Effect of β-1,3/1,6-D-glucan in diet on productivity and humoral and cellular defense mechanisms in sheep

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Abstract

The aim of this study was to determine the effect of β-1,3/1,6-D-glucan, isolated from *Saccharomyces cerevisiae*, on indicators of milk and meat performance in sheep as well as on selected non-specific indicators of humoral and cellular defense. The experiment was carried on 26 suckling ewes divided into 2 equal groups, and their offspring (21 in each group). The ewes were administered concentrate with the addition of β-1,3/1,6-D-glucan at a dose of 3 g/kg. Indicators of milk performance and markers of humoral and cellular immunity were analyzed on days 28 and 70 of lactation; and the indicators of meat performance of lambs on day 28 and 70 of their life. The addition of β-1,3/1,6-D-glucan was observed to cause an increase in milk performance by 13.5–14%. Simultaneously, milk was characterized by a lower somatic cell count. Diet supplementation had a positive effect on the chemical composition of milk, which was manifested by increased percentage contents of fat (by 15– 30%) and protein (by 11%). Lambs were characterized by a higher growth rate and better muscle tissue development. The supplementation caused an increase of gamma-globulin concentration (by 6.33–9.5 g/l), lysozyme activity (by 0.1 mg/l), respiratory burst activity (by 0.11–0.14), potential killing activity (by 0.10–0.12), proliferative response of T-cells stimulated by mitogen concanavalineA (by 0.07–0.09 RI) and proliferative response of B-cells stimulated by mitogen lipopolysaccharide (by 0.13–0.16 RI) in sheep's blood. The activity of β-1,3/1,6-D-glucan as a natural immunostimulator has been studied in many animal species, however, this is the first study conducted on sheep.

Milk yield and composition, musculus longissiumus dorsi, ultrasound scanning, anti-infective immunity

Sheep are animals especially predisposed to utilize natural conditions of the environment. The effectiveness of meat and milk production in their case is lower than in other animal species. For this reason, feed additives are used to increase their productivity (Milewski 2009). In recent years, attention has been paid to specific polysaccharides such as glucans isolated from the yeast *Saccharomyces cerevisiae* (Augustin et al. 2007). The main constituents of their cell wall are: β-1,3/1,6-D-glucan (50 - 60%) and mannanoproteins (40%) (Klis et al. 2006). β-1,3/1,6-D-glucan obtained from the yeast is known as a natural immunostimulant. β-D-glucans affect selected receptors that among others include CD11b/CD18 (CR3), lactosylceramide, scavenger receptors and TLR2 (toll-like receptor 3), TLR4 by activating macrophages, phagocytes and T-helper lymphocytes (Ross et al. 1999). They stimulate phagocytes for the production of cytokines IL-1, IL-9, TNF-α, as well as affect the synthesis of acute-phase proteins, including ceruloplasmin (Małaczewska et al. 2010). Interleukin-1 stimulates T lymphocytes which in a consequence release interleukin-2, which in turn stimulates proliferation of T and B lymphocytes, natural killer (NK) and lymphokine-activated killer (LAK) cells as well as enhances the activity of natural suppressor cells and macrophages (Novak and Vetvicka 2009).

The aim of this study was to determine the effect of dietary β-1,3/1,6-D-glucan on indicators of milk and meat performance of sheep as well as on non-specific humoral and cellular defense mechanisms determining of the anti-infectious immunity of mothers.

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Material and Methods

The study was conducted in a rearing flock of Polish longwool sheep, Kamieniecka variety. The experiment was conducted on 26 suckling ewes divided into 2 equal groups: the control group and the experimental group according to the age of mothers, type of litter and sex of lambs. Each group included 21 lambs. During the 70-day lactation period, ewes of both groups were administered the same sets of feed mixtures: meadow hay at the quantity of 0.5 kg/animal/day, silage from pre-dried grasses and papilionaceous plants at the quantity of 2.4 kg/animal/day, and complete CJ® mixture at the dose of 0.6 kg/animal/day, on the assumption of an equal feeding level. The components of the mixture were the following: ground barley (40%), ground wheat (37.5%), ground maize (10%), soybean meal (10%), mineral premix (2%), fodder chalk (0.2%), dicalcium phosphate (0.2%) and salt fodder (0.10%). Ewes of the experimental group were administered the complete CJ mixture with the addition of Biolex®-Beta S Leiber GmbH preparation containing approximately 70% of β-1,3/1,6-D-glucan in the portion of 3 g/kg of CJ mixture. Glucan supplement did not change the value of the feed ration. During the 70-day lactation nutritional studies were carried out. Glucan supplement did not affect the chemical composition of the feed.

Analyses were carried out for milk performance and its composition on day 28 and 70 of lactation, body weight of lambs on day 2, 28 and 70 of life, daily body weight gains and relative growth rate index of lambs in the periods of 2–28, 28–70 and 2–70 day of life, dimensions of musculus longissimus dorsi (m.l.d.) cross-section and thickness of fat over the "loin eye" in lambs on days 28 and 70 of life and markers of non-specific humoral and cellular immunity of ewes on days 28 and 70 of lactation.

Milk was analyzed to determine the percentage content of dry matter, fat, protein and lactose, and somatic cell counts per ml using the Combi Foss 6000 MilkoScan™ (FOSS Electric). Dimensions of m.l.d. cross-section and thickness of fat over "loin eye" were determined using a SSD-500 ultrasonograph by Aloka Co., Ltd., with a 7.5 MHz linear probe. Measurements were made in live animals behind the last thoracic vertebra.

Blood samples were collected from the jugular vein. Lysozyme activity in the blood plasma was determined by the turbidimetric method described by Siwicki and Anderson (1993), and ceruloplasmin activity by the method proposed by Siwicki and Studnicka (1986). Gamma globulins were determined by the colorimetric micromethod modified by Siwicki and Anderson (1993). Respiratory burst activity (RBA) levels, i.e. the metabolic activity of PMA-stimulated (Phorobol Myristate Acetate) phagocytes, were measured by spectrophotometry (OD 620 nm), using the method modified by Siwicki et al. (1998). The Potential Killing Activity (PKA) of polymorphonuclear and mononuclear phagocytes was determined by spectrophotometry (OD 620 nm) according to Rook et al. (1995). The proliferative response of T cells stimulated with concavalin A (ConA) and B cells stimulated with lipopolysaccharide (LPS) was determined by MTT-based (3-(4,5-dimethylthiazoly-2yl)-2,5-diphenyltetrazolium bromide) spectrophotometry according to Mosmann (1983).

All procedures related to the animals in this study were approved by Local Ethics Committee for Animal Experiments in Olsztyn (28/2008). The results were processed by one-way ANOVA, and the significance of differences between groups was verified with Duncan's test ($P < 0.01$ and $P < 0.05$). Computations were conducted with the Statistica ver. 9.0 software (StatSoft, Inc.)

Results

Results of milking are presented in Table 1. The mean daily milk yield on day 28 of lactation was found to be higher by 13.5% in experimental group ($P < 0.05$). The superiority of the experimental sheep remained until the end of the lactation period. The applied preparation β-1,3/1,6-D-glucan evoked differences in the chemical composition of milk (Table 1). The changes referred, mainly, to the content of fat and, consequently, to that of dry matter. In both terms of analyses, milk of the ewes from experimental group was characterized by a significantly higher ($P < 0.01$) content of fat than milk of the control group. On day 70 of lactation, higher concentration of protein was noted in milk of the experimental group ($P < 0.05$). These changes affected the dry matter content of milk which was higher in the experimental group on both day 28 ($P < 0.01$) and day 70 ($P < 0.05$) of lactation. Milk of the sheep from experimental group had a lower somatic cell count compared to the milk of the control group; however, the significance of differences was confirmed only for the 28th day of lactation ($P < 0.01$).

Results referring to the growth of lambs are presented in Table 2. Lambs of the experimental group were characterized by a higher growth rate than those of the control group; however, no significant differences were demonstrated between these groups in both daily body weight gains and body weight values ($P > 0.06$).

Table 1. Effect of β-1,3/1,6-D-glucan on milk performance traits and composition of ewe's milk

Indicator	Day od life	Control group	Experimental group
Milk yield (ml):	28	1596.15 ± 181.90^{b}	1812.31 ± 231.31^{a}
	70	998.46 ± 187.69^{b}	1138.69 ± 155.46^{a}
Composition of milk (%):			
Fat	28	5.04 ± 0.91^{B}	6.56 ± 1.12^{A}
	70	7.05 ± 1.04^{B}	8.13 ± 1.00^{A}
Protein	28	4.99 ± 0.43	5.02 ± 0.36
	70	5.55 ± 0.88^{b}	6.16 ± 0.53^{a}
Lactose	28	5.17 ± 0.33	5.20 ± 0.14
	70	4.72 ± 0.87	4.70 ± 0.28
Dry matter	28	15.78 ± 0.80^{B}	17.28 ± 1.22^{A}
	70	17.91 ± 1.48^{b}	19.12 ± 1.00^{a}
Somatic cell count (thousand/ml):	28	316.77 ± 699.63^{A}	96.23 ± 25.99^{B}
	70	236.77 ± 296.34	88.92 ± 13.27

Values represent means ± standard deviation. Values within rows with different superscripts are significantly different ^{a,b} ($P < 0.05$), ^{A,B} ($P < 0.01$).

Table 2. Effect of β-1,3/1,6-D-glucan on body weight, daily gains and growth rates of lambs

Indicator	Control group	Experimental group
Body weight (kg) at days of life:		
2	4.91 ± 0.97	4.91 ± 0.49
28	11.84 ± 2.67	12.16 ± 2.60
70	19.75 ± 4.30	20.15 ± 3.00
Daily gains (g) in the period (days):		
2–28	266.48 ± 71.66	278.57 ± 90.26
28–70	188.32 ± 55.32	190.25 ± 47.75
2–70	218.21 ± 53.40	224.02 ± 41.14
Growth rate (%) in the period (days):		
2–28	82.18 ± 8.75	82.91 ± 14.64
28–70	50.27 ± 9.66	50.32 ± 13.79
2–70	119.92 ± 8.77	120.90 ± 8.49

Values represent means ± standard deviation

When analyzing the results of ultrasonographic measurements (Table 3), lambs of the experimental group were characterized by better development of muscle tissue. The area of their m.l.d. was higher by 17.74 % on day 28 and by 10.46% on day 70 of life. Nevertheless, no significant differences were noted in this respect, same as in fat thickness above the "loin eye".

Results describing the immunity markers of sheep are presented in Table 4. It was found that in ewes administered β-1,3/1,6-D-glucan, markers of non-specific humoral immunity (concentration of gammaglobulin, activity of lysozyme, and markers of cellular immunity) were at a higher level than in the control ewes. The differences turned out to be significant in both analytical terms ($P < 0.01$).

Table 3. Ultrasound scanning of *musculus longissimus dorsi* of lambs

Indicator	Day od life	Control group	Experimental group
Depth (cm)	28	1.50 ± 0.37	1.65 ± 0.36
	70	1.75 ± 0.31	1.93 ± 0.38
Width (cm)	28	4.84 ± 0.59	4.93 ± 0.51
	70	5.40 ± 0.45	5.29 ± 0.46
Area (cm2)	28	5.47 ± 1.80	6.44 ± 1.79
	70	7.36 ± 1.77	8.13 ± 2.12
Fat thickness over "eye loin" (cm)	28	0.13 ± 0.03	0.14 ± 0.04
	70	0.16 ± 0.03	0.16 ± 0.04

Values represent means ± standard deviation

Table 4. Effect of β-1,3/1,6-D-glucan on immunology of ewes

Indicator	Day of lactation	Control group	Experimental group
Gamma-globulin content (g/l)	28	35.67 ± 2.87^{B}	42.00 ± 3.46^{A}
	70	31.83 ± 2.23^{B}	41.33 ± 4.32^{A}
Lysozyme activity (mg/l)	28	0.81 ± 0.04^{B}	0.91 ± 0.02^{A}
	70	0.90 ± 0.04^{B}	1.00 ± 0.06^{A}
Ceruloplasmin activity (IU/l)	28	33.00 ± 2.99	31.87 ± 1.65
	70	32.92 ± 2.46	41.57 ± 4.28
Respiratory burst activity	28	0.36 ± 0.02^{B}	0.50 ± 0.01^{A}
	70	0.36 ± 0.02^{B}	0.47 ± 0.03^{A}
Potential killing activity	28	0.32 ± 0.01^{B}	0.42 ± 0.01^{A}
	70	0.32 ± 0.02^{B}	0.44 ± 0.01^{A}
MTT-ConA (RI)	28	0.45 ± 0.02^{B}	0.52 ± 0.02^{A}
	70	0.43 ± 0.02^{B}	0.52 ± 0.02^{A}
MTT- LPS (RI)	28	0.28 ± 0.01^{B}	0.41 ± 0.01^{A}
	70	0.28 ± 0.01^{B}	0.44 ± 0.02^{A}

MTT-ConA - proliferative response of T cells stimulated by mitogen concavalin A,
MTT-LPS - proliferative response of B cells stimulated by mitogen lipopolysaccharide.
Values represent means ± standard deviation, ^{A,B} - values within rows with different superscripts are significantly different ($P < 0.01$)

Discussion

The study demonstrated that β-1,3/1,6-D-glucan exerted a significant impact on indicators of milk performance in sheep. In the experimental group, its administration resulted in increased performance, protein and fat contents of milk, as well as decreased somatic cell count (SCC) in milk, which indicated a better health status of the mammary gland. Mastitis has an adverse effect on the physical indicators of milk, which in a consequence leads to deterioration of its quality and technological usability. Persson-Waller and Colditz (1999) demonstrated that injections of β-1,3/1,6-D-glucan in the udder of ewes manifesting mastitis stimulated migration of monocytes and macrophages to the gland, which resulted in a reduced somatic cell count in milk. Presumably, the improved health status of the

sheep affected a better feed conversion ratio, which consequently contributed to increased milk yield. The higher growth rate and better muscle tissue development in the progeny of ewes fed mixtures with the addition of the analyzed preparation ought to be linked with its stimulating effect on milk production and increased contents of fat and protein in milk. The fact that the effects achieved were not reflected in increased adiposity of the lambs was found beneficial. The increased productivity of sheep, both the mature ones and the lambs, receiving yeast-based preparations was demonstrated in studies by Milewski et al. (2007) and Milewski (2009). The beneficial effect of dietary supplementation on the immunity status of the sheep demonstrated in the present study, was indicated by the enhanced activity of non-specific defense mechanisms, both humoral and cellular. The immunomodulatory properties of β-1,3/1,6-D-glucan were confirmed by results of studies in other animal species, including fish (Sahoo and Mukeherjee 2001), pigs (Li et al. 2005), chicken (Benda et al. 1989) and rodents (Toklu et al. 2006). The efficacy of β-1,3/1,6-D-glucan was also analyzed by Małaczewska et al. (2010). They observed an increase in markers of non-specific humoral immunity (total protein content, activity of lysozyme and ceruloplasmin, concentration of gammaglobulins) as well as non-specific cellular immunity (RBA, PKA, and MTT-ConA). The favourable immunomodulatory properties of β-1,3/1,6-D-glucan administered to lambs were also reported by Wójcik et al. (2007) and Milewski et al. (2010).

Based on results of non-specific humoral and cellular immunity we found significant and positive influence of β-1,3/1,6-D-glucan on milk performance in sheep. These studies suggest to farmers the possibility of using β-1,3/1,6-D-glucan in feeding sheep. This preparation showed a strong stimulating effect without the risk of toxicity and the recommended dose should not exceed 3 g/kg of fodder.

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