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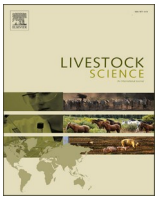

Effect of yeast extracted β-glucans on the immune response and reproductive performance of gilts in the adaptation, gestation, and lactation periods☆

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HIGHLIGHTS

- The beta-glucan supplementation at the adaptation phase did not improve sows' performance and immunity.
- An positive effect of the diet with β-glucan supplementation was observed on the concentration of IgA and IgM in colostrum, and for IgA in the milk of the sows.
- Colostrum from gilts with a diet supplemented with β-glucans showed significantly higher mitogenic activity than colostrum from non-supplemented gilts.

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ABSTRACT

Yeast β-glucans may have beneficial effects on the immune response of gilts and their litter through immunomodulation from the gastrointestinal tract. Thus, the objective of this study was to evaluate the effect of the inclusion of β-glucans in the diet of replacement gilts during the adaptation, gestation and lactation phases on their response and the response of their litter to vaccines against Parvovirus and Leptospirosis, as well as its effect on parity performance and immune parameters of colostrum and milk. In two trials, gilts were randomly assigned to one of two treatments: with or without the inclusion of β-glucans at 300 g/ton in the basal diet. Gilts consumed the experimental diets during the adaptation period in the first experiment (EXP1) and from d 75 of gestation until d 10 of lactation in the second experiment (EXP2). Blood samples were collected from sows and from their piglets. In addition to blood collections, colostrum and milk were also collected in EXP2, and the reproductive performance of sows at the first farrowing was evaluated. Qualitative data were analyzed by chi-square, and quantitative data were evaluated using ANOVA or Kruskal-Wallis, according to their normality. There was an effect of treatment in EXP2 on the IgA and IgM concentrations and mitogenic activity in colostrum ($P < 0.05$), and for IgA in milk from sows ($P < 0.05$). For parvovirus and Leptospira spp. antibodies, in the two experiments, there was no difference between treatments ($P > 0.05$), as well as for reproductive parameters. Thus, under the experimental conditions of this study, it is possible to conclude that including 300 g/ton β -glucans in the diet of first-parity sows can increase the concentration of IgA in their colostrum and milk, as well as the proliferation of intestinal epithelial cells, while decreasing the concentration of IgM in their colostrum, but not in milk.

1. Introduction

The immune system is one of the most complex in higher animal organisms, being divided into innate and adaptive immunity. The innate immune system is historically characterized by promoting a rapid response to pathogens through cytokines, mono- and polymorphonuclear phagocytes, and natural antibodies, among others, but without long-term memory. The adaptive system, on the other hand, promotes a slower, but more specific and memory-based immune response, with the help of T and B lymphocytes and specific antibodies (Hato and Dagher, 2015; Sattler, 2017).

New approaches suggest that the innate immune system can also

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develop an immune response with long-term memory, known as "trained immunity" (Saeed et al., 2014; Netea et al., 2016; Novakovic et al., 2016; Netea and Joosten, 2018). According to Netea et al. (2016), trained immunity is based on an altered functional state of innate immune system cells (myeloid, natural killer and innate lymphoid cells) that lasts for weeks to years after exposure to the initial stimulus, a pathogen or vaccine, for example, and that confers protection to the organism after a re-exposure to the same stimulus.

With this new evidence, β-glucans, glucose polymers naturally occurring in fungi, yeasts, algae, and cereal grains, have been further explored for having an immunomodulatory effect on the innate immune system in animal biosafety (Thompson et al., 2010; Kim et al., 2019). With increasing restrictions on the use of antibiotics in animal production (Boyd et al., 2018), the use of β-glucans can be a good ally to potentiate trained immunity, improve the effect of vaccines and even mitigate the pathogenic effect of biological agents harmful to the animal (Thompson et al., 2010).

Some studies on the inclusion of β-glucans in the diet of suckling and weaning piglets found an effect on their immunity and vaccine response, indicating that this early period in these animals' lives is critical for their later development (Kim et al., 2019; Vries et al., 2020). These works do not explain the effect that β-glucans could have in other phases of swine production, such as in the adaptation period of gilts, gestation, and lactation phases of primiparous sows. These phases are of great importance because they are where the gilts will receive the greatest number of vaccines and will be exposed to the pathogens present in the new environment in which they will be held. In addition, the litter of these gilts tends to have a more weakened immunity due to the immunity passed by the gilt itself, which is also more weakened compared to multiparous sows (Piñeiro et al., 2019; Maciag et al., 2022).

Based on this information, the objective of this study was to evaluate the effect of supplementation of β-glucans, extracted from *saccharomyces cerevisiae* yeast during the periods of gilt adaptation, gestation and lactation of primiparous sows, on their response to vaccines against Parvovirus and Leptospirosis, pathogens that significantly interfere with reproductive parameters and that are achieved with the same vaccine, parameters of immunity of colostrum and milk from these sows, and its effect on performance at parturition as measured by the number of total births, live births, stillbirths and mummified piglets.

2. Material and methods

All animal care and experimental procedures were approved by the Animal Ethics Committee of the Agricultural Sciences Sector of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol 029 - 2019. Two experiments were conducted in the period between January 21, 2020, and August 8 of the same year, on a commercial farm.

2.1. Experiment 1 – gilts treated during the adaptation period

2.1.1. Animals and facilities

96 females of the Camborough genetic lineage (PIC Camborough, Hendersonville, TN) were used, with an average weight of 98.45 kg ± 5.6 kg and 153 ± 1.4 days old. Gilts arrived at the farm in two batches, with a 15-day interval between batches. Each batch was divided into four groups of 12 gilts each, housed in collective pens, forming a total of eight pens of gilts per treatment until the beginning of pre-insemination flushing, when females are fed more nutritionally dense feed, so they are ready for first insemination, which took place at 185 days of life. From the beginning of flushing until two days before parturition, the gilts were housed in individual pens, after that they were transferred to the lactation pen.

For gilts in adaptation period, the collective pens were equipped with a collective funnel-type feeder with a tray, with a capacity of 100 kg of feed, divided between two pens. Each pen had dimensions of 6.0 m (L) x 2.5 m (L). The gestation pens were 2.0 m (L) x 0.6 m (L) and were equipped with ditch-type feeders fed with droppers and the feed was provided by a pipe traction system. The lactation pens were also individual with 2.2 m (L) x 0.6 m (L), equipped with crates that aim to protect the piglets from possible crushing and with concealing boxes with 1.5 m (L) x 2, 0 m (L), heated by incandescent lamps and underfloor heating, and the ration was also provided through droppers in trough-type feeders.

There was no induction of parturition and during farrowing, the sows were monitored by the farm staff. Also, there was no induced health challenges, just the usual ones in a commercial farm.

The temperature of the facilities was controlled by opening and closing curtains and remained on average at 24.3°C ± 6.9°C in the experimental period.

2.1.2. Diets and treatments

The gilts were divided into two treatment groups in a completely randomized design as: CON – control treatment that consisted only of the standard gestation diet of the farm based on corn and soybean meal (Table 1) and TBG – Standard diet of the farm with the addition of a product based on yeast β-glucans, containing 50% of 1,3/1,6 beta-glucans, at 300 g per ton of complete feed. The treatment period took place between the arrival of the gilts at the farm until their first insemination (50 days approximately).

To make the TBG, feed present the β-glucan in the proportion of 300 g/ton, a pre-mix was made as follows: 24 g of product was mixed with 76 g of feed to prepare a pre-mix of 100 g product. The 100 g premix was added to an additional 900 g of feed to form another 1 kg premix. The 1 kg pre-mix was mixed with 9 kg of feed to form 10 kg of pre-mix which was later mixed with 70 kg of feed to obtain the final mixture that was fed to the gilts, and this process was repeated three times to have the enough amount of feed to supply the four treatment pens.

The product for the first pre-mix was weighed on a digital scale and for the other mixtures buckets with the markings for the specific weights

Table 1
Composition of the diets used in the periods of adaptation, gestation, and lactation of the gilts and sows.

Ingredients	Adaptation/Gestation Inclusion g/kg	Lactation Inclusion g/kg
Corn 7.86% CP	684.763	698.434
Soybean hull	180.000	–
Soybean meal 46% CP	86.941	200.900
Viscera meal	15.253	30.000
Poultry fat	–	28.232
Limestone 36% Ca	12.549	12.744
Monocalcium Phosphate	5.104	6.462
Salt	5.000	3.105
Lysine*	2.802	6.587
L-Threonine	0.746	1.661
DL-Methionine 99%	0.562	0.952
Vitamin and Mineral Premix[1]	6.280	10.923
Calculated Nutritional Composition(%)		
Crude Protein	13.800	18.000
Ether Extract	3.610	6.449
Crude Fiber	7.336	2.070
Ash (%)	4.235	4.087
Calcium (%)	0.930	0.970
Total Phosphorus (%)	0.582	0.702
Available Phosphorus (%)	0.420	0.500
Sodium (%)	0.230	0.260
Total lysine (%)	0.779	1.250
Total Methionine (%)	0.279	0.387
Total Met + Cys (%)	0.539	0.697
Total Threonine (%)	0.604	0.852
Total Tryptophan (%)	0.154	0.263
Swine Met. Energy (Kcal/kg)	3.104,56	3.470,00

* BioLys 60.

[1] Contains per kg of complet diet: Vit. A, 16.500 UI; Vit. D3, 3.500 UI; Vit. E, 99.000 UI; Vit. K3, 3.3 mg; Vit. B2, 8.500 mg; Vit. B12, 38.500 mcg; Pantothenic acid, 25.000 mg; Folic acid, 3.850 mg; Se, 0.44 mg; Mn, 60.001 mg; Cu, 22.0 mg; Fe, 121 mg; Zn, 110 mg; I, 1.65 mg.

were used.

From the arrival of the gilts to the farm until the second dose of vaccines (vaccinal program is available in Fig. 1) Farrowsure® Gold (MSD), *Streptococcus suis* (IPEVE) and *E. coli* (IPEVE), where the flushing period began, the feed was provided ad libitum through a traction system by pipes in the feeders for the gilts of the CON and, in the pens of the TBG, the system traction was locked and the feed was supplied in buckets with the β-glucan mixture in the amount of 80 kg per feeder, being filled in the afternoon, when necessary, with approximately 20 kg more so that in the TBG pens the feed was also offered ad libitum.

It is important to note that each feeder was responsible for serving two pens of 12 gilts, that is, each gilt consumed approximately 4 kg of feed per day.

From the beginning of flushing until the first insemination of the gilts, around 20 days, the gilts were transferred to individual pens, where they consumed 2.5 kg of feed per day. The TBG gilts received 0.8 g of β-glucan in the ration, equivalent to 300 g/ton. During the gestation and lactation periods, the gilts received the standard diet of the farm for gestation and lactation (Table 1). Water was provided ad libitum and fresh for pacifier-type drinkers.

2.2. Experiment 2 – gilts treated during gestation and lactation periods

2.2.1. Animals and facilities

40 gilts with 290 ± 1.4 days of age were used. They were housed in individual gestation pens until two days before parturition, when they were transferred to the lactation pens where they remained until weaning (approximately 21 days of lactation). The facilities, management and temperature were as described in experiment 1.

2.2.2. Diets and treatments

The gilts were divided into two treatments in a completely randomized design with 20 gilts each: CON – control treatment (standard farm diet without addition of yeast β-glucans) and TBG – Standard farm diet with addition of yeast β-glucans to 300 g per ton.

During the gestation period, the gilts consumed 1.8 kg of feed until 90 days of gestation in a daily supply, and for this amount of feed, 0.6 g of β-glucan was placed in the droppers for the TBG gilts. From 90 days until the second day before farrowing they consumed 2.5 kg of feed, with the addition of 0.8 g of β-glucan for TBG gilts. In the period between the two days before farrowing until the third postpartum day they consumed 3 kg of feed per day, with the addition of 0.9 g of β-glucan for the TBG sows and about 6 kg per day on average until the tenth postpartum day, with the addition of 1.8 g of β-glucan for TBG sows. After the tenth day postpartum until weaning, all sows consumed only the standard farm diet, without the addition of β-glucan for any of the treatments. For TBG sows, an amount of β-glucan equivalent to the period of consumption was added to the feed at the time of supply.

During gestation, fresh water was provided ad libitum in trough-type drinkers and cup-type in lactation pens. The composition of gestation and lactation diets is described in Table 1.

2.2.3. Blood collections

In the first trial (adaptation), 5 mL blood samples were collected from 8 gilts per treatment in each batch, totaling 32 gilts, 16 from the CON and 16 from the TBG. The samples were collected in four time points, as pointed in Fig. 1. In the first collection, the TBG gilts were consuming the β-glucans for 5 days and in the two following collections they were being supplemented for an average of 32 and 52 days, respectively.

In the second trial (gestation and lactation) 5 mL of blood was collected from 10 gilts per treatment, totaling 20 gilts. The samples were collected in three time points, as pointed in Fig. 1.

Blood collection was performed by puncturing the jugular vein, and the blood was stored in EDTA tubes immediately after collection. Subsequently, the samples were centrifuged at 3000 rpm for five minutes, to obtain the serum. The sera obtained were aliquoted into Eppendorfs® tubes and stored in a freezer at −80 °C until use, to preserve the cytokines.

After collection and centrifugation, which took place on the farm, the serum samples were stored at −4 °C.

Also, for the second trial, colostrum was manually collected in 50 mL sterile conical tubes from all functional teats to a final volume of 10 mL, soon after the birth of the first piglet. To minimize colostrum contamination, the teats were previously washed with water and detergent, then with iodized alcohol, the first jet was discarded, and handling was performed with disposable latex gloves. The samples were stored in flasks at −20°C until analysis. On the tenth day of lactation, milk collection and storage were also performed by manual milking, following the pattern of colostrum collection.

Experimental Schedule				
Animals age (average)	**Time point**	**Vaccine**	**Blood collection**	**Diet program**
153 days	Gilts arrival at the farm		Gilts from 1st experiment	Adaptation diet ad libitum
160 days	First vaccine	Porcilis® PCV M HYO		Adaptation diet ad libitum
165 days	Vaccines	Farrowsure® B Gold		Adaptation diet ad libitum
		Oily Autogenous *E. coli* and *Clostridium*		
		Autogenous *Streptococcus suis*		
185 days	Flushing	Farrowsure® B Gold	Gilts from 1st experiment	2.5kg/day of adaptation diet
		Oily Autogenous *E. coli* and *Clostridium*		
		Autogenous *Streptococcus suis*		
205 days	First insemination		Gilts from 1st experiment	2.5kg/day of adaptation diet
75 days of gestation	Start of the 2nd experiment		Gilts from 2nd experiment	1.8kg/day of gestation diet
80 days of getation	Vaccine	Oily Autogenous *E. coli* and *Clostridium*	Gilts from 1st experiment	1.8kg/day of gestation diet
85 days of gestation	Blood collection			1.8kg/day of gestation diet
100 days of gestation	Vaccine	Oily Autogenous *E. coli* and *Clostridium*		2.5kg/day of gestation diet
114 days of gestation	Farrowing day		Gilts from 2nd experiment	3kg/day of lactation diet
3 days of lactation	Blood collection		Piglets from sows of the 2nd experiment	6kg/day of lactation diet
7 days of lactation	Piglets vaccine	Autogenous *Streptococcus suis*		6kg/day of lactation diet
11 days of lactation	Vaccine	Farrowsure® B Gold		7kg/day of lactation diet
21 days of lactation	Weaning		Gilts from 2nd experiment	7kg/day of lactation diet

Fig. 1. Schedule for both experiments, with vaccination, blood collection and diet program. The vaccines were: Inactivated vaccine (Porcilis® PCV M HYO, MSD, Boxmeer, Netherlands)) against porcine circovirus type 2 and Mycoplasma hyopneumoniae; Inactivated swine parvovirus, *Erysipelothrix rhusiopathiae* and Leptospira (*L. bratislava, L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae and L. pomona*) vaccine (Farrowsure® B *Gold,* Zoetis, Campinas, Brazil), named for this paper as parvovaccine; Oily autogenous vaccines *Escherichia coli* and *Clostridium perfringens* (IPEVE, Belo Horizonte, Brazil); Autogenous vaccine *Streptococcus suis* (IPEVE, Belo Horizonte, Brazil); and Inactivated vaccine (Porcillis® Glasser, MSD, Boxmeer, Netherlands).

2.2.4. Parvovirus and Leptospira spp analysis

Hemagglutination inhibition (HI) analysis was performed with the blood samples, which is the most widely used test for the serological diagnosis of parvovirus infection and is based on the property of parvovirus to agglutinate red blood cells of some species (Fujisaki et al., 1982). If the animal's serum contains antibodies against the virus, this hemagglutinating capacity is blocked, as the antibodies bind to the viral antigens and prevent them from adsorbing to the red blood cells.

For the serological diagnosis of leptospirosis infection, microserum agglutination analysis was performed at CEDISA (Diagnostic Center for Animal Health, internal methodology), also in Concórdia – SC. This analysis is based on the ability of the anti-leptospira antibodies produced by the animal to agglutinate specimens of leptospires belonging to various serovars in a liquid medium. The final titer obtained from each sample corresponds to the highest dilution of serum that causes 50% or more agglutination of leptospires. The samples were tested for nine strains of leptospires: *L. hardjo, L. canícola, L. gryppotyphosa, L. Bratislava, L. icterohaemorrhagiae, L. pomona, L. autumnalis, L. wolffi e L. tarassovi*.

2.2.5. Quantification of IgG and IgA

ELISA analyzes were performed to quantify the concentrations of IgG, IgM and IgA immunoglobulins in colostrum and milk. Before analysis, colostrum samples were centrifuged (1300 x g at 4 °C for 20 min) to remove fat and diluted with a suitable diluent (50 mM Tris buffer, 0.14 M NaCl, 1% BSA and 0.05% Tween 20). ELISA reagents were obtained from Bethyl Laboratories (Montgomery, TX, USA). Briefly, 100 μL of colostrum sample or standard solution was added to each pit and incubated at room temperature for 1 h, then washed 4 times with the wash buffer. The concentrations of IgG, IgM and IgA in the standard solutions were 333.3, 111.1, 37, 12.3, 4.1, 1.37 and 0 ng/mL. All samples were analyzed in duplicate.

After that, 100μL of anti-IgG, anti-IgM or IgA were added and incubated at room temperature for 1 h and washed 4 times with the wash buffer. Then, 100 μL of horseradish peroxidase (HRP) was added. The plates were incubated for 30 min at room temperature and washed 4 times with the wash buffer. TMB substrate (3, 30, 5, 50-tetramethylbenzidine) was added to the plates and the plates were incubated for 30 min in the dark. The reaction was terminated with the addition of 100 μL of stop solution. Plates were read on a microplate reader (Thermolab System, MRX Revelation, Chantilly, VA) at 540 nm. Results were obtained in ng/ml but expressed in mg/ml after appropriate correction of the dilution factor.

2.2.6. Measurement of cell viability

Colostrum and milk cell suspensions were counted in an automated Coulter counter (Orflo Moxi Z, USA) and analyzed on a Neubauer hemocytometer by a single operator. Each sample was mixed with a 0.4% trypan blue solution (Sigma Chemical Co. Germany) in a ratio of 1:2 (V/V). Cell concentration corresponded to the average of all four sets of squares evaluated, considering the Neubauer chamber volume and dilution. Trypan blue-stained cell counts were used to determine the concentration of non-viable cells. The proportion of non-viable cells was calculated based on the number of cells stained with trypan blue (non-viable) compared to the total number of cells.

2.2.7. Cell preparation for flow cytometry

Antibodies produced against porcine leukocyte antigens were purchased from BioRad Serotec (Oxford, UK), and stabilizing fixer (FACSLyse) and compensation beads were purchased from BD (North Ryde, Australia). Flow cytometry buffer was prepared in PBS (Phosphate-Buffered Saline) supplemented with heat-inactivated FBS (Stain Buffer; 2% V/V), bovine albumin (2% W/V, Sigma-Aldrich), and sodium azide (0.01% W/V, Sigma-Aldrich). Antibodies were selected according to Forner et al. (2021). Despite the high homology for some orthologous proteins, there are still uncertainties about their nomenclature. For these clusters, we name them Swine Workshop Clusters (SWC) and their CD-marking orthology is in parentheses.

Colostrum and milk cells (suspended in a flow cytometry buffer at approximately 1×10^6 cells/mL) were incubated for 30 min at room temperature with a specific mAb cocktail. Our panels were designed for a four-color cytometer to assess populations of T and B lymphocytes, macrophages, and granulocytes. The following fluorochrome-conjugated mAbs were used: panel A): panel 7-AAD (BD Biosciences); panel B): FITC-granulocyte (clone 6D10), RPE-CD79a (clone MB-1), PE-Cy7-CD3 (clone PPT3), APC-macrophages (clone BA4D5). To evaluate the nonspecific fluorochrome staining, control isotypes for anti-IgG1, anti-IgG2a and anti-IgG2b were introduced in the preliminary procedure to configure the photomultiplier and technical parameters of the instrument. The antibody dilution for the experiment was established by previous titration.

For intracellular staining panels, cells were resuspended in Cytofix/Cytoperm solution (BD Biosciences) and incubated for 20 min. The samples were then washed twice with BD Perm/Wash (BD Biosciences) to keep the cells permeabilized and make it easier to stain CD79a (the epitope recognized by the mAb is located in the cytoplasmic domain) and CD3 (the PPT3 clone recognizes an epitope extracellular and intracellular in CD3) in the subsequent incubation.

After staining, cells were centrifuged (400 x g at 10 °C for 5 min) and the pellet was washed once with 1 mL of flow cytometry buffer followed by centrifugation (400 x g at 10 °C for 5 min). Cells were resuspended in 300 μL of stabilizing fixative and transferred to a plate, and samples were analyzed by flow cytometry within 2 h.

Cytometry was performed on an Accuri C6 cytometer (BD Biosciences). Fifty thousand events were analyzed (based on FSC and SSC) using Accuri C6 plus software (Becton Dickinson). Compensation beads were used for each antibody in a different reading channel to establish compensation settings. The side scatter (SSC) was set at 8000 gating.

2.2.8. Mitogenic assay

Mice cells of the IEC-6 cell line were suspended (1×10^6 cells/mL) in DPBS and labeled with CFSE (2.5uM; Molecular Probes, USA) for 10 min at 37°C. The labeling process was stopped by adding five times the volume of RPMI 1640 containing 10% FBS (RPMI-SFB) followed by incubation for five minutes on ice, protected from light. The cells were washed twice with 20 ml of RPMI-SFB and then suspended in the same medium. IEC-6 cells were plated in 6-well plates (1×10^6 cells/well), allowed to adhere for 18 h and then washed twice in Hank's balanced salt solution (HBSS, Sigma-Aldrich).

The medium was then changed to 1 mL of serum-free DMEM and cultured with colostrum (100 μL). As a positive control, two pits received the cells with FBS (100 μL), and the cell culture without stimulants represented the negative control (untreated). Cells were cultured for 48 h at 37 °C under 5% CO2. Cells (1×10^6) were transferred to 7-AAD labeled flow cytometry tubes. Samples were tested in triplicate. A total of 50,000 events per tube were acquired on the flow cytometer and analyzed. Cells were recovered after 48 h of cultivation and evaluated for CFSE staining intensity.

The percentage of proliferated IEC-6 cells was determined by CFSE dilution and the geometric mean values of the triplicates stimulated with colostrum were calculated and divided with the geometric mean values of the medium control triplicates to obtain the stimulation index.

2.2.9. Reproductive and productive performance

In addition to blood analyses, birth data were collected from all sows, with total births, live births, stillbirths (animals with perfect external development, but stillborn) and mummified piglets (piglets whose lives were interrupted between the days 35 and 90 of gestation and are born with the appearance of a mummy), to evaluate the performance of the females at farrowing.

2.2.10. Statistical analysis

The collected data were initially divided into qualitative and quantitative. Qualitative data (titration) were submitted to chi-square analysis at 5% significance. Quantitative data were tested for normality using the Anderson-Darling test. IgA, IgG, IEC proliferation, macrophages, granulocytes and B lymphocytes from colostrum, IgM from milk, and total births were the parameters indicated as normal by the Anderson-Darling test ($P < 0.05$). For normal data, analysis of variance was performed, and for the other non-normal quantitative parameters, the Kruskal-Wallis analysis was performed, both at 5% significance. All analyzes were performed in statistical software Minitab 18® (Minitab, Inc. State College, PA).

3. Results

IEC 6 cells stimulated with colostrum from gilts that received β-glucans supplementation showed significantly higher mitogenic activity ($P < 0.008$) than cells stimulated with colostrum from non-supplemented gilts (Fig. 2).

The supplementation of β-glucans for sows did not affect the rates of sows that presented antibodies against Parvovirus ($P > 0.05$) in all blood collections from both experiments, as well as the titers observed ($P > 0.05$). Against Leptospira spp., in the first experiment, the sows showed antibodies only after the second dose of the vaccine, at insemination and at 85 days of gestation, while in the second one, only the sows showed antibodies and only at the end of lactation. Both the rates of positive and negative animals and the titers observed were not affected by the treatment ($P > 0.05$; Tables 2 and 3).

There was no negative effect of the β-glucans on the reproductive and productive performance of the sows and their litters ($P > 0,05$; Table 4).

Trypan blue exclusion staining, and microscopic assessment of viability were performed immediately after colostrum collection and at subsequent times, according to flow cytometry analysis. The colostrum samples evaluated were rich in cells (5–6 × 10^6 for gilts), and their cell viability was greater than 92%. A summary of the cellular components in colostrum from gilts and sows is shown in Table 5. Supplementation of β-glucans for sows did not change the cellular immune composition of their colostrum and milk ($P > 0.05$), the main types of immune cells were granulocytes (neutrophils 34–37%) predominantly followed by T lymphocytes (CD3+ 28–29%), B lymphocytes (CD79a+, 14–15%) and macrophages (9–10%). The population of phagocytic cells in mammary secretions consisted of neutrophils and macrophages.

There is an overall increase of immune cells in the colostrum and milk of sows from β-glucan supplemented group, however, the increase was statistically significant only in IgA concentration in colostrum, and there was a decrease in IgM concentration in colostrum of sows with the β-glucan supplementation in the diet ($P < 0,05$). The level of IgA in the milk of the sows was also significantly higher in the β-glucan supplemented group ($P < 0.05$; Table 5).

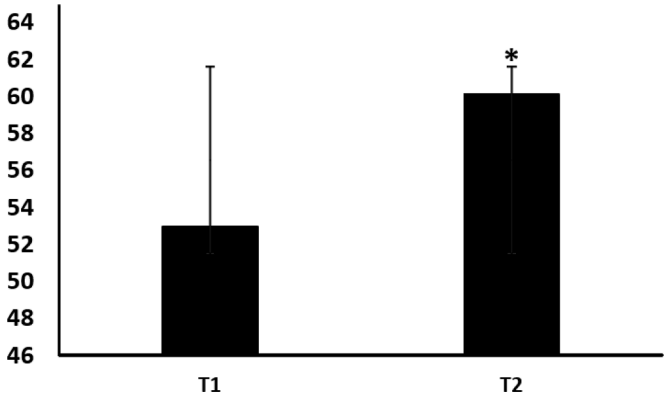

Fig. 2. Mitogenic activity (%) of sow colostrum on intestinal epithelial cells (IEC-6). Data are expressed as mean percentage (± SEM) of cell viability calculated relative to untreated cells ($n = 5$ replicates per treatment). *$p < 0.05$. T1: No Beta-glucan; T2: With Beta-glucan.

Table 2
Rates of first parity sows, with (TBG) and without (CON) supplementation of β-glucans in the diet in the adaptation, gestation and lactation periods, which presented or not antibodies against parvovirus and Leptospira spp. in seven different periods, and of their piglets in experiment 2.

Periods	Treatments	[1]Negative %	[2]Positive %	P-value
Parvovirus				
Experiment 1 - Adaptation				
Before 1st vaccine	CON	56.25	43.75	0.719
	TBG	62.50	37.50	
After 2nd dose of the vaccine	CON	37.50	62.50	0.508
	TBG	37.50	62.50	
Insemination	CON	25.00	75.00	0.233
	TBG	46.15	53.85	
d 85 of gestation	CON	23.08	76.92	0.658
	TBG	30.77	69.23	
Experiment 2 – Gestation and Lactation				
Before 2nd vaccine	CON	20	80	0.528
	TBG	10	90	
Farrowing	CON	20	80	0.592
	TBG	11.10	88.90	
Weaning	CON	10	90	1.053
	TBG	0	100	
3 day-old Piglets	CON	5.88	94.12	0.669
	TBG	3.57	96.43	
Leptospira spp.				
Experiment 1 - Adaptation				
Before 1st vaccine	CON	100.00	0.00	–
	TBG	100.00	0.00	
After 2nd dose of the vaccine	CON	93.75	6.25	–
	TBG	100.00	0.00	
Insemination	CON	81.25	18.75	0.811
	TBG	84.62	15.39	
d 85 of gestation	CON	71.43	28.57	0.662
	TBG	78.57	21.43	
Experiment 2 – Gestation and Lactation				
Before 2nd vaccine	CON	100.00	0.00	–
	TBG	100.00	0.00	
Farrowing	CON	100.00	0.00	–
	TBG	100.00	0.00	
Weaning	CON	50.00	50.00	1
	TBG	50.00	50.00	
3 day-old Piglets	CON	100.00	0.00	–
	TBG	100.00	0.00	

[1] Animals that did not present a positive serological result for parvovirus or Leptospira spp. [2] Animals that tested positive for parvovirus or Leptospira spp. Probability level at 5%.

4. Discussion

The initial hypothesis of this study, based on the literature (Kim et al., 2019; Vetvicka et al., 2020; Vries et al., 2020), was that the inclusion of β-glucans in the diet of first parity sows would demonstrate an increasing effect on the vaccine response of these sows, which could also be passed on to their piglets through colostrum and milk. The results of this work showed that this inclusion in the gestation period increased the IgA concentration and the proliferation rate of intestinal epithelial cells (IEC) from colostrum and milk, but there was no effect on vaccine response, neither on sows nor their piglets to the pathogens Leptospira spp. and Parvovirus, with supplementation during gestation and lactation or during the adaptation phase.

Regarding the results for Parvovirus, which is responsible for reproductive disorders in sows, such as an increase in the stillbirths rate, mummifications, embryonic death and sow infertility (Streck et al., 2020), the animals inside the farm probably will have contact with the field virus during their lifetime, generating a greater and longer-lasting immune response, while vaccination generates a milder response, which

Table 3
Titers observed for the Hemagglutination Inhibition (HI) tests for parvovirus and microserum agglutination for Leptospira spp. from first parity sows, receiving (TBG) or not (CON) β-glucans in the diet in the adaptation, gestation, and lactation periods, in seven blood collections in different production periods and from the piglets of the experiment 2 sows.

Parvovirus	COM	TBG	P-value
Experiment 1 - Adaptation			
Before 1st vaccine	1024	2048	0.506
After 2nd dose of the vaccine	4096	4096	0.935
Insemination	2048	4096	0.305
d 85 Gestation	4096	4096	0.676
Experiment 2 – Gestation and Lactation			
Before 2nd vaccine	4096	4096	0.454
Farrowing	4096	4096	0.699
Weaning	4096	4096	0.698
3 day-old Piglets	4096	6144	0.702
Leptospira spp.			
Experiment 1 - Adaptation			
Insemination	100	150	0.221
d 85 Gestation	200	100	0.307
Experiment 2 - Gestation and Lactation			
Weaning	100	100	0.513

Probability level at 5%. 16 samples per treatment in experiment 1, and 10 samples per treatment in experiment 2.

Table 4
Reproductive performance of sows at first parity, receiving (TBG) or not (CON) β-glucans in the diet during the adaptation, gestation, and lactation periods.

	CON	TBG	P-value
Experiment 1 - Adaptation			
Total born, n	15.39	16.16	0.225
Born alive, n	14.02	14.84	0.234
Stillbirths, n	0.95	0.66	0.482
Deaths, n	2.02	1.95	0.972
Mummifieds, n	0.34	0.36	0.515
Experiment 2 – Gestation and Lactation			
Total born, n	14.85	15.90	0.274
Born alive, n	14.45	15.10	0.280
Stillbirths, n	0.15	0.45	0.207
Deaths, n	2.25	1.80	0.534
Mummifieds, n	0.25	0.29	0.941

Probability level at 5%. 16 samples per treatment in experiment 1, and 10 samples per treatment in experiment 2.

Table 5
Immunoglobulins and concentration of macrophages, granulocytes and B and T lymphocytes from colostrum and milk of first parity sows, receiving (TBG) or not (CON) β-glucans in the diet.

Colostrum	CON	TBG	SEM	P
IgA mg/mL	10.969	12.636	0.372	0.006
IgG mg/mL	79.360	82.890	3.300	0.461
IgM mg/mL	6.949	6.294	–	0.002
Macrophages mg/mL	9.066	10.760	0.792	0.162
Granulocytes mg/mL	34.171	37.000	1.042	0.073
Lymphocytes B mg/mL	14.574	15.083	0.539	0.513
Lymphocytes T mg/mL	28.245	29.455	–	0.450
Milk				
IgG mg/mL	15.192	11.796	–	0.806
IgA mg/mL	10.763	13.452	–	0.008
IgM mg/mL	4.409	4.458	0.168	0.839

SEM: Standard error of the mean.

reduces clinical manifestations, but does not prevent infection, and must be reinforced throughout the animal's life (Streck et al., 2020). That's why β-glucans has been used as vaccine boosters, being able to reduce the number of times the same vaccine must be applied and even the number of vaccines (Vetvicka et al., 2020), but this effect was not evaluated in the present study.

Leptospira spp., like parvoviruses, can cause reproductive problems in sows and the infection can be passed to the litter in the case of pregnant sows (Ellis, 2014). It is important to note that in this study, only a few sows and none of the piglets tested positive for leptospirosis, and all sows were vaccinated. Furthermore, among the positives, some had leptospira serovars not included in the vaccine, and there were persistent post-vaccination agglutinin titers for four months after the application of commercial vaccines (Dobson and Davos, 1975), which may indicate that the positive ones were infected in the environment. The vaccine applied against leptospirosis is the same used against parvovirus, and the results indicate that it was efficient in inducing an immune response only in the second case.

Vries et al. (2020) also evaluated the vaccine response of piglets supplemented with β-glucans to the Salmonella vaccine. They found an increase in the titer of antibodies against Salmonella, but the addition of β-glucans had no effect on this parameter. The answer they found for this result is that the vaccine was very efficient at inducing an immune response, thus, the inclusion of β-glucans in the diet had no noticeable effects, which may also have happened in the current work. The vaccine against parvovirus and leptospirosis may have induced a high immune response in sows to parvovirus and the effect of the inclusion of β-glucans may have been minimal. And for leptospirosis, as there was no effect of the vaccine, the diet had nothing to act on.

Antibody research in unvaccinated gilts (before the first vaccine) showed that most animals were negative, with HI > 8 titers. However, lower titers were also found, confirming the viral presence with a lower degree of infection in some gilts. Others had anti-parvovirus antibody titers of 256, 512 and 1024, showing that the virus is actively circulating in the farm. After vaccination, there was an increase in anti-parvovirus antibody titers in gilts, these titers were higher than those considered protective in the literature, 1:80 (Brown et al., 1987; Barcellos et al., 1998; Sobestiansky et al., 1999). Finally, it is known that the level of neutralizing antibodies to parvovirus increases with the number of vaccinations. However, the level of these antibodies can remain stable or decrease due to a possible saturation of the immune response due to repeated and short gaps between antigenic stimuli, which is also called "immunological paralysis" (Jin et al., 2019).

One of the factors that may have caused the vaccine response of the animals not to be affected by the treatments was the time of exposure to the product before the first vaccine of the gilts, which was five days because it was a commercial farm where the gilts could not go long without being vaccinated. The β-glucan product manufacturer recommends at least 15 days of supplementation before exposing animals to vaccination for greater product efficiency. At the beginning of the tests, this information was still not accurate and, also because of that, this time was not respected.

In addition to the time of inclusion of the product in the diet before the first vaccination, the concentration of the product may also have been insufficient, as despite being greater than the amount used for piglets (108 g/ton in the work by Kim et al. (2019) and 50 – 300 g/ton in de Vries et al. (2020), may have been insufficient for gilts, as there was no other works found by the authors that tested the same product to this specific production phase. Thus, this work is even more important for bringing findings that may guide other researchers in the search for the best use of beta-glucans.

Piglets do not receive antibodies from sows before birth because of the physiology of the swine reproductive system, therefore, colostrum is most responsible for providing the immunity that the newborn piglet needs. In first parity sows, the amount of B and T lymphocyte cells and IgG in colostrum is lower than in multiparous sows (Forner et al., 2021). Hence the interest in working with first-parity sows, an increase in the immune cells of colostrum and milk from these sows may have a more significant effect than it would have in multiparous sows, precisely because of their greater limitations.

IgA is the most abundant immunoglobulin in animals and its main role is to protect mucosal surfaces against infectious microorganisms. It is found in greater amounts following immunization of animals (Sousa-Pereira and Woof, 2019). The IgA concentration in the colostrum and milk of the sows from the second experiment was higher in those that were receiving β-glucans supplementation in the diet, indicating that this additive was able to induce a greater immune response in the sow, which was passed to her colostrum and milk. This is reinforced by the proliferation rate of IECs, which was also higher in the colostrum of sows treated with the addition of β-glucans in the diet.

The intestinal epithelium represents a tissue with rapid cell turnover. An increase in healthy cell proliferation rate in the crypts results in an overall increase in the population of epithelial cells and associated increases in the height of the intestinal villi. Thus, colostrum with higher mitogenic activity or a higher proliferation rate of IECs has the potential to accelerate the maturation of the newborn piglet's gastrointestinal tract and provide piglets with better protection while maintaining the integrity of its intestinal mucosa (Soderholm and Pedicord, 2019; Li et al., 2020).

IgM is the first immunoglobulin secreted after exposure of the organism to a health challenge. In addition to its ability to neutralize pathogens, it also acts as a cell and pathogen signaler for lysis by complementary cells (Keyt et al., 2020). Its concentration was higher in the colostrum of sows treated without the addition of β-glucans, and as it is the first immunoglobulin secreted in the face of a challenge, this may have happened because the sows must have been experiencing some sanitary challenges and their immune systems were less mature compared to the sows treated with β-glucans, causing them to depend more on IgM at that first moment (Walker et al. (2020).

Due to the reproductive disorders that can be caused by parvovirus and leptospirosis (Ellis, 2014; Streck et al., 2020), data on the performance of sows at parturition were also analyzed, including total births, live births, stillbirths, and mummifications. However, these was no effect on these parameters, which is consistent with the data on sow immune response in this study and with studies such as Vries et al. (2020), who found only modest responses when including β-glucans in the diet of lactating piglets.

β-glucans can have a very positive effect on the immunity of gilts, sows and their litters, as can be seen in this work. Even with the shorter supplementation time compared to that currently recommended by the manufacturer. Because this is a new area of research, adjustments such as β-glucans concentration in the diet and supplementation timing will be required in future work to clarify the role of β-glucans as health additives in swine nutrition.

5. Conclusion

Inclusion of β-glucans at 300 g/ton in the diet of primiparous sows during the gestation and lactation periods increases the concentration of IgA in their colostrum and milk. In addition to this, colostrum from β-glucan supplemented sows was able to stimulate proliferation of intestinal epithelial cells in vitro. On the other hand, supplementation of sows' diets with 300 g/ton β-glucans in the adaptation period was not able to modulate the vaccine response against the parvovirus and Leptospira vaccine antigens, nor do they alter the reproductive performance of these sows at the first parturition.

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CRediT authorship contribution statement

M.C. dos Santos: Investigation, Data curation, Formal analysis, Writing – original draft. **K.F. da Silva:** Investigation, Visualization. **A.P. A. Bastos:** Investigation, Data curation, Formal analysis, Writing – review & editing, Visualization. **A.P. Félix:** Conceptualization. **S.G. de Oliveira:** Supervision, Conceptualization. **A. Maiorka:** Conceptualization.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved experimental animals has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from you (marleyconceicaos@ufpr.br).

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