Global Veterinaria 11 (1): 38-43, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.11.1.73188

Effect of Dietary Beta-Glucan Supplementation on Humoral and Cellular Immunologic Factors in Lambs

Aram Saadei Khalkhane, Keyvan Abbasi, Farzin Shokr Zadeh and Amir Hossein Arian

Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran

Abstract: Because of the ban of growth-promoting antibiotics in animal production, there is a need for alternatives to in feed antibiotics. beta-Glucans are indigestible oligosaccharides in the cell wall of yeast, fungi and bacteria. beta -glucans have been shown to stimulate both specific (vaccine adjuvants) and non-specific immune responses. We used pure soluble and particulate beta-glucans derived from the yeast *Saccharomyces cerevisiae* to determine their modulatory effect on innate and adaptive immune responses. A total of 30 male lambs were randomly allocated to six dietary treatment groups. Groups includes: 1) basal diet, 2) basal diet + 50 mg/kg beta glucan, 3) basal diet + vaccine, 4) basal diet + vaccine + 25 mg/kg beta glucan, 5) basal diet + vaccine + 50 mg/kg beta glucan, 6) basal diet + vaccine + 100 mg/kg beta glucan. Leukocytes' differential count was elevated in all experimental groups compared to day 0. beta-glucan dose dependent significant changes were observed for total IgG and interferon gamma levels in lambs. this study demonstrated that oral administration of beta-Glucan affects various aspects of the sheep immune system, including the effects on haematologic parameters, serum IgG and interferon -gamma.

Key words: Beta-Glucan · Leukogram · IgG · IFN-Gamma · Sheep

INTRODUCTION

In animal husbandry the control and prevention of infectious diseases is of great economic importance. Antibiotics have been used in animal production for many years both as prophylactic agents and growth promotor. Because of the ban of growth-promoting antibiotics in animal production, the necessity of controlling animal health to preserve the competitiveness of animal production has brought the need for alternatives to in feed antibiotics. One of the promising alternatives of antibiotics is b-glucans. beta-Glucans are indigestible oligosaccharides in the cell wall of yeast, fungi and bacteria. They consist of beta-1,3-linked beta-D-glucopyranosyl units that forma backbone containing randomly dispersed b-1,6-linked side chains. In humans and mice, beta -glucans have been shown to stimulate both specific (vaccine adjuvants) and non-specific immune responses [1, 2]. As such, beta-glucans stimulate phagocytosis and production of inflammatory cytokines by macrophages [3]. Furthermore, beta-glucans have the ability to stimulate neutrophils and monocytes by production of reactive oxygen species (ROS) [4]. The recognition of fungi and yeast derived particles has been attributed to a variety of pattern recognition receptors (PRRs) including complement receptor 3 (CR3), lactosylceramide and a putativeb-glucan-specific receptor namely Dectin-1. Recently, Dectin-1 was identified in the pig [5]. Only a few studies have been undertaken to analyse the effect of beta-glucan supplementation in pigs, however sometimes with different results [6]. Besides the use of different concentrations in these studies, also physicochemical parameters such as solubility, primary structure, molecular weight, branching and polymer charge, could explain the differences in immune response [7]. In the present study we aimed to increase our insights into the direct effects of beta-glucans on monocytes, neutrophils and lymphocytes of pigs by testing the dose response of different beta-glucans preparations *in vitro*.

Recent studies also demonstrate that beta-glucans can function as potent adjuvants to stimulate innate and adaptive immune responses. beta-glucans are glucose polymers that have a backbone of linear -1,3-linked D-glucose molecules (-1,3-Dglucan). They also have -1,6-

Corresponding Author: Aram Saadei Khalkhane, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran.

linked side chains of 1,3-D-glucan of varied sizes that occur at different intervals along the backbone. Because of the complexity of beta-glucans and different methods performed in the studies, several molecules that could bind beta-glucans have been reported on the membrane of macrophages, monocytes, neutrophils, NK cells, DCs and some T cells. Thus far, the 4 kinds of receptors, complement receptor 3 (CR3, CD11b/CD18, M2-integrin, Mac-1) [8, 9]. Lactosylceramide (LacCer) [10] selected scavenger receptors (SRs) [11] including SR CD36 [12] and dectin-114 [13-15] have been identified as beta-glucan receptors. In addition, TLR2 was also implicated in yeast zymosan beta-glucan induced cytokine production [16]. However, it is unknown how each beta-glucan receptor distinguishes its ligands. Here, we used pure soluble and particulate beta-glucans derived from the yeast Saccharomyces cerevisiae to determine their modulatory effect on innate and adaptive immune responses.

MATERIALS AND METHODS

Animals and Experimental Design: A total of 30 male lambs (ghezel; 33.3±2.25 kg BW and 5 or 6 months age) were randomly allocated to six dietary treatment groups. Lambs were reared in an isolated, disinfected and clean experimental house. Each pen contained dishes to allow *ad libitum* access to feed and water. The room temperature was controlled (20 to 24°C). The whole period includes 10 days adaptation periods and 4 weeks main study period. The experimental diets were formulated to meet the National Research Council (NRC, 1998) [17] requirements for all nutrients. The diets were available twice per day at 8 AM and 4 PM. The water and minerals and vitamins blocks were available *ad libitum*. Lambs feed were monitored every day. All lambs except group 1 and 2 vaccinated on day 0 of study with enterotoxemia vaccine obtained from Razi Vaccine & Serum Research Institute, Iran. The recommended dose for an adult sheep is 2-3ml subcutaneously, repeated 2 weeks later.

Groups includes: 1) basal diet, 2) basal diet + 50 mg/kg beta glucan (Sigma, USA), 3) basal diet + vaccine, 4) basal diet + vaccine + 25 mg/kg beta glucan, 5) basal diet + vaccine + 50 mg/kg beta glucan, 6) basal diet + vaccine + 100 mg/kg beta glucan.

Data and Sample Collection: Peripheral blood was collected into 10 ml with and without heparin containing vacuum tubes on days 0 and 28. Heparinized samples used for determination of leukocytes differential count and none heparinize samples used for determination of serum IgG and INF-gamma concentration. None heparinises samples were centrifuged at 3,000×g for 10 min and the supernatant serum obtained was stored at -20°C.

Leukocytes differential count (leukogram) determined with number of leukocytes (hemocytometric method) and leukocytic formula May-Grunwald-Giemsa method) [18].

Sheep Interferon gamma (IFN gamma) ELISA kit (Cusabio, China) were used to determination of serum IFNgamma concentration as index of cellular immune response. On the other hand, sheep total IgG ELISA kit (Cusabio, China) were used to determination of serum IgG concentration as index of humoral immune response. Minimum detectability was 2.5 pg/ml for IFN gamma and 7.5 µg/ml for IgG. Each assay had a within-assay coefficient of variance of less than 10%. All assays were analysed colorimetrically using a plate reader (Bio-Rad, Model 550, USA).

Statistical Analysis: All data were analyzed using SPSS 16.0 for Windows. Dose effects of dietary beta-glucan on cellular and humoral immunity factors were analyzed by one way ANOVA and Duncan posthoc tests. Effects were considered significant at $p<0.05$.

RESULTS

Differential Leukocytic Count: Leukocytes' differential count (Table 1) was elevated in all experimental groups except groups 1 (fed basal diet only) compared to day 0. Elevation of leukocytes was associated with elevation of lymphocytes (P=0.031) and neutrophils (P =0.022). Monocytes and eosinophils revealed variable changes in different experimental groups.

Humoral Immune Response: Beta-glucan dose dependent significant changes were observed for total IgG levels in lambs (Table 2). Lambs fed a diet supplemented with 50 and 100 mg/kg beta-glucan had increased IgG levels on days 14 and 28 (p=0.023), but no increased effect was observed in lambs IgG levels that fed 25 mg/kg beta-glucan, compared to unsupplemented lambs (P =0.37).

According to Table 2, Interferon gamma levels had significant changes in lambs fed 50 mg/kg beta-glucan on day 28 (P =0.020). However lambs fed 100 mg/kg beta-glucan had higher levels of Interferon gamma on days 14 (P =0.026) and 28 (P =0.005).

Table 1: Effect of different doses of beta-glucan on differential leukocyte count

	Day		
Leukocyte	Treatment	0	28
Neutrophil (%)	1	61 ± 11	62.4 ± 12
	2	60.4 ± 13[a]	61.6 ± 10[b]
	3	58.8 ± 10	62.8 ± 11
	4	61.2 ± 13	62.3 ± 9
	5	58.5 ± 9[a]	64.4 ± 8[b]
	6	59.8 ± 12[a]	64.6 ± 7[b]
Lymphocyte (%)	1	27.8 ± 6	29.5 ± 4
	2	29.4 ± 5	33.7 ± 6
	3	29.7 ± 7	33.3 ± 7
	4	28.6 ± 4[a]	33.8 ± 4[b]
	5	28.4 ± 6[a]	35.2 ± 5[b]
	6	30.9 ± 5[a]	34.4 ± 3[b]
Eosinophil (%)	1	3.2 ± 2	3.3 ± 1
	2	4.4 ± 2	3.4 ± 2
	3	4.1± 1	4.2 ± 1
	4	3± 2	3.5 ± 2
	5	2.8± 2	4.1 ± 2
	6	3.4 ± 1	3.6 ±2
Monocyte (%)	1	5.2 ± 3	5.2 ± 3
	2	4.3 ± 2	6.5 ± 3
	3	6 ± 3	5.2 ±2
	4	5.7 ± 3	5.6 ±3
	5	5.2 ± 3	6.4 ±4
	6	4.5 ± 2	4.4 ±2

a,b: Show significant difference (P>0.05).

Table 2: Effect of different doses of beta-glucan on serum total IgG and interferon gamma (IFN-gamma)

	Day		
Specification	Treatment	0	28
Serum total IgG (µg/ml)	1	19.6 ± 5.5	20.6 ± 4.3
	2	21.3 ± 3.6[a]	20.3 ± 3.2[b]
	3	19.8 ± 4.2	30.6 ± 5.7
	4	20.2 ± 3.3	36.5 ± 5.2
	5	20.8 ± 4.7[a]	40.2 ± 6.4[b]
	6	21.4 ± 3.6[a]	44.8 ± 5.8[b]
Serum IFN-gamma (pg/ml)	1	53.8 ± 6.6	56.4 ± 3.6
	2	55.4 ± 8.4	52.7 ± 6.6
	3	58.7 ± 6.2	54.3 ± 5.2
	4	52.6 ± 5.9	56.7 ± 6.4
	5	54.4 ± 4.8	58.2 ± 5.9
	6	57.9 ± 5.7[a]	62.4 ± 8.3[a b]

a,b: Show significant difference (P>0.05).

DISCUSSION

In present study the effects of dietary supplementation of different doses of beta-glucan (25, 50 and 100 mg/kg of diet) were evaluated on lambs different immunologic factors includes differential leukocyte count serum IgG and IFN-gamma were evaluated. Most of previous studies were about effects of beta-glucan on animal growth performance [19, 20] and there are limited studies on immunologic aspects of beta-glucan on ruminants specially sheep.

Concerning to leukogram, there was significant increase in leukocyte count when beta-glucan was used, this may be attributed to immunostimulatory. The obtained results in agreement with EL-Boshy *et al*. [21] (beta hemato) who reported that, the absolute lymphocyte count (ALC) values on both day 21 and 42 were significantly increased in supplemented group with beta-glucan indicating better immune status.

Total serum IgG concentrations were measured to assess the effects of dietary beta-glucan supplementation on humoral immune competences lambs. We observed that oral supplementation with beta-glucan resulted in increased serum IgG concentrations of lambs. Previous studies also showed that serum IgG levels could be affected by immunomodulatory property of beta-glucan [22, 23]. Therefore, our results indicate that dietary beta-glucan supplementation could enhance humoral immune function of lambs.

In present study Dietary supplementation with beta-glucan resulted in increased serum IFN-gamma concentrations of lambs. This is in agreement with Harada *et al.* [24] that reported beta-glucan induce IFN-gamma production from T cells. Therefore, our results indicate that dietary beta-glucan supplementation could enhance cellular immune function of lambs.

Immune activation is associated with exterior antigen stimulation, for example, in vaccine inoculation, bacterial and viral infections or non-specific immunoregulator inducement. This study was carried out in an isolated and clean experimental house. Moreover, low diarrhea rate stable health status was observed during the experimental period. Therefore, the increases in serum IgG, IFN-gamma, lymphocytes and neutrophil were mostly the result of stimulation by dietary beta-glucan supplementation and not from infection. Based on these findings, our research indicates that beta-glucan might potentiate cellular and humoral immune functions of lambs in an environment with minimal disease challenges. However, the mechanisms underlying the relationship between beta-glucan supplementation and immune response are still unclear. Hence, it is essential that further research is carried ut on the immunomodulatory impact of beta-glucan in models of infection.

Taking into account growth performance and immune response, the most suitable dietary supplementation level of beta-glucan is 50 mg/kg for lambs. This is similar to the level recorded for broilers [23].

Current data suggests that beta-glucans are potent immunomodulators with effects on both innate and adaptive immunity. The ability of the innate immune system to quickly recognize and respond to an invading pathogen is essential for controlling infection. Dectin-1, which is a type II transmembrane protein receptor that binds beta-1,3 and beta-1,6 glucans, can initiate and regulate the innate immune response [25]. It recognizes beta-glucans found in the bacterial or fungal cell wall with the advantage that beta-glucans are absent in mamalian cells. It then triggers effective immune responses including phagocytosis and proinflammatory factors production, leading to the elimination of infectious agents [26]. Dectin-1 is expressed on cells responsible for innate immune response and has been found in macrophages, neutrophils and dendritic cells [27]. The Dectin-1 cytoplasmic tail contains an immunoreceptor tyrosine based activation motif (ITAM) that signals through the tyrosine kinase in collaboration with Toll-like receptors 2 and 6 (TLR-2/6) [34, 37, 38]. The entire signaling pathway downstream to dectin-1 activation has not yet been fully mapped out but several signaling molecules have been reported to be involved. They are NF-κB (through Syk-mediate pathway), signaling adaptor protein CARD9 and nuclear factor of activated T cells (NFAT) [28]. This will eventually lead to the release of cytokines including interleukin (IL)-12, IL-6, tumor necrosis factor (TNF)-α and IL-10.

Beta-glucans can induce peripheral blood mononuclear cells proliferation [29]. It can also enhance phenotypic and functional maturation of monocyte derived dendritic cells with significant IL-12 and IL-10 production. Treatment of dendritic cells with beta-glucan resulted in enhanced T cell-stimulatory capacity and increased T cell secretion of interferon-gamma and IL-10 [30]. This action is at least mediated in part through the Dectin-1 receptor. The potency of such immunomodulating effects differs among beta-glucans and purified polysaccharides of different size and branching complexity.

The adaptive immune system functions through the combined action of antigen-presenting cells and T cells. Carbohydrates have been previously thought to stimulate immune responses independently of T cells [31]. However, zwitterionic polysaccharides (polysaccharides that carry both positive and negative charges) such as beta-glucans, can activate CD4 (+) T cells through the MHC-II endocytic pathway [32].

Another mechanism of beta-glucan action is mediated via the activated complement receptor 3 (CR3, also known as CD11b/CD18), which is found on natural killer (NK) cells, neutrophils and lymphocytes. This pathway is responsible for opsonic recognition of beta-glucans leading to phagocytosis and reactor cells lysis. beta-glucans bind to the lectin domain of CR3 and prime it for binding to inactivated complement 3b (iC3b) on the surface of reactor cells. The reactor cells can be of any cell type including virus infected or cancer cells tagged with monoclonal antibody and coated with iC3b. The beta-glucans-activated circulating cells such as the CR3 containing neutrophils will then trigger cell lysis on iC3b-coated cells [31].

In summary, beta-glucans act on a diversity of immune related receptors in particularly Dectin-1 and CR3 and can trigger a wide spectrum of immune responses. The targeted immune cells of beta-glucans include macrophages, neutrophils, monocytes, NK cells and dendritic cells. The immunomodulatory functions induced by beta-glucans involve both innate and adaptive immune response. beta-glucans also enhance opsonic and non-opsonic phagocytosis. Whether beta-glucans polarize the T cells subset towards a particular direction remains to be explored.

In conclusion, this study demonstrated that oral administration of beta-Glucan affects various aspects of the sheep immune system, including the effects on haematologic parameters, serum IgG and IFN-gamma. These findings demonstrate that beta-Glucan may stimulate both innate and adoptive immune responses in lambs.

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