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Original article

The effect of Biolex-MB40 on the phagocytic activity and oxidative metabolism of peripheral blood granulocytes and monocytes in lambs

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Abstract

The objective of this study was to determine the effect of Biolex-MB40 on the phagocytic activity and oxidative metabolism of peripheral blood granulocytes and monocytes in lambs. The experimental material comprised 32 lambs aged 30 ± 3 days, divided into two equal groups: control and experimental. Experimental group animals were fed a diet supplemented with the Biolex-MB40 (*Saccharomyces cerevisiae*) in the amount of 3 g/kg of the concentrate. At the beginning of the experiment (day 0) and on experimental days 15, 30 and 60, blood was sampled from the jugular vein to determine and compare the phagocytic activity (PHAGOTEST) and oxidative metabolism (BURSTTEST) of peripheral blood granulocytes and monocytes by flow cytometry. Based on the results of an analysis of granulocyte and monocyte phagocytic activity, statistically higher levels of phagocytic activity were observed in the group of lambs administered Biolex-MB40 than in the control animals, expressed in terms of the percentage of phagocytic cells as well as mean fluorescence intensity. Biolex-MB40 also had a positive effect on the oxidative metabolism of both granulocytes and monocytes after stimulation with *Escherichia coli* bacteria and with PMA (4-phorbol-12- β -myristate-13-acetate), expressed in terms of the percentage of oxidative metabolism as well as mean fluorescence intensity.

Key words: Biolex-MB40, flow cytometry, granulocytes, monocytes, lambs

Introduction

Biolex-MB40 is a commercial prebiotic which contains 10-15% mannan oligosaccharide (MOS) and 25-30% 1,3/1,6- β -D-glucan. Oligosaccharides, β -glucans and MOS isolated from the cell wall of *Saccharomyces cerevisiae* yeast exert immunostimulatory effects by binding to specific receptors on the surface of effector cells. β -glucans stimulate a wide range of immune responses, such as cytokine release (Abel and

Czop 1992, Pelizon et al. 2005), generation of ROS (Gallin et al. 1992), generation of NO (Ohno et al. 1996) and release of arachidonic acid metabolites (Czop and Austen 1985). Ozinsky et al. (2000) and Akremiene et al. (2007) demonstrated that TLR-2 and, probably, TLR-4 receptors on macrophages, dendritic cells and lymphocytes are able to bind β -glucans. They activate a cascade of reactions on the surface of those cells to activate nuclear factor κ B (NF- κ B – nuclear factor kappa-light-chain-enhancer

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of activated B cells), which increases the production of proinflammatory cytokines IL-1 (IL – Interleukin), IL-6, IL-8, IL-12 and TNF- α (Tumor Necrosis Factor α). Neutrophils, eosinophils, macrophages, T and B cells and the complement system are activated, which leads to intensified phagocytosis and antibody production to protect the body against pathogens. According to Seifert and Watzl (2007), MOS partially binds to the carbohydrate receptors of intestinal epithelial cells and, after absorption, to the receptors of immune system cells to stimulate the local and systemic immune response. The direct binding of MOS could take place through several receptors, including TLR (Harris et al. 2006), mannose receptors (Netea et al. 2006), galectin family receptors (Almkvist and Karlsson 2004) and transmembrane mucins (MUC1 and MUC40) (Carraway et al. 2003). The above mechanism could explain the high effectiveness of MOS in limiting the colonization of small and large intestines by pathogenic bacteria with type 1 fimbriae which prevent those microorganisms (e.g. *Salmonella*, *Clostridium*, *Escherichia coli*) from becoming attached to mannan-dependent enterocyte receptors in the intestinal epithelium. The fimbriae (lectins) of pathogenic bacteria also bind to mannans and are excreted as undigested particles with feces (Fernandez et al. 2002, Thomas et al. 2004, Yang et al. 2008). In monocytes, macrophages, neutrophils and microglia, dectin-1 plays the role of a carbohydrate receptor that directly binds MOS (Brown 2006), which stimulates non-specific and specific immune responses.

The use of MOS and β -glucans in animal nutrition and their effect on the immune system have been investigated by relatively few studies (Rafstie et al. 2010, Gu et al. 2011, Sadeghi et al. 2013), and no research has been conducted in lambs. The results of previous studies (Milewski et al. 2007, Wójcik et al. 2007, Wójcik et al. 2008, Wójcik 2010) revealed that the supplementation of lamb feed concentrate with β -glucans, alone or in combination with MOS isolated from the cell walls of *Saccharomyces cerevisiae* yeast, delivered significant health benefits.

The objective of this study was to determine the effect of Biolex-MB40 on the phagocytic activity and oxidative metabolism of peripheral blood granulocytes and monocytes in lambs.

Materials and Methods

Experimental design

The experiment was performed on 32 suckling Kamieniec lambs from a conservation herd. Lambs aged 30 \pm 3 days were divided into two equal groups:

control (C) and experimental (B). Both groups were identical in terms of body weight on the second day of life, gender, birth type as well as the age of the ewes, to eliminate possible differences in milk yield. During 60 days of the experiment, control group lambs were fed the CJ[®] mixture without supplementation, whereas experimental group animals were administered the CJ[®] mixture as well as the Biolex-MB40 (Leiber GmbH, Germany) yeast (*Saccharomyces cerevisiae*) extract containing 10-15% MOS and 25-30% 1,3/1,6- β -D-glucan which was added to fodder during the morning feeding at 50 mg/kg BW/day. At the beginning of the experiment (day 0) and on experimental days 15, 30 and 60, blood was aseptically drawn by venipuncture from the jugular vein into heparinized sterile vacutainer tubes (Becton Dickinson Biosciences, San Jose, CA, USA) to determine and compare the phagocytic activity and oxidative metabolism of peripheral blood granulocytes and monocytes by flow cytometry. The experimental design was dictated by the fact that Biolex-MB40 is a natural supplement characterized by relatively low levels of activity, therefore, more frequent evaluations of the analyzed parameters are not required. The experimental design was identical to that applied in the author's previous studies to compare the immunostimulatory effects of Biolex-MB40 with those of previously tested products containing β -glucan (Milewski et al. 2007, Wójcik et al. 2007, Wójcik et al. 2008, Wójcik 2010).

Determination of blood granulocyte and monocyte phagocytic activity in lambs – PHAGOTEST (Orpegen Pharma, Hiedelberg, Germany)

All test reagents were prepared in accordance with the manufacturer's recommendations in the leaflet attached to the product. 100 μ l of whole heparinized blood chilled to 0°C and 20 μ l of chilled *E. coli* bacteria (Orpegen Pharma, Germany) were added to each of the two 5 ml test tubes (blue, Beckman Coulter, Fullerton, CA, USA) (negative control and experimental) and shaken for around 3 s at low speed. The experimental sample was incubated for 10 min at 37°C, and the negative control sample – in an ice bath at 0°C. After incubation, 100 μ l of quenching solution (Orpegen Pharma, Germany) was added to each sample, and the samples were shaken. Three ml of washing solution (Orpegen Pharma, Germany) chilled to 0°C was added, the samples were centrifuged for 5 min at 4°C (250 x g), and the supernatant was removed. The rinsing procedure was performed twice, and 2 ml of lysing solution (Orpegen Pharma, Germany) at room temperature was added to each

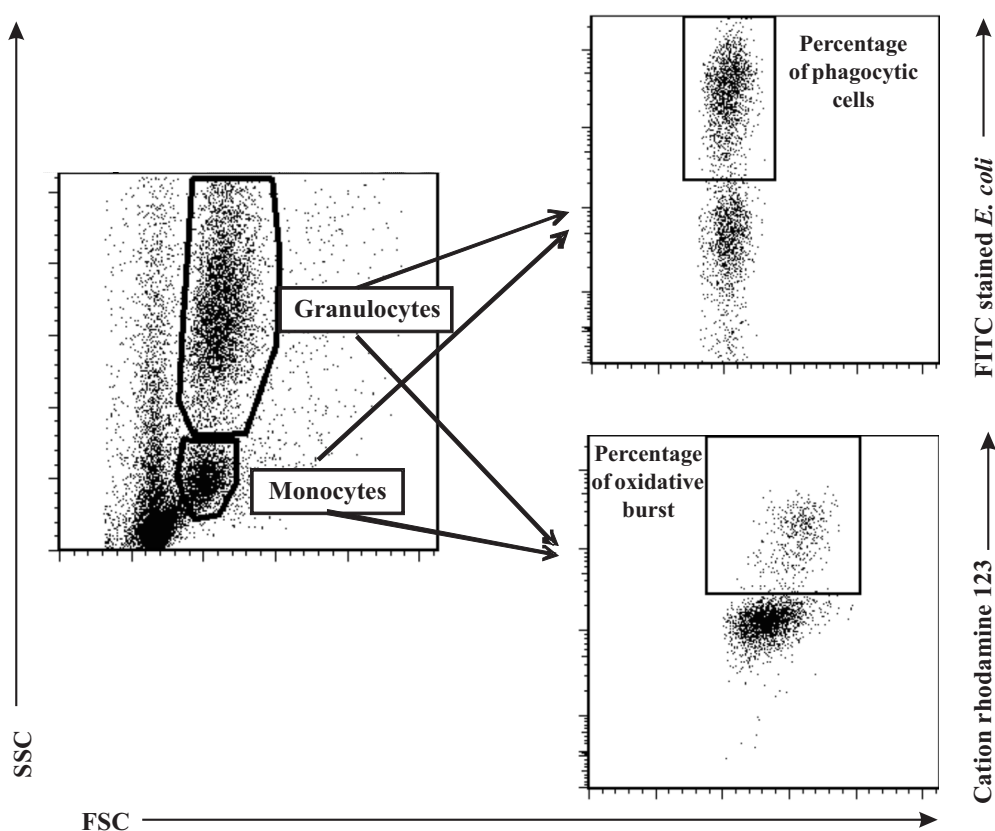


Fig. 1. Gating strategy for flow cytometric data analysis. Granulocytes and monocytes were gated based on forward and side scatter (FSC/SSC) parameters. Each cell subset was analyzed for the relative number of phagocytizing cells and cells stimulated for respiratory burst (fMLP, PMA or *E. coli* bacteria).

sample. The samples were shaken and incubated at room temperature for 20 min. The samples were centrifuged for 5 min at 4°C (250 x g), and the supernatant was removed. Three ml of washing solution (Orpegen Pharma, Germany) chilled to 0°C was added to each sample, the samples were centrifuged for 5 min at 4°C (250 x g), and the supernatant was removed. Two hundred µl of DNA staining solution (Orpegen Pharma, Germany) chilled to 0°C was added, the samples were shaken and incubated for 10 min in an ice bath. Cellular phagocytic activity was determined in a cytometer (Beckmann Coulter, Epics XL, USA) in less than 60 min after the last reagent had been added. A Phagotest (Orpegen Pharma, Germany) is performed with the involvement of fluorescein (FITC)-stained *E. coli* bacteria which are phagocytized by macrophages. Cell nuclei are also stained. The test determines the number of phagocytizing cells, granulocytes and monocytes separately, and their phagocytic activity, i.e. the number of bacteria absorbed by a single cell in terms of fluorescence intensity.

Determination of oxidative metabolism of blood granulocytes and monocytes in lambs – BURSTTEST (PHAGOBURST, Orpegen Pharma, Hiedelberg, Germany)

All test reagents were prepared in accordance with the manufacturer's recommendations in the leaflet attached to the product. Each analyzed sample of whole heparinized blood was divided into four test tubes (blue, Beckman Coulter, Fullerton, CA, USA) of 100 µl each and chilled to 0°C. Twenty fl of chilled *E. coli* bacteria (Orpegen Pharma, Germany) was added to the first sample (experimental), 20 µl of washing solution (Orpegen Pharma, Germany) was added to the second sample (negative control), 20 µl of fMLP (N-formyl-methionyl-leucyl-phenylalanine) (Orpegen Pharma, Germany) was added to the third sample (low control), and 20 µl of PMA (4-phorbol-12-β-myristate-13-acetate) (Orpegen Pharma, Germany) was added to the fourth sample (high control). All test tubes were mixed and incubated for 10 min at 37°C [excluding the fMLP (Orpegen Pharma, Germany) sample which was incubated for 7 min]. After incubation, each test tube was supplemented

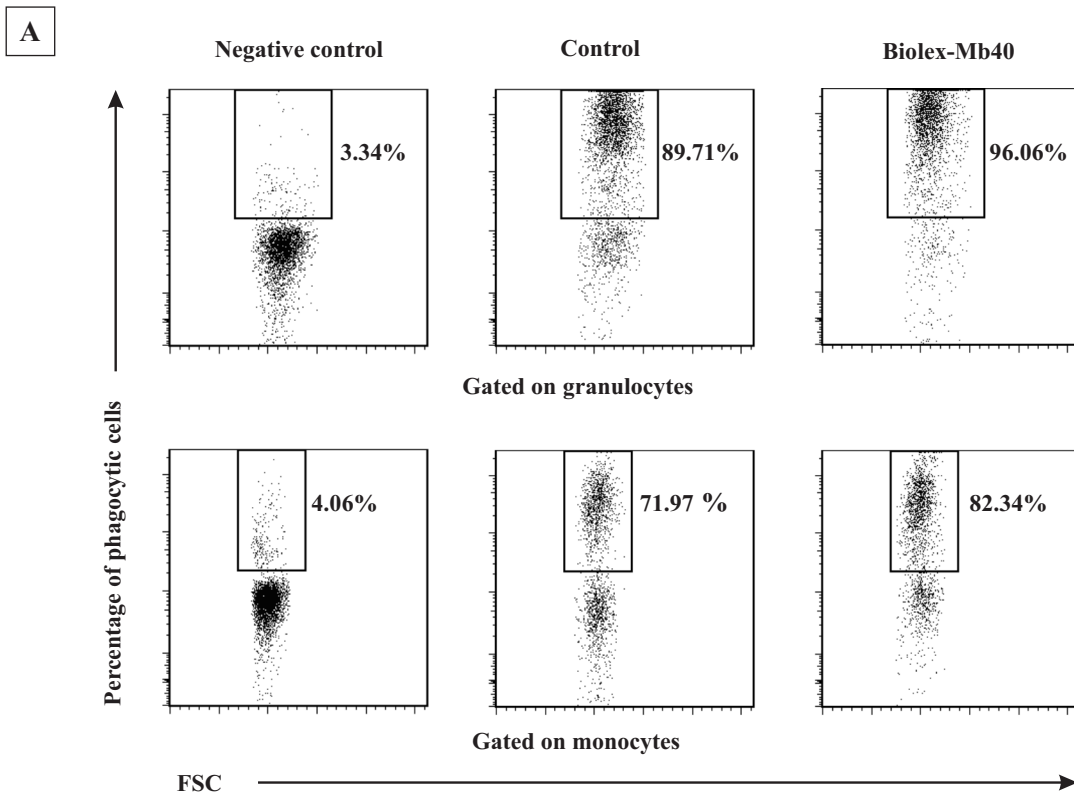
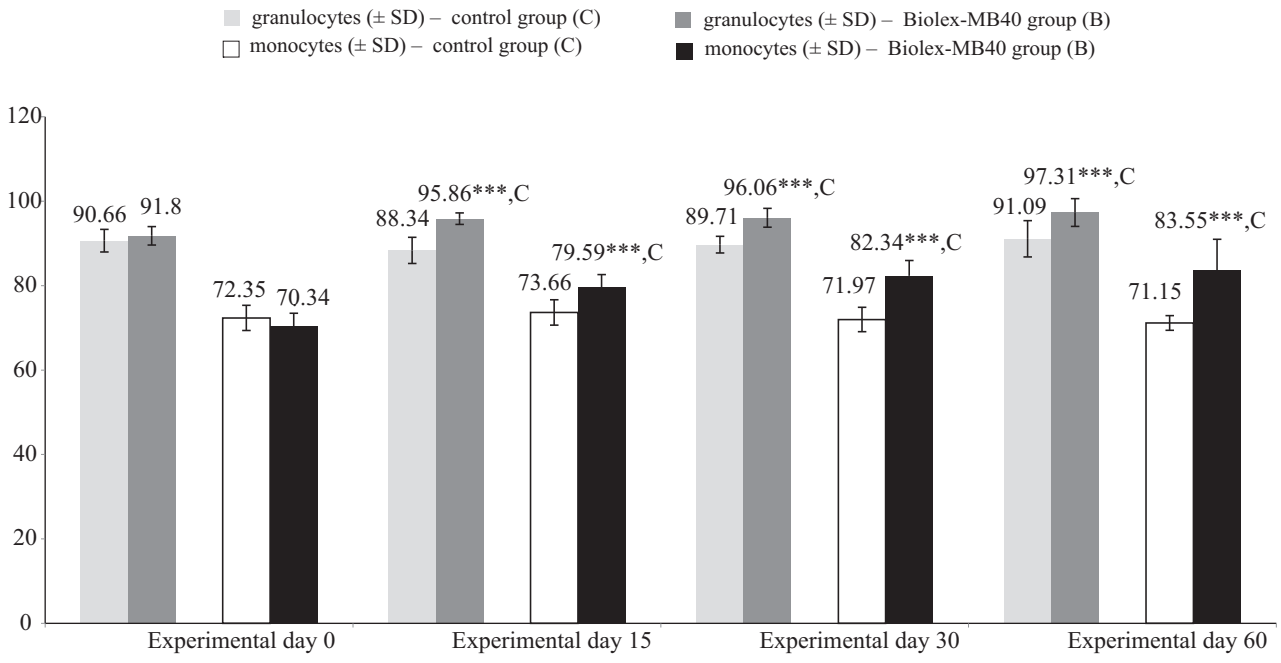


Fig. 2. Percentage of phagocytic granulocytes and monocytes in the lamb groups, as determined in the Phagotest. **A.** Dot plot cytogram showing the percentage of phagocytic granulocytes and monocytes in the control and experimental lambs on experimental day 30. Whole heparinized blood from the control and experimental animals was incubated for 10 minutes with FITC-labeled *E. coli* in an ice bath at the temperature of 0°C (negative control) or in a water bath at the temperature of 37°C (control and Biolex-MB40). The percentages of granulocytes and monocytes with ingested *E. coli* (FITC) bacteria were gated. Key: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; SD – standard deviation. A – $p \leq 0.05$ in comparison with experimental day 0; B – $p \leq 0.01$ in comparison with experimental day 0; C – $p \leq 0.001$ in comparison with experimental day 0.

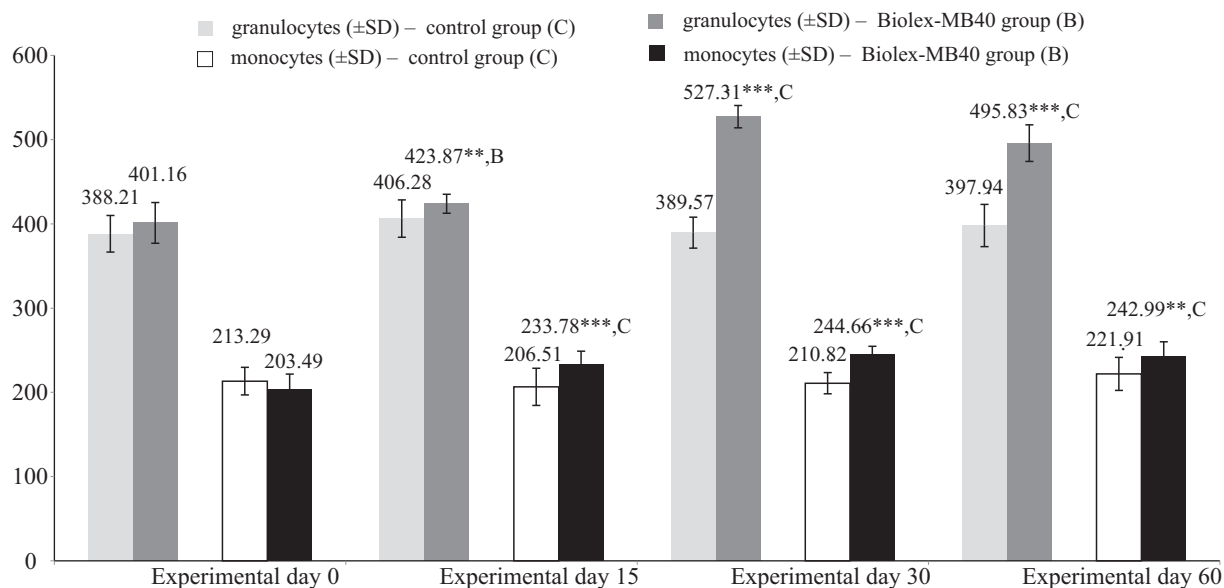


Fig. 3. Mean fluorescence intensity (MFI) of granulocytes and monocytes in lamb groups, as determined in the Phagotest. Key: refer to Fig. 2.

with 20 μ l of substrate solution (Orpegen Pharma, Germany) and was thoroughly shaken. All samples were incubated for 10 min at 37°C. After incubation, 2 ml of lysing solution (Orpegen Pharma, Germany) at room temperature was added. Test tubes were shaken and incubated at room temperature for 20 min. All samples were centrifuged for 5 min at 4°C (250 x g), and the supernatant was removed. All test tubes were rinsed once with 3 ml of washing solution (Orpegen Pharma, Germany), centrifuged for 5 min at 4°C (250 x g), after which the supernatant was removed. Two hundred μ l of staining solution chilled to 0°C was added to each sample, test tubes were shaken and incubated for 10 min in an ice bath. Intracellular killing activity of phagocytes was determined in a cytometer (Beckman Coulter, Epics XL, USA) in less than 30 min after the last reagent had been added. Three activators were used for cell stimulation: *E. coli* bacteria (Orpegen Pharma, Germany), PMA (Orpegen Pharma, Germany) as the strong activator, and fMLP (Orpegen Pharma, Germany) as the weak activator. The added dihydrorodamine (123-DHR) was oxidized in mitochondria by H₂O₂ resulting from cell stimulation and was converted to cation rhodamine 123 (R123), the fluorescent emitter.

FACS acquisition and analysis

The FACS analysis strategy is presented in Fig. 1. Flow cytometry analysis was performed using the Epics XL cytometer (Beckman Coulter) equipped with

a single 488 nm argon ion laser. Data were acquired using System II 3.0 software (Beckman Coulter) and analyzed with FlowJo software (Tree Star Inc., Stanford, CA, USA). Unstained control cells and single stain controls for every fluorochrome were prepared and used to set up flow cytometric compensation.

Statistical analysis

The results obtained were processed statistically by one-way analysis of variance for orthogonal designs. The significance of differences between groups was verified by the Student's t-test, one-way ANOVA and the Bonferroni test with the use of the GraphPad Prism application (v. 2.0; GraphPad Software, Inc., USA).

Results

Throughout the experiment, a significant increase ($p \leq 0.01$ or $p \leq 0.001$) was observed in the average phagocytic activity of peripheral blood granulocytes in the group of lambs fed the Biolex-MB40 prebiotic (experimental group) in comparison with animals whose diets were not supplemented (control group) and relative to average initial values (day 0). An increase was also found in the average percentage of phagocytic cells (Fig. 2) and mean fluorescence intensity (MFI) (Fig. 3), which describes the number of bacteria phagocytized by a single phagocyte. Similar

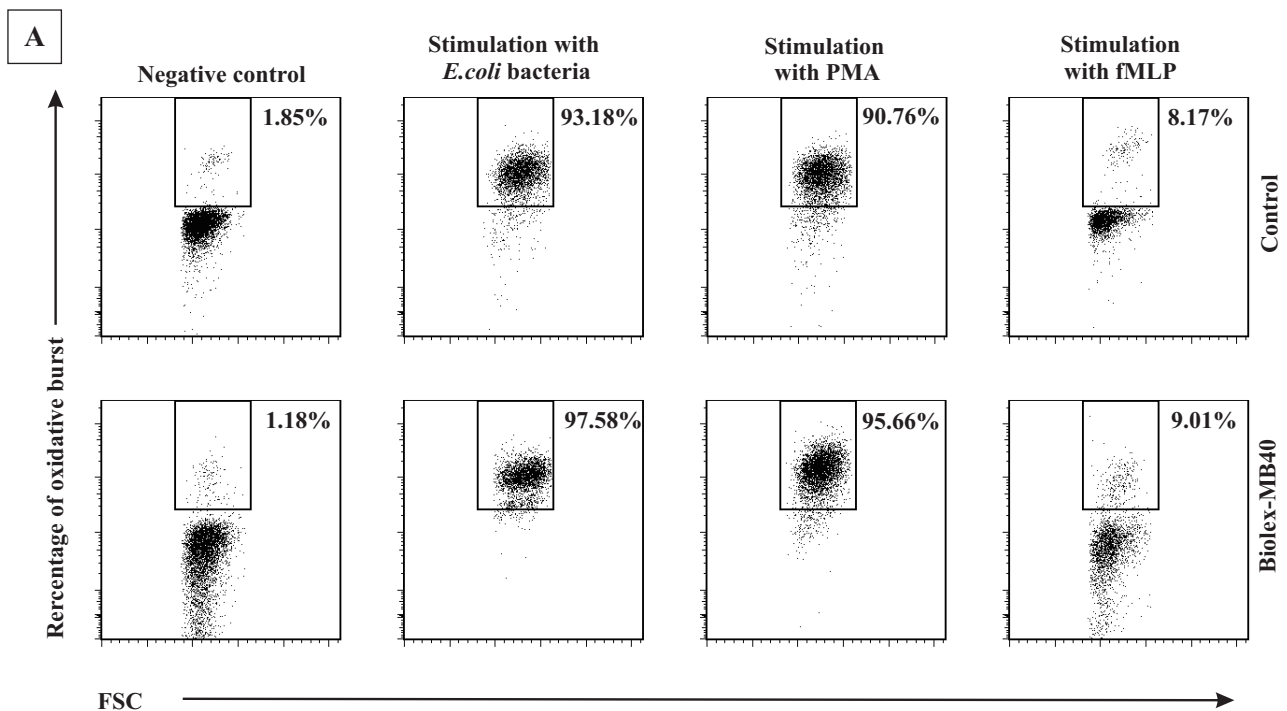
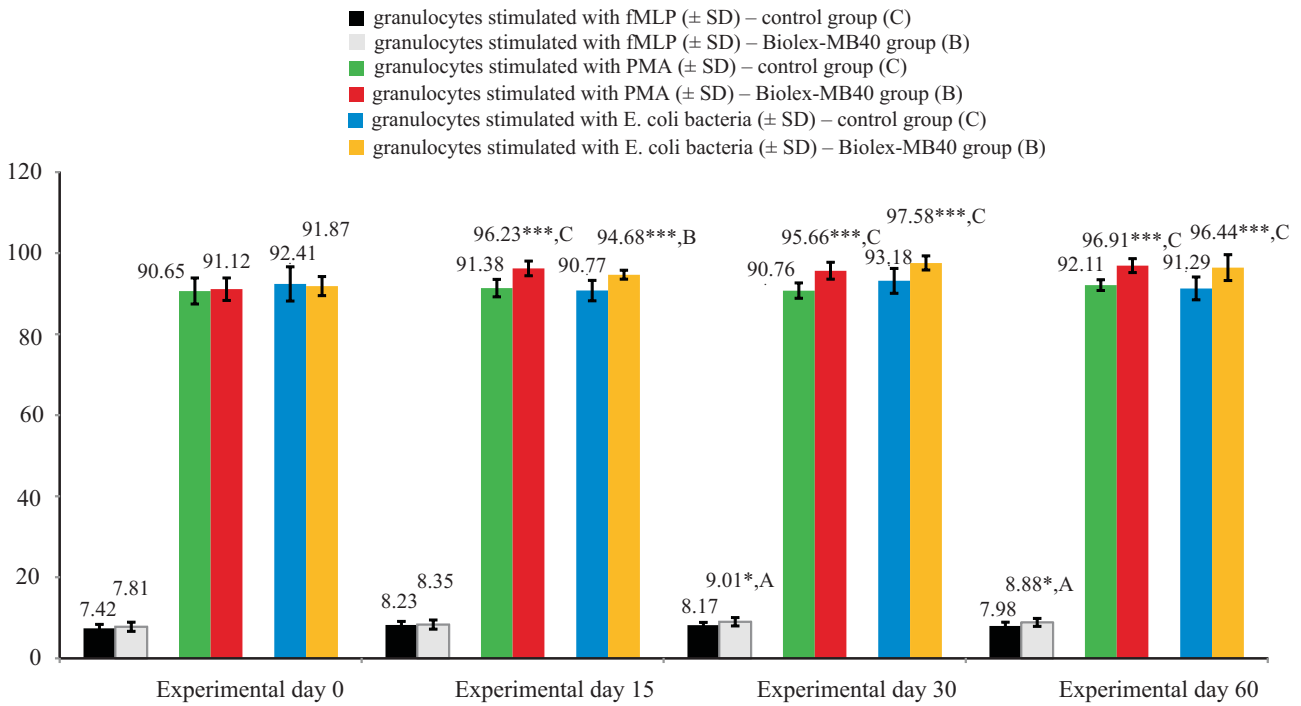


Fig. 4. Percentage of granulocytes stimulated to undergo respiratory burst in the lamb groups after stimulation with fMLP, PMA and *E. coli*, as determined in the Phagoburst test. **A.** Dot plot cytogram showing the percentage of granulocytes stimulated to undergo respiratory burst in the control and experimental lambs on experimental day 30. Whole heparinized blood from the control and experimental animals (control and Biolex-MB40) was divided into four test tubes. The samples were combined with the washing solution (negative control), *E. coli* bacteria (opsonizing stimulus), PMA (strong stimulus) or fMLP (weak stimulus) and incubated with dihydrorhodamine 123 in a water bath at the temperature of 37°C. After incubation, cells were lysed and DNA staining solution was added. The percentages of granulocytes stimulated to undergo respiratory burst (conversion of dihydrorhodamine 123 to rhodamine 123) were gated. Key: refer to Fig. 2.

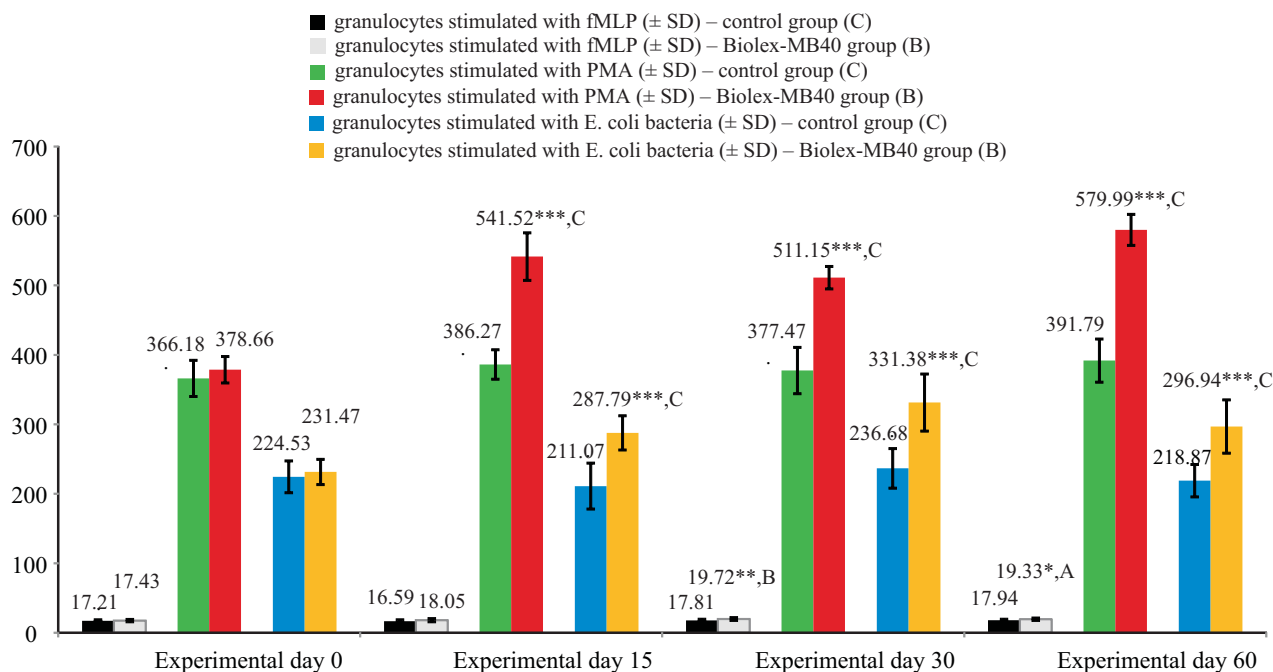


Fig. 5. Mean fluorescence intensity (MFI) of granulocytes in the lamb groups after stimulation with fMLP, PMA and *E. coli*, as determined in the Phagoburst test.

Key: refer to Fig. 2.

results were obtained throughout the experiment for average phagocytic activity (Fig. 2) and MFI (Fig. 3) of peripheral blood monocytes in the control and experimental group lambs, relative to average initial values (day 0).

Biolex-MB40 had a stimulatory effect on average oxidative metabolism (respiratory burst) of peripheral blood granulocytes in the experimental animals in comparison with the control group and relative to average initial values (day 0). An increase was observed in the average percentage of stimulated cells (Fig. 4) and MIF (Fig. 5) that describes respiratory burst intensity in different granulocytes. Stimulation with PMA, an effective activator of respiratory burst activity, and *E. coli* bacteria led to a significant increase ($p \leq 0.001$) in the above parameters throughout the experiment. As a result of stimulation with fMLP, a weaker activator of respiratory burst activity, significant differences ($p \leq 0.05$) were observed only between days 30 and 60.

Peripheral blood monocytes were also stimulated with *E. coli* and PMA, and both activators produced a significant increase ($p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$) in the percentage of stimulated cells (Fig. 6) and average respiratory burst intensity (Fig. 7) in the group of lambs fed Biolex-MB-40 in comparison with the control animals and relative to average initial values (day 0). Different results were reported when respiratory burst in monocytes was stimulated by fMLP. Despite an increase in the average percentage

of stimulated monocytes in experimental animals in comparison with control and relative to average initial values (day 0), no significant differences were observed throughout the experiment (Fig. 6). Although fMLP stimulation of monocytes increased average fluorescence intensity in the experimental animals in comparison with the control and relative to average initial values (day 0), significant differences ($p \leq 0.001$) were observed only between days 30 and 60 (Fig. 7).

Discussion

The immunostimulatory effects of prebiotics have been widely documented in recent years (Bland et al. 2004, Hoyles and Vulevic 2008, Gu et al. 2011). Due to substantial differences in the activity of prebiotics isolated from different sources, their biological properties should be evaluated before any suggestions can be formulated regarding the use of any particular prebiotic in clinical practice. The present study has analyzed the biological activity of Biolex-MB40 containing MOS and 1,3/1,6- β -D-glucan.

The ability of prebiotics to stimulate phagocytosis has been well documented (Tzianabos 2000, Bland et al. 2004), therefore, a phagocytosis assay should be the first test to investigate the immunological characteristics of Biolex-MB40. In this experiment, a significant increase in the phagocytic activity, expressed as

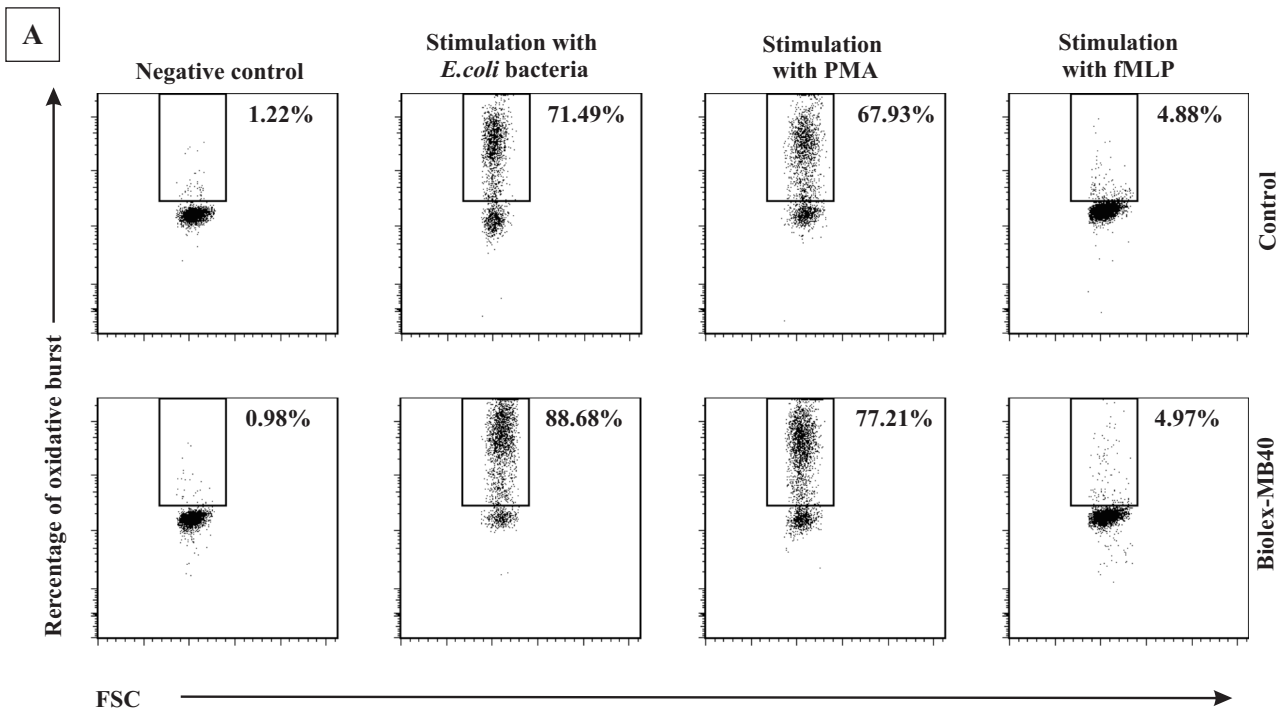
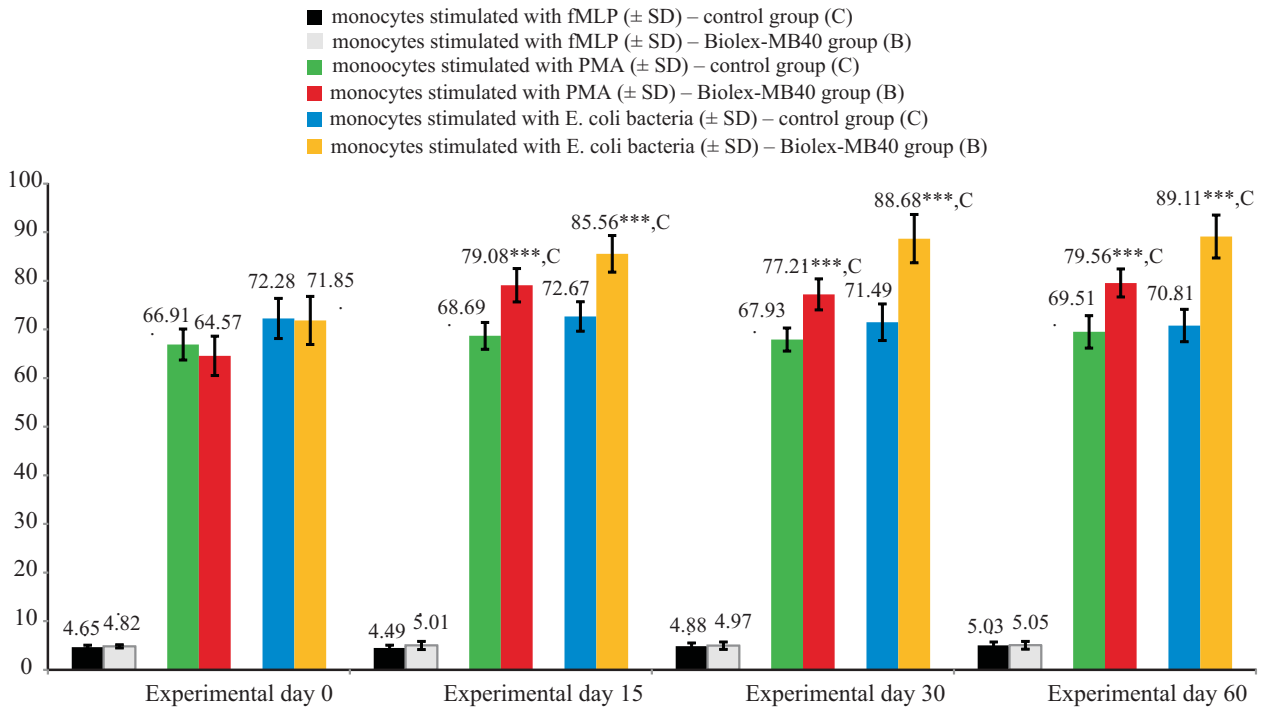


Fig. 6. The percentage of monocytes stimulated to undergo respiratory burst in the lamb groups after stimulation with fMLP, PMA and *E. coli*, as determined in the Phagoburst test. **A.** Dot plot cytogram showing the percentage of monocytes stimulated to undergo respiratory burst in the control and experimental animals on experimental day 30. The percentages of granulocytes stimulated to undergo respiratory burst were gated. Key: refer to Fig. 2.

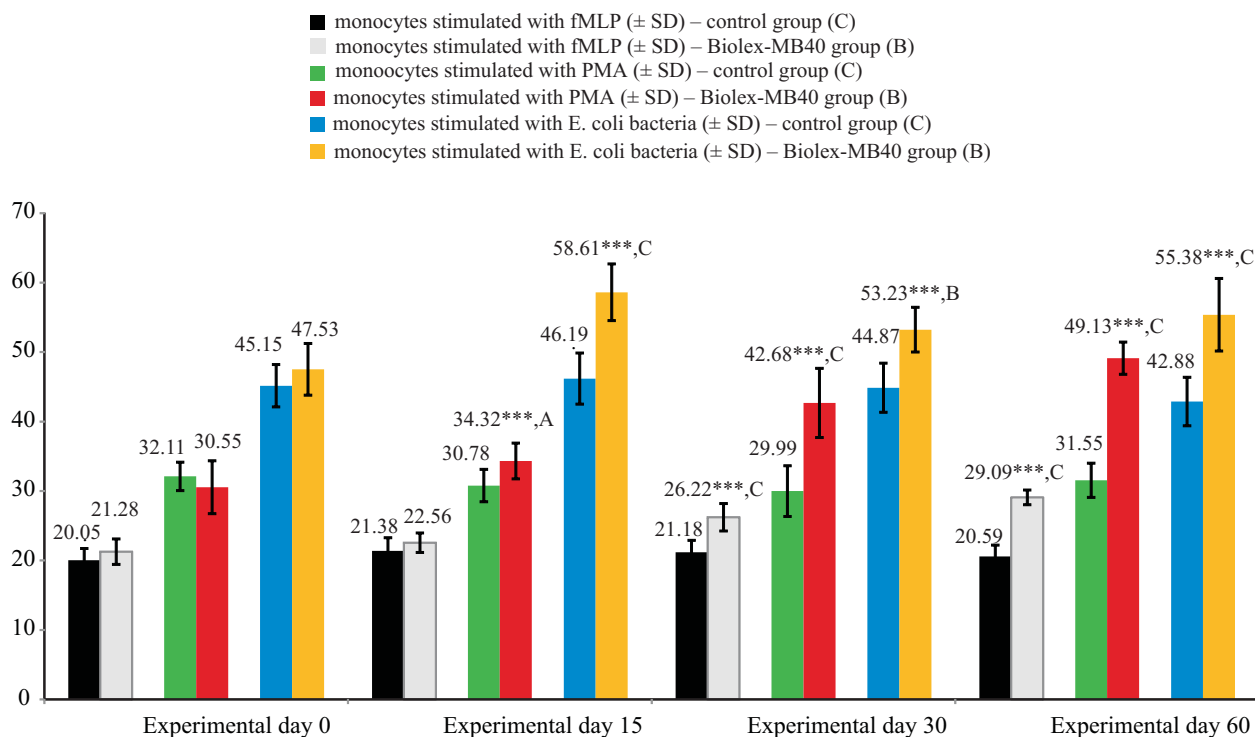


Fig. 7. Mean fluorescence intensity (MFI) of monocytes in the lamb groups after stimulation with fMLP, PMA and *E. coli*, as determined in the Phagoburst test.

Key: refer to Fig. 2.

the percentage of phagocytes, and a significant increase in the average number of bacteria eliminated by one phagocyte, expressed as average fluorescence intensity of monocytes and granulocytes, were observed in the experimental animals administered Biolex-MB40 in comparison with the control lambs. Similar results were reported by Gu et al. (2011) in sea cucumbers (*Apostichopus japonicus*) whose diets were supplemented with β -glucan, MOS and both substances for 29 days. The highest increase in the phagocytic activity of coelomocytes was found in the group of animals fed both MOS and β -glucan in comparison with the animals whose diets were supplemented with MOS or β -glucan. According to Underhill and Ozinsky (2002), the observed increase in the phagocytic activity could be attributed to MOS binding by mannose receptors (MRs), whereas Borchers et al. (2004) attributed the above to β -glucan binding by specific receptors on macrophages/monocytes and granulocytes, which increases the production of proinflammatory cytokines such as the tumor necrosis factor alpha (TNF- α), interleukin (IL)-1 β , IL-6, IL-8, IL-12, IFN- γ and IFN- β 2 (Rasmussen and Seljelid 1991, Abel and Czop 1992, Che et al. 2012). IL-12 seems to play a special role in this process by promoting type 1 T-helper cell (Th1) responses and cell-mediated immunity, and acting as a potent inducer of IFN- γ and IL-2 produced by Th1 cells (Lackovic et al.

1970, Gately et al. 1998, Ranta et al. 2013). IFN- γ and IL-2 stimulate macrophage activation and differentiation into subpopulations of conventionally activated macrophages that are characterized by greater ability to ingest bacteria, produce ROS and other toxins and release antibacterial proteins such as defensins (Ganz 1987, Mosmann and Coffman 1989, Vetvicka et al. 2008). The above contributes to the elimination of microorganisms or their fragments from the body, and it prevents the progression of inflammatory processes. In the present study, Biolex-MB40 also intensified the intracellular killing activity of granulocytes and monocytes stimulated with PMA and *E. coli*, expressed by the percentage of stimulated cells and average fluorescence intensity. Higher values of the analyzed parameters point to greater effectiveness of phagocytic cells and more effective pathogen elimination. A similar effect was observed by Sakurai et al. (1996) in mice, where the phagocytes of the animals receiving β -glucans were characterized by intensified oxidative metabolism and produced more hydrogen peroxide (H_2O_2) than the macrophages of the non-stimulated animals. In a study of *Apostichopus japonicus*, Gu et al. (2011) observed the synergistic effect of combined dietary supplementation with MOS and β -glucan on increased production of superoxide anions. In the animals whose diets were supplemented only with MOS, superoxide anion levels decreased after a brief initial

increase. Enhanced ROS production results from the activation of granulocytes and monocytes/macrophages. In an in vitro study conducted by Fernhndez et al. (2005), human mononuclear phagocytes were activated after the administration of α -mannan or zymosan. Kennedy et al. (2007) demonstrated that human granulocytes were activated after the administration of zymosan. Gow et al. (2007) reported higher expression of dectin-1 receptors on human monocytes and murine macrophages after MOS or β -glucan binding, following the administration of $\beta(1,3)$ -glucan. In an in vitro study of mouse splenocytes treated with β -glucan (Kim and Joo 2012), the said activation was induced by higher expression of IL-2 receptor CD25+ and phagocyte stimulation by IL-2. Hino et al. (2012) demonstrated that macrophages released soluble glucans into the medium after phagocytizing insoluble β -glucan particles. They found that those particles were fragmented by macrophage-produced ROS, and that the released soluble β -glucan was dectin-1-reactive and biologically active in terms of macrophage activation.

The results of this study of lambs and the author's previous work (Wójcik 2010) investigating the immunomodulatory properties of Biolex MB40 indicate that the analyzed product can be safely and effectively used to stimulate the immune system of animals after birth, during weaning, under poor housing conditions or during changes in dietary regime. The present findings encourage further research into the use of prebiotics in lambs and sheep to boost immune functions that were impaired by environmental and infectious factors.

References

- Abel G, Czop JK (1992) Stimulation of human monocyte beta-glucan receptors by glucan particles induces production of TNF-alpha and IL-1 beta. *Int J Immunopharmacol* 14: 1363-1373.
- Akramiene D, Kondrotas A, Didziapetriene J, Kevelaitis E (2007) Effects of β -glucans on the immune system. *Medicina (Kaunas)* 43: 597-606.
- Almkvist J, Karlsson A (2004) Galectins as inflammatory mediators. *Glycoconj J* 19: 575-581.
- Bland EJ, Keshavarz T, Bucke C (2004) The influence of small oligosaccharides on the immune system. *Carbohydr Res* 339: 1673-1678.
- Borchers AT, Keen CL, Gershwin ME (2004) Mushrooms, tumors, and immunity: an update. *Exp Biol Med (Maywood)* 229: 393-406.
- Brown GD (2006) Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat Rev Immunol* 6: 33-43.
- Carraway KL, Ramsauer VP, Haq B, Carothers Carraway CA (2003) Cell signaling through membrane mucins. *Bioessays* 25: 66-71.
- Che TM, Johnson RW, Kelley KW, Dawson KA, Moran CA, Pettigrew JE (2012) Effects of mannan oligosaccharide on cytokine secretions by porcine alveolar macrophages and serum cytokine concentrations in nursery pigs. *J Anim Sci* 90: 657-668.
- Czop JK, Austen KF (1985) Generation of leukotrienes by human monocytes upon stimulation of their beta-glucan receptor during phagocytosis. *Proc Natl Acad Sci USA* 82: 2751-2755.
- Fernández N, Alonso S, Valera I, Vigo AG, Renedo M, Barbolla L, Crespo MS (2005) Mannose-containing molecular patterns are strong inducers of cyclooxygenase-2 expression and prostaglandin E2 production in human macrophages. *J Immunol* 174: 8154-8162.
- Fernandez F, Hinton M, Van Gils B (2002) Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to Salmonella Enteritidis colonization. *Avian Pathol* 31: 49-58.
- Gallin EK, Green SW, Patchen ML (1992) Comparative effects of particulate and soluble glucan on macrophages of C3H/HeN and C3H/HeJ mice. *Int J Immunopharmacol* 14: 173-183.
- Ganz T (1987) Extracellular release of antimicrobial defensins by human polymorphonuclear leukocytes. *Infect Immun* 55: 568-571.
- Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH (1998) The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 16: 495-521.
- Gow NA, Netea MG, Munro CA, Ferwerda G, Bates S, Mora-Montes HM, Walker L, Jansen T, Jacobs L, Tsoni V, Brown GD, Odds FC, Van der Meer JW, Brown AJ, Kullberg BJ (2007) Immune recognition of *Candida albicans* beta-glucan by dectin-1. *J Infect Dis* 196: 1565-1571.
- Gu M, Ma H, Mai K, Zhang W, Bai N, Wang X (2011) Effects of dietary β -glucan, mannan oligosaccharide and their combinations on growth performance, immunity and resistance against *Vibrio splendidus* of sea cucumber, *Apostichopus japonicus*. *Fish Shellfish Immunol* 31: 303-309.
- Harris G, KuoLee R, Chen W (2006) Role of Toll-like receptors in health and diseases of gastrointestinal tract. *World J Gastroenterol* 12: 2149-2160.
- Hino S, Kito A, Yokoshima R, Sugino R, Oshima K, Morita T, Okajima T, Nadano D, Uchida K, Matsuda T (2012) Discharge of solubilized and dectin-1-reactive β -glucan from macrophage cells phagocytizing insoluble β -glucan particles: involvement of reactive oxygen species (ROS)-driven degradation. *Biochem Biophys Res Commun* 421: 329-334.
- Hoyle L, Vulevic J (2008) Diet, immunity and functional foods. *Adv Exp Med Biol* 635: 79-92.
- Kennedy AD, Willment JA, Dorward DW, Williams DL, Brown GD, DeLeo FR (2007) Dectin-1 promotes fungicidal activity of human neutrophils. *Eur J Immunol* 37: 467-478.
- Kim JM, Joo HG (2012) Immunostimulatory effects of β -glucan purified from *Paenibacillus polymyxa* JB115 on mouse splenocytes. *Korean J Physiol Pharmacol* 16: 225-230.
- Lackovic V, Borecky L, Sikl D, Masler L, Bauer S (1970) Stimulation of interferon production by mannans. *Proc Soc Exp Biol Med* 134: 874-879.
- Milewski S, Wójcik R, Małaczewska J, Trapkowska S, Siwicki AK (2007) Effect of β -1,3/1,6-D-glucan on meat

- performance and non-specific humoral defense mechanisms in lambs. *Med Weter* 63: 360-363.
- Mosmann TR, Coffman RL (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 7: 145-173.
- Netea MG, Gow NA, Munro CA, Bates S, Collins C, Ferwerda G, Hobson RP, Bertram G, Hughes HB, Jansen T, Jacobs L, Buurman ET, Gijzen K, Williams DL, Torrensma R, McKinnon A, MacCallum DM, Odds FC, Van der Meer JW, Brown AJ, Kullberg BJ (2006) Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J Clin Invest* 116: 1642-1650.
- Ohno N, Egawa Y, Hashimoto T, Adachi Y, Yadomae T (1996) Effect of beta-glucans on the nitric oxide synthesis by peritoneal macrophage in mice. *Biol Pharm Bull* 19: 608-612.
- Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB, Schroeder L, Aderem A (2000) The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci USA* 97: 13766-13771.
- Pelizon AC, Kaneno R, Soares AM, Meira DA, Sartori A (2005) Immunomodulatory activities associated with beta-glucan derived from *Saccharomyces cerevisiae*. *Physiol Res* 54: 557-564.
- Ranta K, Nieminen K, Saariaho T, Kortekangas-Savolainen O, Kumpula EK, Kosonen J, Pasanen AL, Savolainen J (2013) Evaluation of fungal extracts to determine immunomodulatory properties. *J Investig Allergol Clin Immunol* 23: 226-233.
- Rasmussen LT, Seljelid R (1991) Novel immunomodulators with pronounced in vivo effects caused by stimulation of cytokine release. *J Cell Biochem* 46: 60-68.
- Refstie S, Baeverfjord G, Seim RR, Elvebø O (2010) Effects of dietary yeast cell wall β -glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunower and soybean meal. *Aquaculture* 305: 109-116.
- Sadeghi AA, Mohammadi A, Shawrang P, Aminafshar M (2013) Immune responses to dietary inclusion of prebiotic-based mannan-oligosaccharide and β -glucan in broiler chicks challenged with *Salmonella enteritidis*. *Turk J Vet Anim Sci* 37: 206-213.
- Sakurai T, Ohno N, Yadomae T (1996) Effects of fungal β -glucan and interferon- γ on the secretory functions of murine alveolar macrophages. *J Leukoc Biol* 60: 118-124.
- Seifert S, Watzl B (2007) Inulin and oligofructose: review of experimental data on immune modulation. *J Nutr* 137: 2563S-2567S.
- Thomas WE, Nilsson LM, Forero M, Sokurenko EV, Vogel V (2004) Shear-dependent "stick-and-roll" adhesion of type 1 fimbriated *Escherichia coli*. *Mol Microbiol* 53: 1545-1557.
- Tzianabos AO (2000) Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. *Clin Microbiol Rev* 13: 523-533.
- Underhill DM, Ozinsky A (2002) Phagocytosis of microbes: complexity in action. *Annu Rev Immunol* 20: 825-852.
- Vetvicka V, Vetvickova J, Frank J, Yvin JC (2008) Enhancing effects of new biological response modifier β -1,3 glucan sulfate PS3 on immune reactions. *Biomed Pharmacother* 62: 283-288.
- Wójcik R, Małaczewska J, Trapkowska S, Siwicki AK (2007) Influence of β -1,3/1,6-D-glucan on non-specific cellular defence mechanisms in lambs. *Med Weter* 63: 84-86.
- Wójcik R, Milewski S, Małaczewska J, Tański Z, Brzostowski H, Siwicki AK (2008) Defence mechanisms of the offspring of ewes fed a diet supplemented with yeast (*Saccharomyces cerevisiae*) during pregnancy and lactation. *Centr Eur J Immunol* 33: 197-201.
- Wójcik R (2010) Effect of brewer's yeast (*Saccharomyces cerevisiae*) extract on selected parameters of humoral and cellular immunity in lambs. *Bull Vet Inst Pulawy* 54: 181-187.
- Yang Y, Iji PA, Kocher A, Mikkelsen LL, Choct M (2008) Effects of mannanoligosaccharide and fructooligosaccharide on the response of broilers to pathogenic *Escherichia coli* challenge. *Br Poult Sci* 49: 550-559.