Hematological and biochemical parameters of dairy calves submitted to pegbovigrastim administration

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ABSTRACT

This study was designed to evaluate the response of hematological and biochemical parameters submitted to pegbovigrastim administration and postchallenge with lipopolysaccharide (LPS). In experiment 1, 20 newborn Holstein calves were divided into 2 groups: the Imrestor (Elanco Saúde Animal, São Paulo, Brazil) group (IMR, $n = 10$), which received a 25 $\mu\text{g/kg}$ of body weight (BW) subcutaneous administration of pegbovigrastim, and the control group (CTR, $n = 10$), which received a subcutaneous administration of 0.9% saline solution. Blood samples were collected on d 0, 10, 12, and 14 relative to birth to analyze the biochemical and hematological parameters. Moreover, growth measurements were taken on d 0, 7, 14, 21, and 60 relative to birth. The number of total leukocytes in the IMR group increased on d 12 and 14 in comparison to the CTR group, as well as the counts of segmented neutrophils, band cells, and monocytes. No differences were observed in the other hematological, biochemical, and growth parameters. In experiment 2, 20 Holstein calves from 30 to 60 d old were divided into 4 groups: group 1 (LPS, $n = 5$) received a 0.25 $\mu\text{g/kg}$ of BW single intravenous dose of *Escherichia coli* LPS at d 0; group 2 (IMR, $n = 5$) received a 25 $\mu\text{g/kg}$ of BW subcutaneous dose of pegbovigrastim at d 1; group 3 (IMR + LPS, $n = 5$) received a 0.25 $\mu\text{g/kg}$ of BW intravenous LPS dose at d 0 and a 25 $\mu\text{g/kg}$ of BW subcutaneous dose of pegbovigrastim at d 1; and group 4 (CTR, $n = 5$) received an intravenous dose of 0.9% sodium chloride at d 0 and a subcutaneous dose of 0.9% sodium chloride at d 1. For the analysis of biochemical and hematological parameters, blood samples were collected on d -1, 0, 1,

2, 3, 4, 8, 14, and 21 relative to LPS administration. An increase in the number of total leukocytes was observed in the IMR, IMR + LPS, and LPS groups, and the IMR group remained as the highest from d 2 to 21. The levels of paraoxonase 1 were higher in the IMR group compared with all the others. The administration of pegbovigrastim in the dairy calves increased the number of circulating leukocytes, especially neutrophils, with an increase in paraoxonase 1, without altering the metabolites for the hepatic function.

Key words: dairy calf, lipopolysaccharide, pegbovigrastim

INTRODUCTION

The role of peripheral blood phagocytes in preventing infections is one of the most important nonspecific defense mechanisms of dairy calves. Neutrophils are the major immune cells that fight pathogens, and their production is regulated by granulocyte colony-stimulating factor (**G-CSF**), which induces differentiation from myeloblast to neutrophil, reduces maturation time in the bone marrow, and alters its functionality by increasing phagocytosis and cytotoxicity mediated by antibody-dependent cells and by starting oxidation (Mayadas et al., 2014; McDougall et al., 2017).

Recently, a bovine G-CSF was developed through covalent binding of polyethylene glycol (**PEG**) to G-CSF to increase its lifespan around 7 to 14 d and allow a reduction in first-pass renal clearance and proteolytic degradation (Kehrli et al., 1991; Molineux, 2003). Bovine G-CSF has been shown to significantly increase neutrophil concentration and function in peripartum cows, increase production of milk, and reduce susceptibility to clinical mastitis during lactation (Canning et al., 2017; Ruiz et al., 2017). However, no information is available regarding the effectiveness of the response to PEG bovine G-CSF on calves' immune system and their metabolic profile.

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The high incidence of diarrhea in this bovine class has been associated with a great number of infectious agents, such as the *Escherichia coli*, which is one of the most prevalent. Lipopolysaccharide is a constituent of its outer membrane capable of eliciting an inflammatory response by increasing the production of proinflammatory cytokines (Crisan et al., 2016). Neutrophils have an important role in reducing these effects through the acyloxyacyl hydrolase. This key enzyme is responsible for detoxifying lipid A, which is a component of bacterial endotoxins and is also associated with most of its toxic effects (McDermott et al., 1991; McDermott and Fenwick, 1992). Also, Reber et al. (2017), studying the role of neutrophils in LPS-induced endotoxemia in a new mouse model that allows selective and inducible ablation of neutrophils, found that neutrophil myeloperoxidase enhances the innate host resistance to the toxic effects of LPS, once the group of mice with neutrophil depletion had an increased mortality and were more susceptible to endotoxemia at a 10 times reduced dose of LPS compared with the control group.

We proposed that subcutaneous administration of pegbovigrastim at 25 µg/kg of BW may reduce the negative effects on the liver function caused by an endotoxemia, which was mimicked by an LPS challenge in our study; also, it would not represent a metabolic challenge capable of impairing calves' health and growth. Thus, the aim of our study was to evaluate the short-term effects of the administration of PEG bovine G-CSF (pegbovigrastim) on circulating leukocyte counts and plasma biochemical markers, including the response to an inflammatory challenge with LPS.

MATERIALS AND METHODS

The experiments were approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (no. 9382). Two studies were conducted in 2 different commercial properties in the south of Rio Grande do Sul: 31°44'S, 52°28'W (experiment 1) and 32°16'S, 52°33'W (experiment 2). In both experiments, the immunostimulating PEG bovine G-CSF (pegbovigrastim, Imrestor, Elanco Saúde Animal, São Paulo, Brazil) was administered subcutaneously at a dose of 25 µg/kg of BW.

Animals and Experimental Groups

Experiment 1. Twenty newborn Holstein calves from a commercial farm were assigned to this experiment. The calves were housed in individual stalls, and only those with total plasma protein (**TPP**) levels above 5.5 g/dL at 12 to 24 h after colostrum intake

and without clinical changes until d 10 of life were considered for entry in the experiment. The animals received milk twice a day (0800 and 1600 h), totaling 4 L per day, and water and concentrate were available ad libitum, being supplied at up to 3% of live weight, adjusted weekly according to NRC (2001).

The calves were randomly assigned into 2 groups: the Imrestor group (**IMR**, n = 10), with an average birth weight (kg) of 41.70 ± 1.94 , which received a 25 µg/kg of BW s.c. administration of pegbovigrastim; and the control group (**CTR**, n = 10), with an average birth weight (kg) of 42.05 ± 1.94 , which received the placebo (0.9% saline solution). Both administrations were performed at d 10 of life.

Experiment 2. Twenty Holstein calves aged between 30 and 60 d old from a commercial farm were assigned to this experiment. The animals were housed in individual stalls, and they were enrolled into 4 groups: group 1 (LPS, n = 5), which received a single intravenous dose of 0.25 µg/kg BW of *Escherichia coli* LPS (Sigma-Aldrich, St. Louis, MO) at d 0; group 2 (IMR, n = 5), which received a 25 µg/kg of BW s.c. dose of pegbovigrastim at d 1; group 3 (IMR + LPS, n = 5), which received a 0.25 µg/kg of BW LPS dose at d 0 and a 25 µg/kg of BW pegbovigrastim dose at d 1; group 4 (CTR, n = 5), which received an i.v. placebo dose of 0.9% NaCl at d 0 and another subcutaneous dose of saline at d 1. All the doses were calculated according to the animals' weights.

Clinical Evaluations

The animals were monitored daily, and in the case of clinical symptomatology manifestation, a clinical examination was performed. The main vital signs analyzed were heart rate through auscultation of the cardiac area; respiratory rate by pulmonary auscultation; body temperature, measured by insertion of a mercury thermometer into the rectal ampulla; mucous membrane color; and capillary refill time evaluated by compressing the oral mucosa, considering the physiological parameters proposed by Feitosa (2000).

Blood Tests

Blood samples were collected by jugular vein puncture using the vacutainer system (Vacuplast, Shandong, China). The samples were collected in 2 tubes: one containing EDTA to perform hematological analysis and another containing silicon (clot activator) to obtain serum and perform biochemical analyses.

In experiment 1, the samples were taken at 0, 10, 12, and 14 d of age. The samples of d 0 were used only for

the addition or exclusion of the animals, whereas, in experiment 2, the samples were taken at -1 , 0 , 1 , 2 , 3 , 4 , 8 , 14 , and 21 d relative to LPS administration.

After collection, the samples in the clot activator tube from both experiments were centrifuged (Centrifuge Sirius 4000, Sieger, Parana, Brazil) at $1,800 \times g$ for 15 min at $24 \pm 3^\circ\text{C}$ to obtain the serum. Samples were then transferred to Eppendorf tubes and stored at -20°C for further analysis of albumin (**ALB**), aspartate aminotransferase (**AST**), creatinine (**CREAT**), gamma glutamyl transferase (**GGT**), TPP, and urea using commercial kits (Labtest Diagnostica SA, Minas Gerais, Brazil). In addition, the C-reactive protein (**CRP**) analysis was also performed in experiment 2. The samples analyses were performed by using an automatic biochemical analyzer Labmax Plenno (Labtest Diagnostica SA, Minas Gerais, Brazil). The globulin concentration was determined by subtracting the ALB from the TPP (Silva et al., 2008).

The analyses of the inflammatory parameters were performed at d 10, 12, and 14 in the first experiment and at d 0, 2, and 8 in the second experiment. The colorimetric method described by Jones and Mould (1984) and adapted by Schneider et al. (2013) was used to measure haptoglobin (**HP**) concentration, using a microplate reader (Thermo Plate TP-Reader, São Paulo, Brazil). Moreover, these samples were also used to analyze the serum activity of paraoxonase 1 (**PON1**) as the protocol described by Browne et al. (2007). The reading was performed using a UV-visible spectrophotometer (Femto, São Paulo, Brazil) with a wavelength of 270 nm and a reading time of 30 s. The activity of the enzyme determined by the formula Δ absorbance $\times 115 \times 4$.

The d 0 samples from experiment 1 were submitted for centrifugation in digital microhematocrit (Benfer, São Paulo, Brazil) with 1.0 -mm internal capillary tubes (Perfecta, São Paulo, Brazil) at $2,650 \times g$ for 5 min at $24 \pm 3^\circ\text{C}$. The serum of capillary tube was then read into the refractometer for TPP analysis (Atago, Tokyo, Japan). The other EDTA samples from this experiment were sent to a commercial laboratory to perform the blood count with erythrogram and leukogram evaluation.

To perform the hemogram of experiment 2, the red blood cell and white blood cell (**WBC**) counting was performed by an automatic cell counter, CC-530 (CELM, São Paulo, Brazil) using the impedance method. Initially, the samples were diluted in the automatic diluent DA-500 (CELM) at ratios of $1:500$ for WBC and $1:49,999$ for red blood cells. The leukocyte cell differential was determined by counting 100 cells in blood smears stained with Panótico Rápido (Labor-

Clin, Paraná, Brazil) and read in an optical microscope (Nikon Eclipse E200, Nikon Instruments Inc., Tokyo, Japan) by a single evaluator.

The hematocrit of each sample from the second experiment was determined in duplicate using a digital microhematocrit centrifuge (Benfer, São Paulo, Brazil) with internal 1.0 -mm capillary tubes (Perfecta, São Paulo, Brazil) subjected to 5 min of centrifugation at $2,650 \times g$ and measured with a micro-hematocrit reader. After this analysis, one of the capillary tubes was used to determine TPP by refractometry (Atago), whereas the other was placed in a water bath (Quimis, São Paulo, Brazil) for 5 min at 56°C , then centrifuged again in the same conditions for the determination of plasma fibrinogen using the method based on the precipitation of fibrinogen recommended by Jain and Jain (1993), and it was measured by refractometry.

The intra- and interassay coefficients of variation were, respectively, 1.82% and 4.76% for ALB, 3.18% and 5.33% for AST, 3.28% and 7.36% for CREA, 1.70% and 8.20% for GGT, 3.27% and 7.98% for urea, 1.95% and 5.23% for CRP, 1% and 3.14% for HP, 4.97% and 9.8% for PON1, and 2.23% and 4.93% for TPP. The maximum and minimum quantification limits of the assays were, respectively, 0.2 to 6 g/dL for ALB, 3.5 to 400 U/L for AST, 0.2 to 12 mg/dL for CREAT, 2 to 700 U/L for GGT, 2 to 300 mg/dL for urea, 2 to 100 mg/dL for CRP, 0.1 to 4 g/L for HP, 10 to 200 U/mL for PON1, and 1 to 14 mg/dL for TPP.

Growth Measurements

Growth was evaluated through ADG and some body measurements at d 0, 7, 14, 21, and 60 in the first experiment. The weight was measured with a specific weight measuring tape for large dairy breeds (Bovitec, São Paulo, Brazil), whereas thoracic perimeter, wither height, and rump width were measured using a measuring tape.

Statistical Analyses

The sample sizes in both experiments were calculated a priori: computation required sample size power analyses test (G*Power 3.1.9.2 for Windows, 2010–2018 Heinrich-Heine-Universität Düsseldorf). In the both experiments the animals were grouped according to neutrophil count (d 10 and -1 in experiment 1 and 2, respectively). The dependent variables in both experiments were ALB, AST, CREAT, GGT, TPP, urea, CRP, globulin concentration, haptoglobin, serum activity of paraoxonase, blood cells, white blood cells, hematocrit, and plasma fibrinogen. In the first

experiment the dependent variables weight, thoracic perimeter, wither height, and rump width were also included, which were measured using a measuring tape. The dependent variables were submitted for Shapiro-Wilk test ($P > 0.90$), and those considered with normal distribution had their means analyzed using ANOVA proc mixed models in the SAS statistical program (SAS Studio 3.5, SAS Institute Inc., Cary, NC). The model used was as follows: proc mixed method = reml plots = (residualpanel) $\alpha = 0.05$ covtest; class time group calf_id; dependent variable = calf_id(group) time group time $\times$ group/solution cl $\alpha = 0.05$ alphap = 0.05 covb. The GLMM and Tukey-Kramer test was used to compare the main effects, and their interactions were included in the models and the P -values less than 0.05 were considered significant. The calf_id was a nested group, which was treated as a random effect.

The precision was evaluated by 6 analyses of the same sample at the same day (intraassay precision) and at different days (interassay precision). The precision calculus was expressed as a function of the coefficient of variation. The covariance structure selected was the variance component because it achieved the lowest Bayesian information criterion value of the studied variables.

RESULTS

Experiment 1

We observed an increase in the number of total leukocytes ($P < 0.01$; Figure 1A) in the IMR group 48 h (d 12) after the administration of pegbovigrastim, and it remained high until 96 h (d 14) after treatment compared with the CTR group. At the same time, an increase in the segmented neutrophil count ($P < 0.01$; Figure 1B), band cells ($P < 0.01$; Figure 1C), and monocytes ($P < 0.01$; Figure 1D) was observed in the IMR group. No differences were observed in the other hematological and biochemical parameters analyzed ($P > 0.05$, Tables 1 and 2, respectively).

When we analyzed the growth parameters, we observed no differences between the groups ($P > 0.05$; Table 3). The ADG ($P > 0.05$; Figure 2) differed only among the evaluated days; however, no differences were observed between groups ($P < 0.01$, Table 3; Figure 2).

Experiment 2

We observed an increase in the number of total leukocytes ($P < 0.01$; Figure 3A) in the IMR, IMR + LPS, and LPS groups, and the IMR group remained with the highest number of cells 24 h after the administration of

pegbovigrastim (d 2) up to 312 h after treatment (D14). The CTR group had lower levels of total leukocytes ($P > 0.05$; Figure 3A); however, it remained stable at basal levels. The LPS group had a higher number of total leukocytes ($P < 0.01$; Figure 3A) 24 h after the administration of LPS (d 1), being higher than the other groups for this period. This pattern was observed in the same proportions by segmented neutrophils ($P < 0.01$; Figure 3B) and to a lesser extent by lymphocytes ($P < 0.01$; Figure 3D). For band cells ($P < 0.01$; Figure 3C), a significant increase of the IMR and IMR + LPS groups occurred 24 h after the administration of pegbovigrastim (d 2). No differences were observed among the groups for the other hematological parameters ($P > 0.05$; Table 4).

In the biochemical parameters, we observed that the IMR group maintained ALB levels equal to CTR, whereas the IMR + LPS group had smaller levels than the IMR group ($P < 0.01$) but equal to the CTR group. Moreover, PON1 levels were higher in the IMR group ($P = 0.02$) compared with all the others. As for the CRP levels, the CTR group differed from the LPS group obtaining lower concentrations ($P = 0.03$). For AST, the IMR group did not differ from the CTR group, and the IMR + LPS and LPS groups differed between themselves and from IMR and CTR ($P < 0.01$), whereas the IMR + LPS group had the lowest levels of this enzyme. Regarding GGT, we observed that the IMR + LPS group had the highest values ($P < 0.01$), differing from the other groups. For the levels of urea, the IMR + LPS group had the lowest values, differing from the IMR and CTR groups ($P = 0.02$). In addition, for CREAT, the IMR and LPS groups showed lower levels ($P = 0.02$), and the IMR group differed from the CTR and IMR + LPS groups but did not differ from the LPS group. The other metabolites analyzed (globulins, HP, and TPP, $P > 0.05$, Table 5) showed no differences between the groups.

DISCUSSION

The number of total leukocytes and segmented neutrophils in experiment 1 was higher in the IMR group than in the CTR group, reaching a peak at 24 h (d 2) after the administration of pegbovigrastim and decreasing 48 h after (d 3), but nevertheless maintaining a neutrophilia up to 312 h (d 14) after treatment. Similar results were found in peripartum cows receiving pegbovigrastim 7 d before calving and 24 h postpartum, in which neutrophils remained elevated for at least 7 d after the second dose (Canning et al., 2017). These results could justify the use of this drug in newborn calves because it increases the number of circulating

Figure 1. Mean ± SE of leukocytes, segmented neutrophils, band cells, and monocytes of calves evaluated on the day of treatment administration (d 10), 48 h (d 12), and 96 h after (d 14), for the control (CTR) and Imrestor (IMR; Elanco Saúde Animal, São Paulo, Brazil) groups. *Differences between groups ($P < 0.05$).

Table 1. Mean ± SE of hematological parameters of calves evaluated on the day of treatment administration (d 10), 48 h (d 12), and 96 h after (d 14), for the control (CTR) and Imrestor (IMR; Elanco Saúde Animal, São Paulo, Brazil) groups

Variable ¹	Group		P-value		
	IMR	CTR	Group	Day	G × D ²
MCHC (%)	33.26 ± 1.15	32.07 ± 1.11	0.46	0.26	0.29
Eosinophils (10 ⁹ cells/L)	0.0127 ± 0.008	0.00763 ± 0.00762	0.65	0.39	0.10
Red blood cells (10 ¹² /L)	7.83 ± 0.32	7.95 ± 0.31	0.78	0.91	0.87
Hemoglobin (g/L)	102.4 ± 6.3	99.9 ± 6.1	0.78	0.64	0.85
Lymphocytes (10 ⁹ cells/L)	4.632.14 ± 0.829.4	5.4338 ± 0.7896	0.49	0.25	0.36
RDW (%)	18.67 ± 0.63	18.12 ± 0.62	0.54	0.01	0.27
MCV (fL)	39.22 ± 0.78	39.10 ± 0.75	0.91	0.63	0.69

¹MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; MCV = mean corpuscular volume.

²G × D = group by day interaction.

Figure 2. Mean $\pm$ SE of the mean ADG of calves evaluated in the first 60 d of life, for the control (CTR) and Imrester (IMR; Elanco Saude Animal, São Paulo, Brazil) groups.

cells of innate immunity. In the early weeks of life, it could provide an advantage for some individuals during this crucial period, where the main challenges caused by pathogenic agents occur.

In the same way, in experiment 2, both groups that received PEG had a higher number of total leukocytes and segmented neutrophils than the CTR group. However, the IMR + LPS had a lower peak than the IMR group. Other studies investigating the effects of LPS on the WBC found an LPS-time interaction with neutrophils, in which this leukocyte showed an increase at 12 h after LPS administration (Yates et al., 2011). The LPS exposition 24 h before PEG administration could have modulated the leucocyte recruitment, which

could explain why the IMR + LPS group had a smaller peak of neutrophils than the IMR group. In rats, high levels of dietary LPS have been associated with the decrease of neutrophils in the lymphatic vessels, so another blood collection procedure a few hours after LPS injection and 24 h again to evaluate the response could be proposed (Chakraborty et al., 2013). Despite this, the PEG in the IMR + LPS group has shown to be able to maintain a significant effect on neutrophil recruitment, which could be considered a potential strategy to decrease the toxic effects of LPS. Recently, McDougall et al. (2017) showed that the PEG effects were not influenced by energetic status. In this sense, even in inadequate nutritional conditions, such as in a convalescent patient, PEG would be able to recruit the neutrophils, and its myeloperoxidase action could be maintained, reducing the LPS toxic effects (Reber et al., 2017).

Although the hematological PEG effects have been seen in adults, our study was proposed to evaluate the possible metabolic effects on the protein metabolism parameters because PEG induces a quick inflammatory cell release. In lactating cows, it was recently demonstrated that an acutely activated immune system uses >1 kg of glucose within 720 min to respond to an LPS challenge (Kvidera et al., 2017). Based on this, we supposed that PEG and endotoxemia combined could stimulate an exacerbated metabolic response, and this would impair the hepatic function.

However, PEG administration did not significantly alter most of the parameters related to hepatic and protein metabolism in both experiments, showing that in these conditions, PEG administration was not a metabolic challenge to these animals. On the other hand, PON1 activity was lower in the animals of experiment 1, which may be explained by the difference in the ani-

Table 2. Mean $\pm$ SE of biochemical parameters of calves evaluated on the day of treatment administration (d 10), 48 h (d 12), and 96 h after (d 14), for the control (CTR) and Imrester (IMR; Elanco Saude Animal, São Paulo, Brazil) groups

Variable ¹	Group		P-value		
	CTR	IMR	Group	Day	G $\times$ D ²
ALB (mmol/L)	0.4093 $\pm$ 0.0105	0.3882 $\pm$ 0.0105	0.15	0.26	0.13
AST (U/L)	29.63 $\pm$ 1.54	28.99 $\pm$ 1.57	0.77	0.77	0.56
CREAT (mmol/L)	0.1441 $\pm$ 0.0044	1.56 $\pm$ 0.05	0.29	0.82	0.60
GGT (U/L)	115.80 $\pm$ 29.97	178.88 $\pm$ 30.52	0.15	0.66	0.95
GLO (mmol/L)	0.6214 $\pm$ 0.0331	0.6530 $\pm$ 0.0346	0.51	0.27	0.83
HP (μ mol/L)	5.6 $\pm$ 0.5	6.4 $\pm$ 0.5	0.33	0.62	0.95
PON1 (U/L)	28,930 $\pm$ 2,420	29,090 $\pm$ 2,240	0.96	0.14	0.23
TPP (g/L)	68.6 $\pm$ 2.1	69.2 $\pm$ 2.1	0.82	0.47	0.95
Urea (mmol/L)	4.6903 $\pm$ 0.1782	4.3989 $\pm$ 0.1815	0.26	0.28	0.11

¹ALB = albumin; AST = aspartate aminotransferase; CREAT = creatinine; GGT = gamma glutamyl transferase; GLO = globulins; HP = haptoglobin; PON1 = paraoxonase 1; TPP = total plasma proteins.

²G $\times$ D = group by day interaction.

Table 3. Mean $\pm$ SE of the development of calves evaluated at d 0, 7, 14, 21, and 60 in relation to birth, for the control (CTR) and Imrestor (IMR; Elanco Saúde Animal, São Paulo, Brazil) groups

Variable	Group		P-value		
	IMR	CTR	Group	Day	G $\times$ D ¹
Height of withers (cm)	80.11 $\pm$ 0.41	80.52 $\pm$ 0.42	0.49	<0.01	0.73
Thoracic perimeter (cm)	81.10 $\pm$ 0.48	81.42 $\pm$ 0.48	0.64	<0.01	0.99
Width of rump (cm)	21.38 $\pm$ 0.17	21.45 $\pm$ 0.17	0.76	<0.01	0.75

¹G $\times$ D = group by day interaction.

mals' age in the experiments, which was around 40 d because it was already observed that PON1 activity is lower in newborns compared with adults (Sumegová et

al., 2007). Moreover, we observed that in experiment 2, the IMR group had higher PON1 activity, which could suggest that in addition to the increased number of

Figure 3. Mean $\pm$ SE of leukocytes, segmented neutrophils, band cells, and lymphocytes evaluated 1 d before administration of LPS (d -1), on the day of LPS administration (d 0), 24 h (administration of pegbovigrastim, d 1), 48 h (d 2), 72 h (d 3), 96 h (d 4), 192 h (d 8), 336 h (d 14), and 504 h after (d 21), for the control (CTR), Imrestor (IMR; Elanco Saúde Animal, São Paulo, Brazil), IMR + LPS, and LPS groups. *Differences between groups ($P < 0.05$).

Table 4. Mean ± SE of hematological parameters of calves evaluated 1 d before administration of LPS, on the day of LPS administration, 24, 48, 72, 96, 192, 336 and 504 h after, for the control (CTR), Imrestor (IMR; Elanco Saúde Animal, São Paulo, Brazil), IMR + LPS, and LPS groups

Variable	Group				P-value	
	CTR	IMR	IMR + LPS	LPS	Group	G × D ¹
Eosinophils (10 ⁹ cells/L)	0.0925 ± 0.0745	20.2142 ± 0.0755	0.301 ± 0.0745	0.1383 ± 0.0745	0.22	<0.01
Fibrinogen (g/L)	2.2111 ± 0.2299	2.0222 ± 0.2299	1.6167 ± 0.2299	1.7333 ± 0.2299	0.25	<0.01
Hematocrit (%)	35.78 ± 0.75	34.09 ± 0.75	34.11 ± 0.75	36.31 ± 0.75	0.08	<0.01
Monocytes (10 ⁹ cells/L)	0.2408 ± 0.07476	0.393 ± 0.0758	0.538 ± 0.0748	0.4015 ± 0.0748	0.05	<0.01
Red blood cells (10 ¹² /L)	11,210 ± 420.46	10,453 ± 420.46	10,719 ± 420.46	11,557 ± 420.46	0.25	<0.01

¹G × D = group by day interaction.

Table 5. Mean ± SE of biochemical parameters of calves evaluated 1 d before administration of LPS, on the day of LPS administration, 24, 48, 72, 96, 192, 336, and 504 h after for the control (CTR), Imrestor (IMR; Elanco Saúde Animal, São Paulo, Brazil), IMR + LPS, and LPS groups, respectively

Variable ¹	Group				P-value	
	CTR	IMR	IMR + LPS	LPS	Group	G × D ²
ALB (mmol/L)	0.3762 ± 0.0075 ^{ab}	0.3867 ± 0.0075 ^a	0.3581 ± 0.0075 ^{bc}	0.3491 ± 0.0075 ^c	<0.01	<0.01
AST (U/L)	58.84 ± 2.38 ^a	62.86 ± 2.40 ^a	41.09 ± 2.37 ^c	48.42 ± 2.40 ^b	<0.01	0.13
CREAT (mmol/L)	0.0813 ± 0.0027 ^a	0.0716 ± 0.0027 ^b	0.0813 ± 0.0027 ^a	0.0751 ± 0.0027 ^{ab}	0.02	<0.01
GGT (U/L)	27.00 ± 3.04 ^b	26.12 ± 3.09 ^b	38.78 ± 3.04 ^a	24.41 ± 3.09 ^b	<0.01	0.59
GLO (mmol/L)	0.4093 ± 0.0226	0.3611 ± 0.0226	0.4002 ± 0.0226	0.4228 ± 0.0226	0.24	0.11
HP (μmol/L)	6.6 ± 0.8	7.6 ± 0.8	7.2 ± 0.8	7.5 ± 0.8	0.82	0.06
CRP (nmol/L)	5.8095 ± 0.4762 ^b	6.7619 ± 0.4762 ^{ab}	6.8571 ± 0.4762 ^{ab}	7.9048 ± 0.4762 ^a	0.03	<0.01
PON1 (U/L)	84,000 ± 6,240 ^b	105,530 ± 6,240 ^a	73,770 ± 6,240 ^b	82,030 ± 6,240 ^b	0.02	0.10
TPP (g/L)	52.3 ± 1.2	49.7 ± 1.3	50.5 ± 1.2	51.3 ± 1.3	0.52	0.03
Urea (mmol/L)	5.6427 ± 0.1798 ^a	5.6210 ± 0.1815 ^a	4.9434 ± 0.1798 ^b	5.4962 ± 0.1815 ^{ab}	0.02	<0.01

^{a-c}Different superscript letters indicate differences between groups ($P < 0.05$).

¹ALB = albumin; AST = aspartate aminotransferase; CREAT = creatinine; GGT = gamma glutamyl transferase; GLO = globulins; HP = haptoglobin; CRP = C-reactive protein; PON1 = paraoxonase 1; TPP = total plasma proteins.

²G × D = group by day interaction.

defense cells, this drug could help increase antioxidant proteins and then reduce the damage of inflammatory processes generated by reactive oxygen species. Nevertheless, this effect was not evidenced in the IMR + LPS group.

Although higher GGT levels in the IMR + LPS group were observed, which could indicate a metabolic challenge, the values of GGT for all groups were maintained among the reference values for this category (Trefz et al., 2017). Nonetheless, the GGT activity could have been influenced by colostrum ingestion until approximately 40 d of age (Knowles et al., 2000).

In experiment 1, we recorded the withers height, thoracic perimeter, rump width, and ADG to evaluate the growth of the animals (Frenau et al., 2007). The treatment did not have a significant effect on the growth parameters analyzed. Apparently, PEG administration did not impair the energetic balance of the calves, although additional studies should be conducted to study this effect. As described by Ingvarsten and Moyes (2013), an exacerbated neutrophil recruitment requires a fairly amount of energy, thus PEG administration could represent a high energy cost; however, our results suggest that PEG treatment do not impair the energetic balance of newborn calves.

CONCLUSIONS

The results of this study showed that PEG administration in dairy calves increased the number of circulating leukocytes, especially segmented neutrophils, even when the calves were challenged with LPS. An increase in PON1 was also observed but did not alter the fundamental parameters of hepatic function. These results suggest that PEG could be used to elevate immune status in dairy calves, which could improve their response to infectious agents.

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