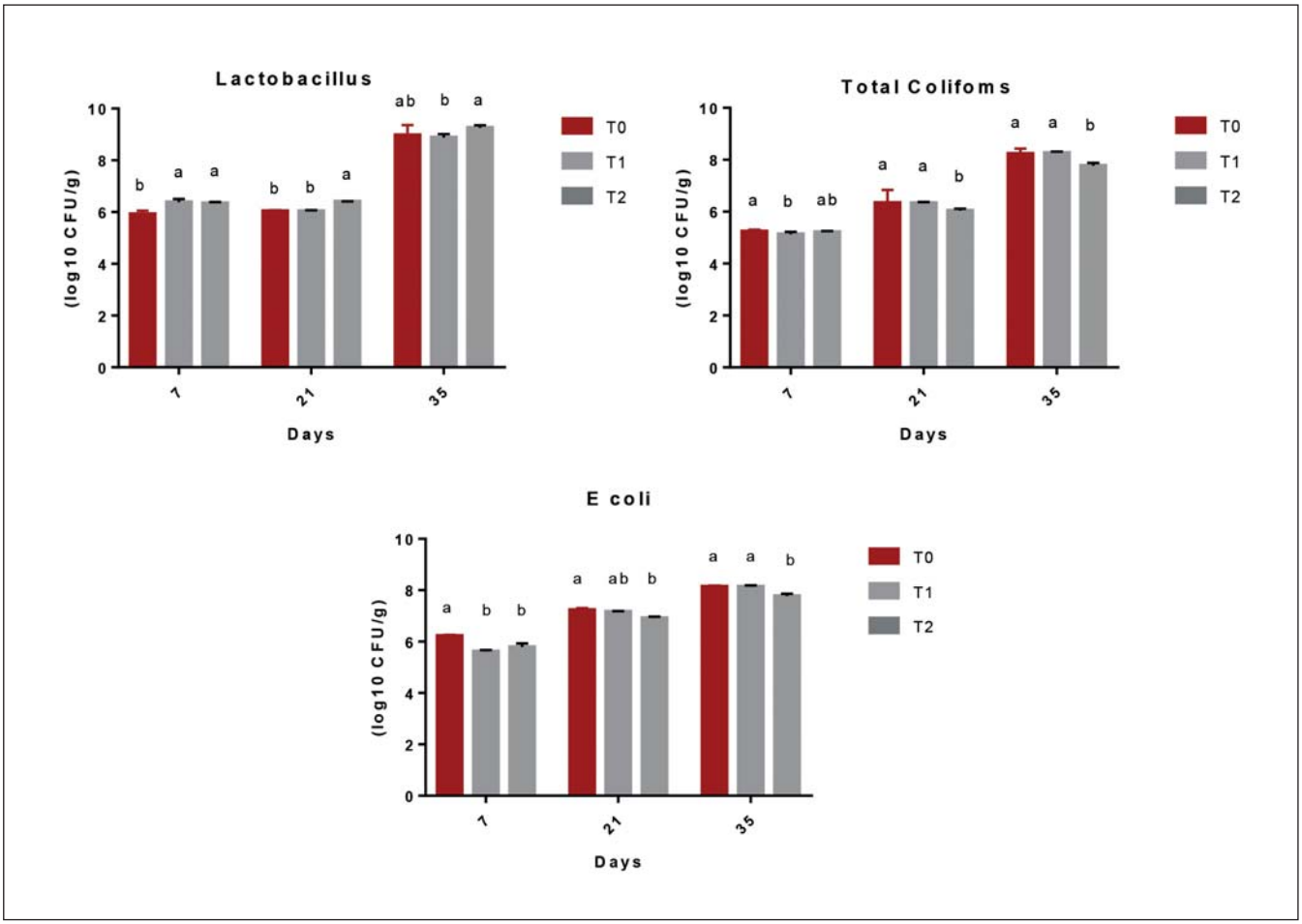

Figure 1 - Effect of breeding stages prebiotic supplementation on caecal microflora population of broilers (log10 CFU/g).

the microbial population of *Lactobacillus* spp, *E. coli,* and coliforms in the ileum.

Our results pointed out that the effects of prebiotic on *Lactobacillus* and total Coliforms were related to its administration. The withdrawal of prebiotic in the T1 group significantly reduced *Lactobacillus* loads and increased total Coliforms at days 21 and 35 when compared to the T2 group still receiving the prebiotic. Teng et al. (26) have reported direct or indirect mechanisms by which prebiotics could improve the ecosystem of the chicken gut. In particular, prebiotics directly promote the growth of *Lactobacillus*, which then prevents enteropathogen colonization. Similarly, prebiotic RFC induced a reduction in *Salmonella*, and *E. coli* loads in broiler caecas (13). Prebiotic RFC, FOS, GOS, and raffinose were shown to inhibit *in vitro* adhesion of *Salmonella Typhimurium* and *Campylobacter jejuni* pathogens to chicken epithelial cells (27). Additionally, MOS can also hamper the attachment to and colonization of intestinal epithelia by certain pathogenic bacteria, such as *Escherichia coli* and total coliforms. This action was partially due to the binding between Type 1 fimbriae of some Coliforms and MOS. Seifert and Watzl (28) have suggested a mechanism based on a direct effect of oligosaccharides with carbohydrate receptors on intestinal epithelial cells and immune cells, or possibly even partial absorption of the oligosaccharides, resulting in local and systemic responses. Several mechanisms are anticipated explaining the mutual relation between the positive effects of prebiotics on broiler performance and gut health. Their role in facilitating the competitive exclusion of potential pathogens could be related to the fact that *Saccharomyces* MOS can block pathogen binding to mannan receptors on the mucosal surface. One of the main mechanisms of prebiotics is the production of short-chain fatty acids (SCFA), mainly butyrate, propionate, and acetate as a part of the fermentation process (26). After prebiotic supplementation, fermentation products such as SCFA increased and modified the bacterial ecosystem by lowering the pH that inhibits pathogens growth like *Salmonella* and *Campylobacter* and stimulates the growth of beneficial bacteria like *Bifidobacterium* and *Lactobacillus* (LAB), and the process is the most effective in the caecum (29). Due to other fermentation products, like bacteriocin produced by LAB and organic acids produced by *Bifidobacteria*, colonization of pathogenic bacteria is reduced from the gut. Also, the production of SCFA could be the reason behind better growth performance, since they provide energy to epithelial cells and increase the partition of nutrients into other tissues of the body (30).

CONCLUSION

The present study showed that the duration of *Saccharomyces cerevisiae*-derived prebiotic supplementation can produce varying responses in performances and caecal microflora composition. A longer period of supplementation (35 d) was needed to elicit beneficial responses. These findings suggest that the modulation of the microbial community by *Saccharomyces cerevisiae*-derived prebiotic led to improvement in animal health via increasing *Lactobacillus* count and by reducing Coliforms load. Further researches are needed to increase knowledge regarding the effect of prebiotic administration on the other detrimental microorganisms of broiler chickens such as *E.coli*, *Salmonnella* and *Campylobacter*, as well as investigating the direct *in-vitro* antimicrobial actions of *Saccharomyces cerevisiae*-derived prebiotic against these detrimental microorganisms.

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Conflict of interest

The authors declare that they do not have any competing interest

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