The effect of Leiber Beta-S on selected immunity indicators in calves

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Abstract

A wide variety of dietary supplements containing polysaccharides are being introduced on the market. One of them is Leiber Beta-S (β-1,3/1,6-D-glucan) whose immunostimulatory effects have not yet been fully evaluated, in particular in polygastric animals. The aim of this study was to evaluate the effect of this supplement on selected indicators of cellular and humoral immunity in calves. The experiment was performed on 14 calves aged 30 ± 2 days, divided into two equal groups of control and experimental animals. The feed administered to calves of the experimental group was supplemented with Leiber Beta-S at 50 mg/kg body weight, whereas control calves were administered standard farm-made feed without supplementation. Blood was sampled before the experiment (day 0) and on days 15, 30 and 60 to determine the immunity indicators (proliferative response of lipopolysaccharide- and concanavalin A-stimulated lymphocytes, respiratory burst activity, potential killing activity of phagocytes, gamma globulin concentrations, lysozyme activity, ceruloplasmin activity) and biochemical indicator (total protein concentrations). Diet supplemented with Leiber Beta-S stimulated the immune system of calves. Significant differences between the experimental and the control group were found in lysozyme and ceruloplasmin activity, gamma globulin concentrations, potential killing activity of phagocytes, proliferative response of lymphocytes ($P < 0.001$) and respiratory burst activity of phagocytes ($P < 0.05$). No differences were found in the serum total protein between the experimental and the control group. This study reports for the first time the effect of Leiber Beta-S (β-1,3/1,6-D-glucan) on selected biochemical and immunity indicators in calves.

β-1,3/1,6-D-glucan, humoral and cellular immune response, cattle

Beta-glucans synthesized by prokaryotic and eukaryotic cells have been studied extensively in recent years (Akramiene et al. 2007; Milewski et al. 2007; Wójcik et al. 2007; Wójcik et al. 2008). The main source of β-glucans are fungi, in particular Basidiomycota and yeasts of the genus *Saccharomyces cerevisiae* which produce soluble and branched β-1,3/1,6-D-glucans. The molecular structure, type and number of side chains, tertiary structure, molecular mass and solubility of β-glucans determine their physicochemical and immune properties. Product purity is also an important determinant of β-glucans' quality and effectiveness. Only pure β-glucan has immunomodulating effects, and the process of glucan extraction by hydrolysis should yield a minimum of 70% pure β-glucan. Unlike whole cells of *S. cerevisiae* yeast and the isolated mannan-oligosaccharides, free β-1,3/1,6-D-glucan molecules are able to penetrate the intestinal endothelial barrier with the use of specialized surface epithelial (M) cells. In gut-associated lymphoid tissue (GALT), β-glucans act as antigens by stimulating specific macrophage receptors (Engstad and Robertsen 1993).

Disease and death of calves in the first weeks *post partum* generate the greatest financial losses in cattle breeding. Most diseases are caused by respiratory and gastrointestinal infections as well as non-infectious factors such as the season of birth, low birth weight, unsupportive environment or failure to supply the calf with colostrum within the recommended time after birth. The most serious health risks are posed by diarrhoea (more than 50% of calf deaths) caused by infectious factors (*Escherichia coli, Salmonella,*

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Clostridium, rotaviruses and coronaviruses) and non-infectious factors (poor hygiene, feeding the milk of cows diagnosed with mastitis, low quality of milk replacers), bronchial pneumonia (caused by more than 10 viruses as well as mycoplasma and chlamydophila bacteria), and calf diphtheria (caused by *Bacillus necrophorus* that abundantly colonizes soil and fertilizers). Treatment is difficult and prognosis is doubtful, which is why a preventative use of biostimulators, including Leiber Beta-S which contains 80% pure β-1,3/1,6-D-glucan, is recommended to mobilize the immune system. Its activity has been tested on selected animal species (Siwicki et al. 2011; Frericks 2013), but to date, the product effectiveness has not been investigated in cattle, in particular in calves.

The aim of this study was to evaluate the effect of feed supplementation with Leiber Beta-S on concentrations of total proteins and selected immunity indicators in calves.

Materials and Methods

Experimental design

The experiment was carried out on 14 one-month-old Polish Holstein-Friesian calves (including 7 males and 7 females) in July and August 2011 at a private farm in Księży Dwór, Poland. The calves were administered colostrum at the amount of 2 kg/animal/day from 1 h after birth to 5 days of age. After that, the animals were fed the Polmass Mlekowit milk replacer (a standard farm-made diet) at the amount of 4 liters/day/animal in two portions until the age of 8 weeks. The animals had unlimited access to solid feed (maize silage, meadow hay and Jösera Kälberkost prestarer). Fresh water was supplied *ad libitum* throughout the experiment. At the age of 1 month, the animals were weighed and divided into two groups (control and experimental) by the analog method. The feed administered to calves of the experimental group was supplemented with Leiber Beta-S (β-1,3/1,6-D-glucan) (Leiber GmbH, Bramsche, Germany) at 50 mg/kg body weight/day, whereas control calves were administered standard farm-made feed without supplementation. At the beginning of the experiment (day 0) and on days 15, 30 and 60, blood was sampled from the jugular vein of calves to determine biochemical and immunity indicators.

Cellular immunity indicators

The metabolic activity of blood phagocytic cells was determined based on measurements of intracellular respiratory burst activity after stimulation with phorobol myristate acetate, (Sigma, USA) based on the method developed by Chung and Secombes and modified by Siwicki et al. (2004). The potential killing activity of mononuclear phagocytes and polymorphonuclear phagocytes was determined in isolated blood leukocytes stimulated with killed microorganisms, according to the method by Siwicki et al. (2004).

The proliferative response of blood lymphocytes after stimulation with mitogens, concanavalin A (ConA) and lipopolysaccharide (LPS) was determined by the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay that was first described by Mosmann and used in animals by Wagner et al. (1999).

Humoral immunity indicators

Lysozyme activity in blood plasma was determined by the turbidimetric method developed by Siwicki and Anderson (1993). Ceruloplasmin activity in blood plasma was evaluated by the method proposed by Siwicki and Studnicka (1986), and γ-globulin concentrations in blood serum were determined by the precipitation method modified by Siwicki and Anderson (1993).

Biochemical indicator

Concentrations of total proteins in blood serum were determined by spectrophotometry according to the method described by Siwicki and Anderson (1993).

Statistical analysis

The results were processed statistically by one-factorial analysis of variance in an orthogonal design. The significance of differences ($P < 0.05$, $P < 0.01$, $P < 0.001$) between groups was verified by Student's *t*-test and Bonferroni test with the use of GraphPad Prism 5 software.

Results

In the evaluated cellular immunity indicators (Table 1), a significant ($P \le 0.05$) increase in respiratory burst activity and potential killing activity was observed on days 15 and 30 ($P \le 0.01$ and $P \le 0.001$, respectively), and a significant ($P \le 0.001$) increase in the proliferative response of lymphocytes stimulated with lipopolysaccharide (B lymphocytes) and ConA (T lymphocytes) was noted on days 15, 30 and 60 in animals from the experimental group

Table 1. The effect of Leiber Beta-S on selected indicators of cellular immunity in calves.

Indicator	Group	Experimental day			
		0	15	30	60
Respiratory burst activity (OD 620 nm)	Control	0.37 ± 0.07	0.33 ± 0.08	0.37 ± 0.04	0.29 ± 0.06
	Experimental	0.32 ± 0.06	$0.42 \pm 0.05^{a, A}$	$0.45 \pm 0.08^{a, B}$	0.30 ± 0.05
Potential killing activity (OD 620 nm)	Control	0.2 ± 0.04	0.19 ± 0.03	0.15 ± 0.04	0.13 ± 0.01
	Experimental	0.17 ± 0.05	$0.25 \pm 0.04^{b, B}$	$0.26 \pm 0.03^{c, C}$	0.19 ± 0.01
Proliferative response of lymphocytes (-ConA)	Control	1.21 ± 0.18	0.86 ± 0.12	1.04 ± 0.22	0.96 ± 0.10
	Experimental	1.31 ± 0.21	$2.01 \pm 0.3^{c, C}$	$2.78 \pm 0.35^{c, C}$	1.33 ± 0.19^{c}
Proliferative response of lymphocytes (-LPS)	Control	0.94 ± 0.31	0.81 ± 0.04	0.96 ± 0.24	0.93 ± 0.08
	Experimental	1.19 ± 0.22	1.37 ± 0.17^{c}	$1.52 \pm 0.21^{c, A}$	1.22 ± 0.09^{c}

Data are expressed as mean ± SD. ConA – concanavalin A, LPS – lipopolysaccharide, OD – optical density, [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ difference between control group (without supplementation) and experimental group (supplementation with Leiber Beta-S), [A] $P \leq 0.05$, [B] $P \leq 0.01$, [C] $P \leq 0.001$ in comparison with day 0.

Table 2. The effect of Leiber Beta-S on selected indicators of humoral immunity in calves.

Indicator	Group	Experimental day			
		0	15	30	60
Lysozyme activity (mg/l)	Control	0.51 ± 0.17	0.33 ± 0.09	0.38 ± 0.12	0.41 ± 0.09
	Experimental	0.48 ± 0.12	0.57 ± 0.11^{c}	$0.70 \pm 0.16^{b, A}$	0.50 ± 0.08
Ceruloplasmin activity (mg/l)	Control	48.43 ± 4.30	45.20 ± 2.45	53.03 ± 8.44	49.31 ± 2.86
	Experimental	49.07 ± 2.60	$52.74 \pm 1.99^{c, A}$	52.36 ± 1.98	49.87 ± 2.15
γ-globulin levels (g/l)	Control	8.17 ± 0.51	9.09 ± 1.65	9.07 ± 0.63	10.34 ± 0.73
	Experimental	7.63 ± 0.97	10.53 ± 1.26^{C}	$11.81 \pm 0.72^{c, C}$	$12.92 \pm 0.66^{c, C}$

Data are expressed as mean ± SD. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ difference between control group (without supplementation) and experimental group (supplementation with Leiber Beta-S), [A] $P \leq 0.05$, [B] $P \leq 0.01$, [C] $P \leq 0.001$ in comparison with day 0.

compared to animals from the control group. Cellular immunity indicators increased significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) in animals from the experimental group on days 15 and 30 compared to day 0 (Table 1).

Humoral immunity indicators are summarized in Table 2. Compared to control, a significant increase was noted in animals from the experimental group for these indicators: lysozyme activity on day 15 ($P \leq 0.001$) and day 30 ($P \leq 0.01$), ceruloplasmin activity

Table 3. The effect of Leiber Beta-S on total protein concentrations in calves.

Indicator	Group	Experimental day			
		0	15	30	60
Total protein concentrations (g/l)	Control	48.29 ± 2.81	49.66 ± 4.09	50.27 ± 3.98	55.89 ± 6.2
	Experimental	46.96 ± 2.23	51.57 ± 3.59	52.76 ± 6.40	56.07 ± 2.77^{B}

Data are expressed as mean ± SD. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ difference between control group (without supplementation) and experimental group (supplementation with Leiber Beta-S), [A] $P \leq 0.05$, [B] $P \leq 0.01$, [C] $P \leq 0.001$ in comparison with day 0.

on day 15 ($P \leq 0.001$), gamma globulin concentrations on days 30 and 60 ($P \leq 0.001$). Compared to day 0, a significant increase in the experimental group was recorded for lysozyme activity on day 30 ($P \leq 0.05$), for ceruloplasmin activity on day 15 ($P \leq 0.05$) and for gamma globulin concentrations ($P \leq 0.001$) throughout the experiment.

Despite a minor increase in total protein concentrations (Table 3) in animals from the experimental group compared to control, no significant variations were noted in this biochemical indicator on different days of the experiment. A significant ($P \leq 0.01$) increase in total protein values was observed only in the experimental group on the last day of the experiment in comparison with day 0.

Discussion

Leiber Beta-S was found to exert positive effects on fish (Siwicki et al. 2011) and pigs (Frericks 2013), but its effectiveness in calves has not been investigated to date.

In our study, a 27% and 22% increase in respiratory burst activity values and a 32% and 73% increase in potential killing activity values were observed in animals of the experimental group on days 15 and 30, respectively, compared to control. Similar growth trends were reported in the author's previous studies where calf diets were supplemented with other products containing β-glucans: Biolex-Beta HP (Wójcik et al. 2007) and Biolex MB40 (Wójcik 2010). After the administration of whole glucan particles and zymosan in an *in vitro* study, Qi et al. (2011) observed an increase in the production of reactive oxygen species (ROS) and enhanced phagocytic activity of macrophages and dendritic cells. Borchers et al. (2004) suggested that higher concentrations of phagocytic activity could result from β-glucan binding by Toll-like receptor 2 (TLR-2), dectin-1, complement receptor 3 (CR3), lactosylceramide (LacCer), scavenger receptors and probably other receptors on macrophages/monocytes and granulocytes, which leads to their activation. The above process leads to chemokinesis, chemotaxis and cell degranulation in phagocytes, leading to increased expression of adhesion molecules on the surface of macrophages, intensified formation of reactive oxygen species, reactive nitrogen species and free radicals, higher concentrations and activity of hydrolytic and metabolic enzymes and induction of inflammatory cytokines (Schepetkin and Quinn 2006; Goodridge et al. 2007; Qi et al. 2011). Beta-glucans enhance the phagocytic activity of leukocytes which constitute the first line of defense against pathogens. Supplementation diet with β-glucans contributes to effective elimination and degradation of pathogens. Antigens are presented to T lymphocytes, thus further stimulating T-cell-mediated immunity.

In our study, a significant increase in the proliferative activity of B lymphocytes stimulated with LPS (by 69%, 58% and 31%, respectively) and T lymphocytes stimulated with ConA (by 134%, 167% and 39% respectively) was observed in experimental animals compared to control on days 15, 30 and 60, respectively. Wang et al. (2008) observed in mice similar increase in the proliferative activity of lymphocytes induced by only three of the tested compounds: Astragalan (extract from *Astraglus membranaceus*), Lentinan (extract from *Lentinula edodes*) and Ganoderan (extract from *Ganoderma lucidum*). Sonck et al. (2010) evaluated seven β-glucans of different origin and structure and found out stimulating effects on lymphocyte proliferation in porcine leukocytes (*in vitro* study) only in three of them (Curdlan - bacteria, Zymosan and β-glucans produced by *Euglena gracilis*).

In our study, β-1,3/1,6-D-glucans stimulated lysozyme activity, ceruloplasmin activity and gamma globulin concentrations. The stimulating effects of Leiber Beta-S were significantly less profound than those noted in the author's previous studies on calves whose diets were supplemented with Biolex-Beta HP (Milewski et al. 2007) and Biolex MB40 (Wójcik 2010). Higher concentrations of IgG secretion and a significant increase

in gamma globulin concentrations compared to control were observed by Wang et al. (2008) in an *in vitro* study of murine spleen lymphocytes after the administration of Astragalan, Lentinan and Ganoderan and by Soročinová et al. (2013) in an *in vivo* study of suckling pigs whose diets were supplemented with β-glucan (Immunol P). In contrast, Hiss and Sauerwein (2003) did not report an increase in specific antibody concentrations in pigs fed with β-1,3/1,6-D-glucan after vaccination against the porcine reproductive and respiratory syndrome. It should be stressed that gamma globulins include not only specific antibodies, but also haemaglutinin and haemolysin (first class antibodies that play a very important role in the non-specific humoral immune response). A similar increase in lysozyme activity to that noted in our study was reported by Ząbek et al. (2013) in the blood serum of sheep administered β-1,3/1,6-D-glucan (Biolex®-Beta S). Cleary et al. (1999) demonstrated in mice that serum concentrations of lysozyme do not always increase in response to the administration of β-glucans. Ząbek et al. (2013) did not observe an increase in ceruloplasmin activity in sheep fed Biolex®-Beta S, whereas Milewski et al. (2013) reported such increase in lambs whose diets were supplemented with *S. cerevisiae* (Inter Yeast S). This could be attributed to the stimulation of proinflammatory cytokines (IL-1, IL-6, TNF-α) after β-glucan binding by glucan receptors and the stimulation of acute-phase protein genes in hepatocytes including ceruloplasmin (Schepetkin and Quinn 2006). This is an important observation because the stimulation of acute-phase proteins which determine innate mechanisms of humoral immunity could suggest a quick return to homeostasis.

The noted variations in immune responses of different animal species to products containing β-glucans indicate that the biological activity of β-glucans is determined by their origin, structure and purity. Further research is needed to test the effectiveness of β-glucan supplementation in other animal species.

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