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β(1-3)(1-6)-D-glucans Modulate Immune Status and Blood Glucose Levels in Dogs

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The objective of this study is to evaluate the effects of adding two different glucans coded as BGO1 and BGO2 into commercial feed of dogs.
Study Design: We measured changes in phagocytosis, levels of IL-2 in blood, antibody formation and level of blood sugar (normal homeostasis and experimentally-induced hyperglycaemie with streptozotocin).
Place and Duration of Study: Department of Pathology, University of Louisville, and Department of Research and Development, Biorigin, June 2012 and May 2013.
Methodology: The technique employing phagocytosis of synthetic polymeric microspheres was used for evaluation of phagocytic activity. IL-2 production was evaluated in serum by an ELISA kit. Formation of antibodies was tested using ovalbumin as an antigen, level of specific antibodies was measured by ELISA. Blood sugar evaluation was done both in normal animals and in animals with experimentally-induced hyperglycaemie. The level of glucose in serum was measured by Antech Diagnostics.
Results: In phagocytosis, both glucans significantly (P ≤0.05 level) increased the phagocytic activity of blood monocytes and neutrophils. Both glucans were active in IL-2 tests, but BG01 activity was 160% of that of BG02. Similar data were achieved in evaluation of effects on antibody response – BG01 reached OD of 0.717, whereas BG02 reached OD 0.411. Blood sugar evaluations showed no effect of glucan on normal dogs, but significant (P ≤0.05 level) reduction in dogs with hyperglycaemie (both glucans showed same activity).

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Conclusion: Our data showed that both glucans had significant immunomodulating effects in immune reactions in the dog model, strongly suggesting that supplementation of feed with these glucans results in improvement of biological and immunological conditions of dogs.

Keywords: Dogs; glucan; phagocytosis; immunity; blood sugar; IL-2; diabetes.

1. INTRODUCTION

It is generally agreed that dogs were the first species of animal to be domesticated and archaeological evidence suggests it happened about 14,000 years ago [1]. To some extent, the dogs have been following some of the human evolution risk factors, such as inadequate feeding behavior, inadequate diets, obesity, physical inactivity, or aging. These factors may predispose them to chronic diseases and metabolic syndrome. When diabetes is considered, epidemiological factors closely match those of the latent autoimmune diabetes in the adult form of human type1diabetes. At least 50% of diabetic dogs have diabetes based on the immune destruction of beta cells. In addition, extensive pancreatic damage, most likely from chronic pancreatitis, causes about 28% of canine diabetes cases [2]. For both conditions, there are opportunities for dietary therapy with immunomodulators.

Glucan's role as a biologically active immunomodulator has been well documented for over 50 years. Initial interest in the immunomodulatory properties of polysaccharides was raised after experiments showing that a crude yeast cell preparation stimulated macrophages via activation of the complement system [3]. β(1-3)-D-Glucans belong to a group of physiologically active compounds called biological response modifiers and represent highly conserved structural components of cell walls of fungi (yeasts, molds and mushrooms) and seaweed. Since glucans are active throughout the entire animal kingdom [4], it is not surprising that attention has recently focused on dogs as well.

In addition to stimulating the immune system, glucan-like oligosaccharides were also found to influence ileal nutrient digestibility, microbial populations and concentrations of protein catabolites in the bowel of dogs [5]. In an older study, glucan helped dogs against acute radiation sickness [6]. In puppies, glucan was found to increase the effects of vaccines against canine parvovirus and rabies [7]. Similar results were achieved when glucan was used with polyvalent vaccines [8]. Using glucan alone, the effects on antigen-specific antibody response were less clear [9]. Glucan was also tested as a potential treatment for inflammatory bowel disease. The addition of glucan to the feed caused significant improvements in histopathological and immunological parameters of dogs [10].

Beyond of the long term relationships with humans (from the initial partnership for hunting to the nowadays family relationship), dogs have provided an enormous contribution to the study of diabetes mellitus. In 1889, Joseph von Mering and Oskar Minkowski created an animal model of diabetes based on dog's pancreatectomy and, in 1921, a diabetic dog became the first recipient of insulin therapy [11]. Fortunately non-invasive methods, based on temporary change on homeostasis, as experimentally induced hyperglycaemie with streptozotocin, have replaced invasive methods as pancreatectomy. Streptozotocin was originally used as an antibiotic and after that it was found to be selectively toxic to the beta cells of the pancreatic islets. Since then, it has been used on a limited basis for certain

treatment of cancers of the pancreatic islets and in research to produce an animal model for diabetes.

Based on the significance of what is mentioned above, we decided to evaluate the effects of adding two different glucans into commercial feed of dogs. These glucans differ in purity and in biotechnological processes used in their isolation. We measured changes in phagocytosis, levels of IL-2 in blood, antibody formation and level of blood sugar (normal homeostasis and experimentally-induced hyperglycaemie with streptozotocin). The objective of this study was to compare the effects of these two glucans on biological characteristics important for canine health.

2. MATERIALS AND METHODS

2.1 Animals and Diet

Male and female dogs (Marshall Farms, North Rose, NY, USA) were used in this study. At the onset, the animals were examined for signs of any disease and all were considered healthy on the basis of a lack of clinically relevant abnormalities. The animals were then randomly assigned to individual groups. During this study, none of the dogs used developed any disease—including lethargy, fever or gastrointestinal problems. Similarly, none of the dogs died or were euthanized during this study. The use of animals was approved by the University of Louisville IACUC committee (#10080).

2.2 Materials

Wright stain, sodium azide and streptozotocin were purchased from Sigma (St. Louis, MO, USA). Rat anti-CD4-FITC antibody was purchased from Serotec (Kiddlington, Great Britain), rat anti-CD8-FITC antibody from (MyBiosource, San Diego, USA) and mouse anti-CD19-FITC from Abcam (Cambridge, MA, USA).

2.3 Glucan

Biorigin has developed two different samples of insoluble glucans (BG 01 is 68.5% pure; BG 02 is 55.5% pure) both from Saccharomyces cerevisiae, using two different biotechnological processes. Based on different purities and immune effects, previously demonstrated in mice model (unpublished results), different amounts of samples were added to the feed.

Dogs were given regular food (standard Purina Dog Chow; Purina, USA) or food enhanced with 15 mg/kg/day of the BG 01 or 25 mg/kg/day of the BG 02.

2.4 Phagocytosis

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier [12,13]. Briefly: peripheral blood cells or isolated peritoneal cells were incubated *In vitro* with 0.05 ml of 2-hydroxyethyl methacrylate particles (HEMA; $5x10^8$/ml). The test tubes were incubated at 37°C for 60 min., with intermittent shaking. Smears were stained with Wright stain. The cells with three or more HEMA particles were considered positive. Mice were injected with either glucan or PBS (control). All experiments were performed in triplicate. At least 300 cells in 60 high power fields were examined in each experiment.

2.5 IL-2 Production

Sera obtained from animals fed with glucans or control feed were collected, filtered through 0.45 μm filters and stored at -80°C till experiment. The presence of IL-2 was evaluated using a Dog IL-2 ELISA kit (Bethyl Laboratories, Montgomery, TX, USA) as recommended by the manufacturer.

2.6 Antibody Formation

Formation of antibodies was evaluated using ovalbumin as an antigen. Animals were s.c. injected twice (two weeks apart) with 400 μg of albumin and the serum was collected 7 days after last injection. Experimental groups were fed with either glucan or normal feed. Level of specific antibodies against ovalbumin was detected by ELISA [14].

2.7 Blood Sugar Evaluation

For blood glucose evaluations, dogs were not fed for 24 hr prior to measurement of blood glucose levels. In additional experiments, we measured the effect of glucan feeding in animals with experimentally-induced hyperglycaemie. Dogs were pretreated (i.v.) with streptozotocin (250 mg/kg) 12 days before the start of feeding with the glucan [15]. The level of glucose in serum was measured by Antech Diagnostics (Louisville, KY, USA) using an glucose oxidase evaluation.

2.8 Statistics

Student's t-test was used to statistically analyze the data.

3. RESULTS AND DISCUSSION

3.1 Phagocytosis

Glucans show notable physiological effects, which is the main reason they are of such great interests throughout the world. Glucans belong to a class of biologically active natural compounds, usually addressed as natural immunostimulators or biological response modifiers. Thus far, among numerous immunomodulators of the first order, natural polysaccharides are the most researched molecules with over 10, 000 published studies. One of the strengths of glucan is that it affects numerous cell types and biological processes. In addition to the effects on various immune cells (such as macrophages, neutrophils or natural killer cells), it affects various physiological functions such as regulation of stress or cholesterol levels.

Glucans are well-established activators of cellular immunity, particularly macrophages. Phagocytosis is therefore one of the main defense reactions, where a stimulating effects of glucan are measured. Binding of glucan to specific receptors activates macrophages. These processes include migration, degranulation leading to increased expression of adhesive molecules, stimulation of respiratory burst, secretion of cytokines and phagocytosis (for review see [16]). For evaluation of the potential effects of our two glucan samples on phagocytosis, we used synthetic polymer microparticles based on hydroxyethyl methacrylate. These particles are known for their low negative charge and therefore minimal

subjective error during evaluation [13]. Our results showed that both types of glucan significantly potentiated phagocytic activity of both peripheral blood monocytes and neutrophils (Fig. 1). Glucan BG 02 was more active. Significant stimulation of phagocytosis by both peripheral blood monocytes and neutrophils shown in our study is in agreement with previous finding in mice [17]. As phagocytosis is the first defense reaction occurring after infection, our findings represent significant proof of activity of both glucans.

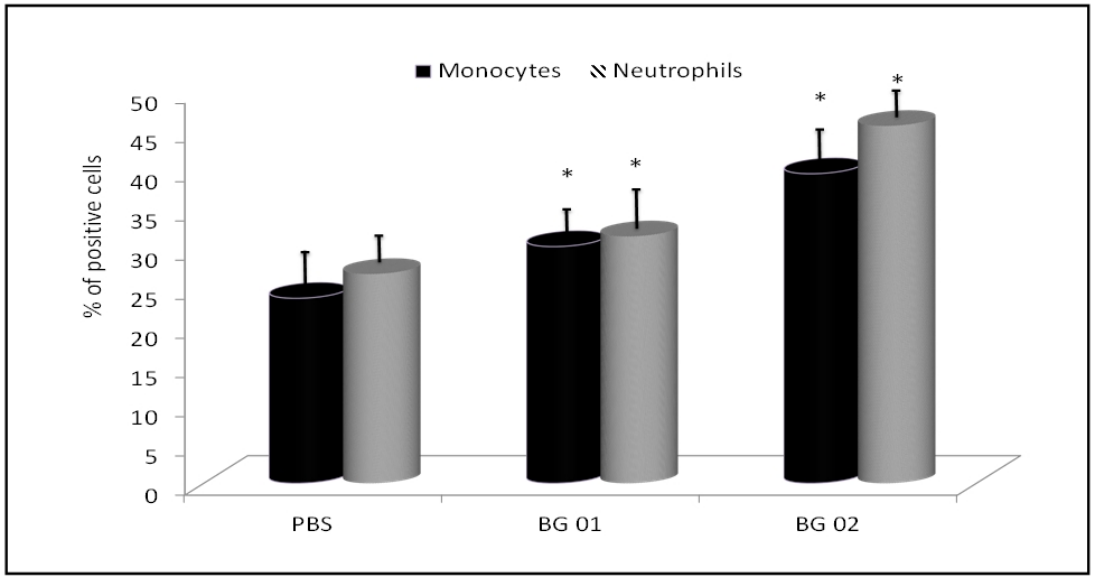

Fig. 1. Effect of dietary supplementation with glucan samples on phagocytosis by peripheral blood monocytes and neutrophils in dogs. Each value represents the mean±SD. *Represents significant differences between control (PBS) and tested samples at P ≤0.05 level

3.2 IL-2 Production

It is widely assumed that glucan application results in signaling processes leading to significant modulation of cytokines and other biologically active substances such as IL-1, IL-2, IL-6 and TNF-α [18,19]. Among them, IL-2 regulates the growth, proliferation, and differentiation of T cells and represent one of the most important cytokines. We next measured the effects of glucans on IL-2 production in blood. We found that, whereas both glucans stimulated IL-2 secretion, the effects of BG 01 were almost twice as strong as effects of BG 02. These experiments showed that feeding with glucan significantly increased the level of IL-2, but this time glucan BG 01 was more active (Fig. 2).

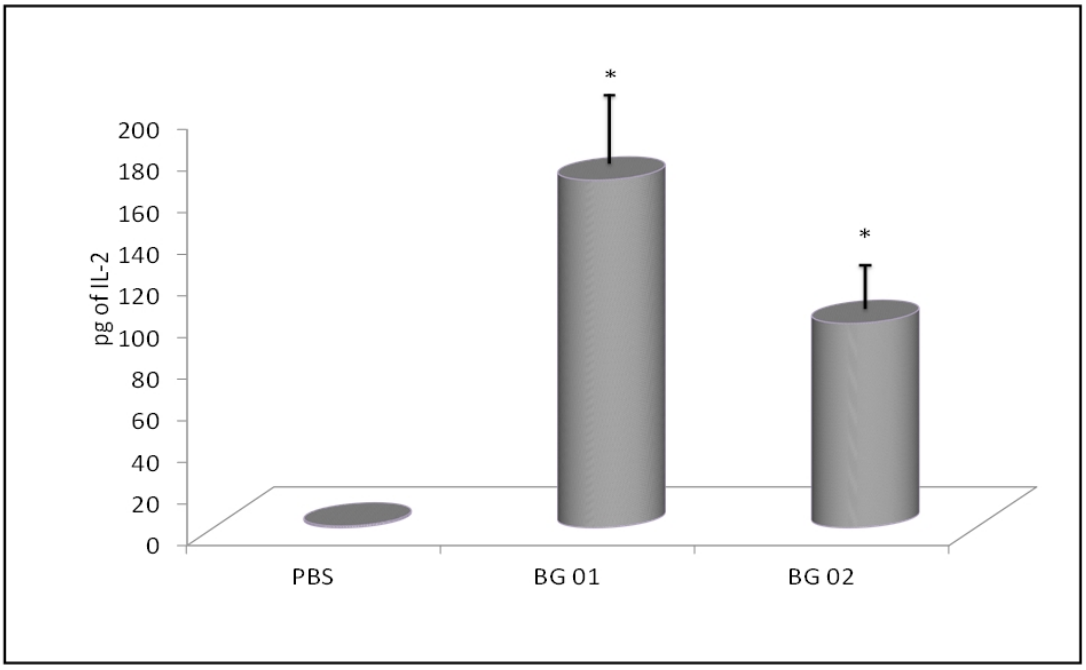

Fig. 2. Effect of dietary supplementation with glucan samples on IL-2 level in serum of dogs. Each value represents the mean±SD. *Represents significant differences between control (PBS) and tested samples at P ≤0.05 level. All experiments were performed in triplicates

3.3 Antibody Response

In addition to the well-established effects on macrophages and the whole cellular immunity, glucans were lately shown to affect also antibody response [20]. The finding of other laboratories of glucan's effects on antibody response in dogs [7,8,9] led us to also test this activity of our glucans. For evaluation of the possible stimulation of antibody response, we used a model of ovalbumin as an antigen. Our data showed that both glucans were active (again, sample BG 01 was more active), but a classical Freund's adjuvant caused stronger stimulation (Fig. 3). These data were similar to the results found in mice [21], with BG 01 showing stronger effects. In all cases, Freund's adjuvants were stronger. From our results we can conclude that both glucans stimulate both cellular and humoral branch of immune reactions.

3.4 Blood Sugar

The final part of our project was focused on effects of glucan addition on levels of blood sugar. The effects of glucans on blood sugar levels are less known. Some studies of glucan's effects on levels of blood glucose were done in humans. In a randomized blind study, the addition of oat glucan to food resulted in the significant lowering of blood glucose [22,23]. A study that included obese women demonstrated that barley glucan improved insulin response and postprandial glycemic response [24]. Recent experiments showed than oat glucan increased insulin sensitivity index and Na^+ K^+ -ATPase activity in rat ileum [25]. But all of them used high doses of vegetal-derived glucans.

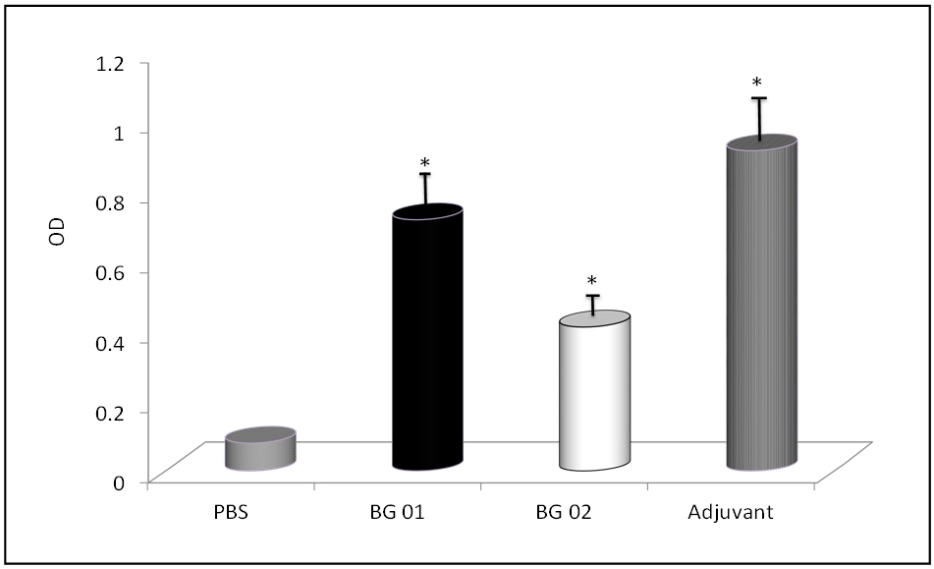

Fig. 3. Effects of dietary supplementation with glucan on formation of anti-ovalbumin antibodies. Dogs were injected twice (two weeks apart) with antigen and the serum was collected 7 days after last injection. Level of specific antibodies against ovalbumin was detected by ELISA. As positive control, Freund's adjuvant was used. *Represents significant differences between control (ovalbumin alone) and samples at P ≤ 0.05 level. All experiments were performed in triplicates

An older mouse study, using modest doses of yeast-derived glucan, demonstrated that there are no direct effects on blood glucose levels in a normal homeostasis status. However, it significantly lowered level of glucose in mice with experimentally-induced hyperglycaemie [26]. This current data showed the same results in dogs. However the mechanisms of these effects remain unknown, as the amount of glucan used would not be enough to produce any significant effect in viscosity of lumen content in intestine. High viscosity of upper gut content was related to prolonged gastric emptying and slower transit time.

In normal animals, feeding glucan did not significantly affect the sugar levels even after 14 days (Fig. 4). However, the different situation was found when we used animals with experimentally induced hyperglycaemie. After 7 days of feeding, both glucans reduced the sugar levels to normal values (Fig. 5). A longer application of glucan showed identical effects. No differences between the two glucan used were found, suggesting that neither the purity or isolation differences play role.

A canine model experiment demonstrated that even a modest increase in the fat content of the diet, without increasing calories, results in visceral fat deposition and insulin resistance [27]. It also supported the role of a family of inflammatory cytokines and adipokines, including free fatty acids (FFAs), in the development of insulin resistance.

The base of experimentally-induced hyperglycaemie is the selectively toxic effect of streptozotocin to beta cells of the pancreatic islets. Thus, added to insulin sensitivity increasing hypothesis, faster beta cells renewal and inflammatory modulation and must be considered. The potential importance of this latter hypothesis is the fact that pancreatitis

inflammatory response in dogs also may affect endocrine function; beta cells, in particular, seem to be sensitive to the deleterious effects of inflammatory mediators including IL-10 and TNF-α [11]. Our previous experiments with chicken and pigs showed the benefic effects of the inflammatory modulation of the same glucans in LPS inflammatory challenge [28].

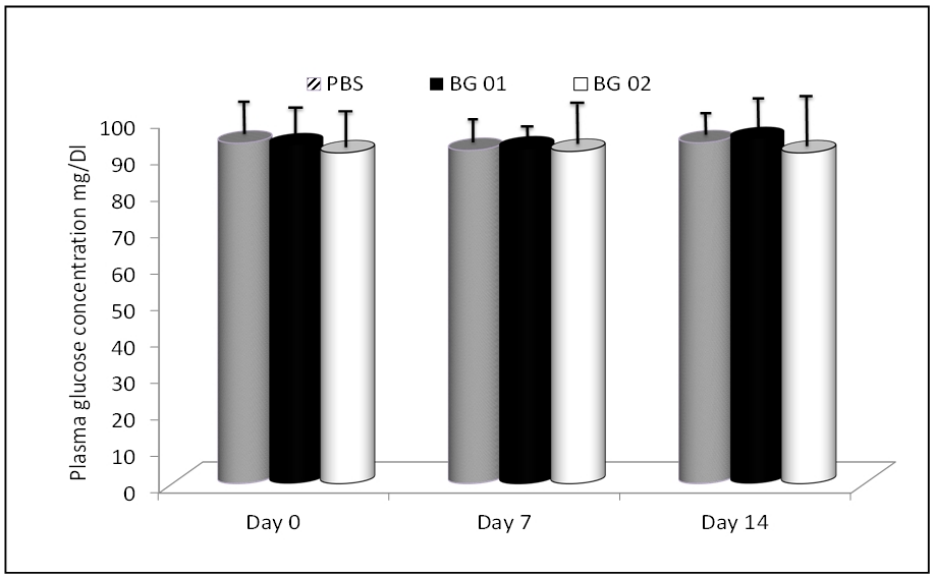

Fig. 4. Effects of dietary supplementation with glucan on blood glucose levels. *Represents significant differences between control (PBS) and tested samples at P ≤0.05 level. All experiments were performed in triplicates

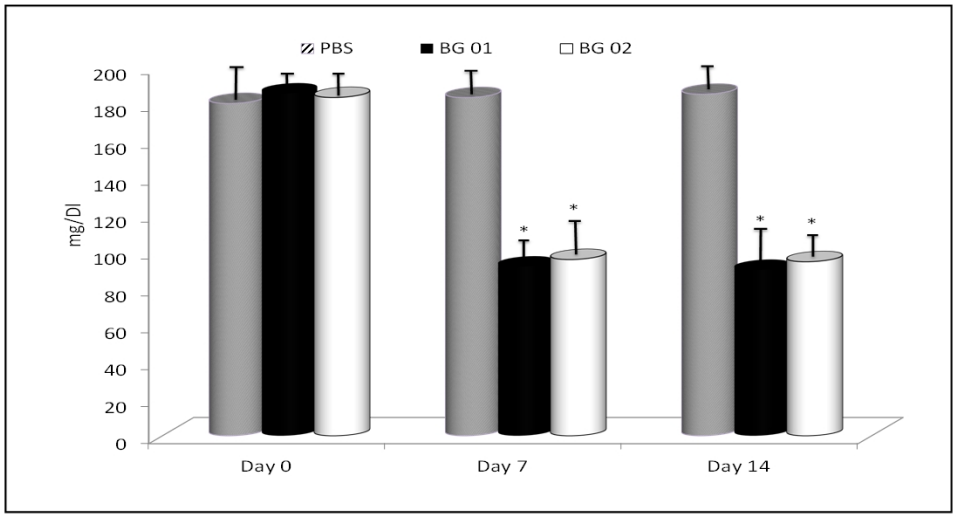

Fig. 5. Effects of dietary supplementation with glucan on blood glucose levels in animals with experimentally-induced hyperglycaemie. *Represents significant differences between control (PBS) and tested samples at P ≤0.05 level. All experiments were performed in triplicates

4. CONCLUSION

In conclusion, the two glucans used had significant immunomodulating effects in dog model, strongly suggesting that supplementation of feed with these glucans results in improvement of biological and immunological conditions of dogs.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. The use of animals was approved by the University of Louisville IACUC Committee (#10080).

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V.V. has no conflict of interest, C.O. is employed by Biorigin. The study was financed by Biorigin (preparation, isolation and characterization of samples). The funders had no role in study resign, data collection and analysis, or decision to publish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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