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Probiotic effect on calves infected with *Salmonella* Dublin: haematological parameters and serum biochemical profile

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Abstract

The aim of this study was to evaluate the effect of a probiotic/lactose inoculum on haematological and immunological parameters and renal and hepatic biochemical profiles before and during a *Salmonella* Dublin DSPV 595T challenge in young calves. Twenty eight calves, divided into a control and probiotic group were used. The probiotic group was supplemented with 100 g lactose/calf/d and 10^{10} cfu/calf/d of each strain of a probiotic inoculum composed of *Lactobacillus casei* DSPV318T, *Lactobacillus salivarius* DSPV315T and *Pediococcus acidilactici* DSPV006T throughout the experiment. The pathogen was administered on day 11 of the experiment, at an oral dose of 10^9 cfu/animal (LD_{50}). Aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), urea, red blood cells, haemoglobin, haematocrit, mean cell haemoglobin (MCH), mean corpuscular volume, mean corpuscular haemoglobin concentration (MCHC), white blood cells, lymphocytes, neutrophils, band neutrophils, monocytes, eosinophils, basophils and the neutrophils/lymphocytes ratio were measured on days 1, 10, 20 and 27 of the experiment. In addition, animals were necropsied to evaluate immunoglobulin A (IgA) production in the jejunal mucosa. The most significant differences caused by the administration of the inoculum/lactose were found during the acute phase of *Salmonella* challenge (9 days after challenge), when a difference between groups in neutrophils/lymphocytes ratio were detected. These results suggest that the probiotic/lactose inoculum administration increases the calf's ability to respond to the disease increasing the systemic immune response specific. No differences were found in haemoglobin, haematocrit, MCH, MCHC, AST, urea, GGT, band neutrophils, eosinophils, monocytes and IgA in the jejunum between the two groups of calves under the experimental conditions of this study. Further studies must be conducted to evaluate different probiotic/pathogens doses and different sampling times, to achieve a greater understanding of the effects of this inoculum on intestinal infections in young calves and of its mechanism of action.

Keywords: probiotic, lactic acid bacteria, immune system, *Salmonella* spp.

1. Introduction

Salmonellosis is one of the most common diarrhoeal diseases affecting pre-weaned dairy calves. Intensive rearing systems increase infection frequency especially when there are deficiencies in hygienic practices during rearing (Frizzo *et al.*, 2012). In cattle, the most common serotypes are *Salmonella* Typhimurium, *Salmonella* Dublin and *Salmonella* Newport. Infection in young calves cannot be limited only to the intestinal tract and often septicaemia and premature death occurs. After bacteraemia, *Salmonella* spp. is primarily located in mesenteric lymph nodes, liver, spleen and gallbladder (Radostis *et al.*, 1999), which is why hepatic enzymes and metabolites are reliable indicators of

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damage caused by the pathogen. Moreover, the presence of leukopenia, neutropenia and the presence of immature cells in blood is indicative of the host defence response.

This pathogen causes morbidity or mortality, and consequently great economic losses (Brewer *et al.*, 2014). To control this infectious disease, antibiotics at sub-therapeutic doses have been used in feed for calves since 1950s, and many researchers have reported that this practice improves the calves' performance (Heinrichs *et al.*, 1995; Monticello and Rusoff, 1961), phagocytic efficiency, and decreases the faecal score (Monticello and Rusoff, 1961; Quigley *et al.*, 1997) and mortality (Berge *et al.*, 2005). However, the massive use of antibiotics in sub-therapeutic dose has undesirable consequences, such as the development of resistant bacterial populations (Langford *et al.*, 2003) and retention of antibiotic residues in meat for consumption and its products (Jones and Seymour, 1988; Seymour *et al.*, 1988), which has a direct effect on human health. The use of antibiotics as growth promotors should thus be totally eliminated in the near future (Abu-Tarboush *et al.*, 1996). As a result, the demand for non-traditional alternative treatments to control acute enteric infections has increased (Ewaschuk *et al.*, 2004).

One alternative treatment is the use of probiotics, which are defined as 'live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host' (FAO/WHO, 2001). Probiotics can inhibit pathogens by production of inhibitory compounds such as organic acids, hydrogen peroxide, bacteriocins or by competitive exclusion by adhesion to the intestinal epithelium or by nutrients (Knap *et al.*, 2011; O´Shea *et al.*, 2012; Zhang *et al.*, 2010). They can also prevent systemic infections avoiding translocation of pathogens to internal organs (Copeland *et al.*, 2009; Luyer *et al.*, 2005; Perdigón *et al.*, 2001). Some probiotic strains cause an increase in the innate and specific immune response: bacteria bind to receptors expressed on the surface of epithelial cells in the intestinal lumen and trigger a cascade of defence mechanisms, resulting in an innate and/or specific immune response (Britti *et al.*, 2006). Also, they can produce an increased mucosal and/or systemic immune response, indicating that probiotics exert a 'barrier effect' against colonic pathogens (Perdigón *et al.*, 2001). Other effects on the host such as influence on some haematological parameters and blood biochemistry of the host have also been reported (Aboderin and Oyetayo, 2006; Çetin *et al.*, 2005).

In previous studies, lactic acid bacteria (LAB) have been isolated from young calves (Schneider *et al.*, 2004). An inoculum was settled by selection of three strains with *in vitro* competitive advantages over other strains studied (Frizzo *et al.*, 2006). Probiotic properties *in vivo* of this inoculum include capacity to colonise and remain in the mouse gastrointestinal tract (Frizzo *et al.*, 2007), capacity to protect against *S.* Dublin DSPV 595T in the intestinal tract in mice (Frizzo *et al.*, 2010a), and capacity to improve growth performance in young calves under nutritional stress (Frizzo *et al.*, 2010b) and by modifying the intestinal microbiota (Soto *et al.*, 2014). Other studies have been done evaluating the effect of this inoculum with lactose. This probiotic/lactose inoculum administered to young calves showed to have beneficial effects. These included increases the *Lactobacillus* count, and can thus help to improve the health status of calves (Frizzo *et al.*, 2011) and stimulating the production of immunoglobulin A (IgA) in the jejunum (Frizzo *et al.*, 2009). Furthermore, in calves challenged with *S.* Dublin, the probiotic/lactose decreases some effects of the infection, such as the levels of haptoglobin (Frizzo *et al.*, 2010c), the injury of the internal organs (Frizzo *et al.*, 2012), the severity of diarrhoea, and more stable maintains the intestinal microbiota (Soto *et al.*, 2015).

To further confirm the effects of this probiotic/lactose inoculum, the aim of this study was to evaluate the immunological response against *Salmonella* infection and the effect of *Salmonella* infection on haematological and hepatic behaviour of young calves before and during a *S.* Dublin DSPV 595T challenge.

2. Materials and methods

Animals

A total of 28 Holstein calves (*Bos taurus*) with an average age of 5 days were used. Artificial rearing was carried out on dirt floor covered with natural grass. Each animal was confined to its individual feeder by a 3 m-long chain, with a limited space to move. Animals were weekly moved to a new site with the same characteristics and free of manure. Animals were divided into two experimental groups: the control group (CG, n=15) and the probiotic group (PG, n=13). All animals were fed with starter supplied *ad libitum* throughout the experiment and with milk replacer (4 l/d) rationed directly into the feeder twice a day. The milk replacer was reconstituted to 11% dry matter and delivered to calves at 6:00 h and at 18:00 h at a temperature of about 38 °C. Spray-dried lactose was also provided to the PG (50 g in each administration) together with the milk replacer, and 150 ml of probiotic inoculum delivered together with the milk replacer only at 18:00 h. Calves were evaluated for 27 days. Animal care was provided following the guidelines for the care and use of animals in research and teaching (FASS, 1998). This study was reviewed by the bioethics committee of the Catholic University of Córdoba.

Feed composition

The feeds used were not supplemented with antibiotics. The milk replacer (AF 80®, Asociación Cooperativas Argentinas, Buenos Aires, Argentina) contained 230 g/kg protein, 150 g/kg fat, 10 g/kg crude fibre, 10 g/kg Ca, 8 g/kg P and 8 g/kg total minerals. Lactose (Milkaut®, Franck, Argentina) contained 990 g/kg lactose. The starter (Arranque Terneros®, Asociación Cooperativas Argentinas) was formulated with the following ingredients: 180 g/kg crude protein, 29 g/kg ethereal extract, 60 g/kg total minerals, 16 g/kg Ca, 10 g/kg P and 50 g/kg crude fibre. All feed composition data were indicated in the label provided by the feed supplier.

Microorganisms

The inoculum used consisted of *Lactobacillus casei* DSPV 318T, *Lactobacillus salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T. Their accession numbers of ribosomal DNA 16S genes at GenBank were FJ787305, FJ787306, and FJ787307, respectively. The pathogen used (*S.* Dublin DSPV 595T, accession number of ribosomal DNA 16S gen at GenBank FJ997268) was obtained at a necropsy of a calf in the Animal Health Hospital (FCV, UNL, Esperanza, Argentina).

Preparation and administration of the inoculum

The bacteria were multiplied in skim milk (10%, w/v) supplemented with casein hydrolysate (5%, v/v) for 18-20 h at 37 °C. The bacterial concentration was estimated by plate count. The culture was dispersed into containers and frozen at -20 °C until use in artificial rearing of calves. Rifampicin-resistant strains were members of the probiotic inoculum, which consisted of a total dose of 10^{10} cfu/calf/d of each strain. This inoculum was administered to the calves of the PG together with the milk replacer throughout the experiment. The viability of the inoculum under freezing storage in a milk matrix during the experimental period was above the minimum level suggested (Soto *et al.*, 2009). The CG was inoculated in the same way but with 150 ml of skim milk (10%, w/v), which served as a placebo.

Pathogen inoculation

S. Dublin DSPV 595T, cultured in brain-heart infusion broth (Oxoid, Basingstoke, UK) for 18 h at 37 °C, was administered together with the milk replacer, on day 11 of the experiment to all calves of the two groups. The pathogen was given at a dose of 10^9 cfu/animal. The infective dose of *S.* Dublin DSPV 595T used in this work corresponds to LD_{50} (Soto *et al.*, 2015).

Blood count and blood biochemical profile

Blood samples (10 ml) were taken before the beginning of the experiment (day 1) and on days 10, 20 and 27 of the experiment, from the jugular vein. A part of the sample (2 ml) was processed with EDTA as anticoagulant for blood leukocyte count in a Neubauer chamber. Differential leukocyte count was performed in a microscope, from smears stained with Giemsa. We analysed the following parameters: red blood cells (RBC), haemoglobin, haematocrit, mean cell haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes, neutrophils, band neutrophils, monocytes, eosinophils, basophils, and the lymphocytes/neutrophils relationship.

Blood (5 ml) was also processed without anticoagulant to determine uraemia, aspartate aminotransferase (AST), and gamma-glutamyltranspeptidase (GGT). Urea was measured with a spectrophotometer (Wiener Lab CM 200; Wiener Lab, Rosario, Argentina) reagents through the urease test (570 nm). The liver enzymes AST and GGT were determined by enzymatic methods using Wiener Lab reagents.

Survival

Survival was measured daily and was calculated based on the proportion of live animals in each group.

Necropsies

All the animals that died following *Salmonella* challenge were necropsied at the time of death, whereas all the animals that survived the infection were necropsied at the end of the experiment. For necropsy, animals were euthananised using Euthanyle™ (Brouwer S.A., Buenos Aires, Argentina) administered under aseptic conditions. The jejunum was removed to assess production of s-IgA in mucosa.

IgA quantification in the jejunum

Jejunum samples were analysed by immunohistochemical analysis. The samples were fixed in buffered formalin (10%, v/v) for 12 h, washed with phosphate-buffered saline, processed with routine histological protocols, and embedded in paraffin (Woods and Ellis, 1994). Finally, serial sections 5 µm thick were performed with a rotary microtome, mounted on slides pretreated with 3-aminopropyltriethoxysilane (Sigma-Aldrich, St. Louis, MO, USA) and dried in an oven at 37 °C for 24 h. Immunostaining was performed following the protocol previously described by Salvetti *et al.* (2007), but using anti-bovine/ovine IgA Clon 842F9

Serotec as primary antibody. The results obtained were analysed with immunohistochemistry. The images generated with a microscope Olympus CH2 (Tokyo, Japan) were digitised using a Sony CCD-IRIS camera (Tokyo, Japan) connected to a PC. The immunostained tissues were evaluated with the digital image analyser Image Pro-Plus 3.0 (Media Cybernetics, Inc., Rockville, MD, USA). Immunohistochemistry was evaluated using a colour segmentation analysis in the tones and intensities corresponding to areas of reactivity of the chromogen used, consistent with that observed in positive controls. The area immunohistochemically marked was evaluated as a percentage of total area evaluated through colour segmentation, extracting the brown objects. The brown colour was selected with a sensitivity of 4 (maximum 5) and a mask was then applied for the permanent separation of colours. A histogram analysis was performed to determine the percentage of the marked area (Salvetti *et al.*, 2004) which corresponds to the presence of IgA.

Statistical analysis

The effect of probiotic administration was analysed in a factorial ANOVA (treatment × time) and Duncan`s test with a *P*-value set at <0.05. T-student test was performed at day 0, 10, 20 and 27 of the experiment to determine differences between experimental groups at different times. One way ANOVA was performed to evaluate the changes within each group through the trial and Duncan´s test for differences with a $P<0.05$. Statistical tests were performed using the software SPSS 11.0 for Windows (SPSS, Chicago, IL, USA).

3. Results

Survival

On day 20 (i.e. 9 days after the inoculation of the pathogen), the surviving animals (60% in CG and 53% in PG) still showed clinical signs of salmonellosis (data not shown). At the end of the study (day 27), 72 h after no more deaths were observed, most of the surviving animals (47% in PG and 47% in CG) appeared to be recovering from the acute phase of salmonellosis, showing no characteristic clinical signs of this disease (data not shown). Survival rates are shown in Table 1.

Table 1. Survival of calves at days 1, 10, 20 and 27.

	Time (days)			
	1	10	20	27
Control group				
No. of live calves	15	15	9	7
Proportion of live calves (%)	100	100	60	47
Probiotic group				
No. of live calves	13	13	7	6
Proportion of live calves (%)	100	100	53	47

Red blood cell count, haemoglobin and haematocrit

The haematocrit of PG was higher ($P=0.035$) than GC at the beginning of the experiment. Before and after the challenge, the haematocrit showed no differences between groups. No difference within each group were observed through time (Figure 1C). Haemoglobin showed no differences between groups along the experiment ($P=0.670$). Differences in haemoglobin values were found when the time factor was analysed: levels in CG increased before challenge and decreased after infection ($P=0.001$); on the other hand, levels in PG decreased at the end of the experiment ($P=0.004$) (Figure 1B). The RBC count of PG was higher than GC at the beginning of the experiment ($P=0.011$) and during the rest of the experiment, no differences were observed between groups. Time factor showed no differences in RBC count for GC ($P=0.909$), but GP decreased ($P=0.005$) after the infection with respect to the count at the start of the experiment. These differences in the behaviour of RBC between the PG and CG yielded a significant difference ($P=0.027$) in the time×treatment interaction (Figure 1A).

MCV was higher ($P=0.043$) for PG than GC after the infection (day 20). Also, differences in MCV values were found when the time factor was analysed for PG: values increased significantly ($P=0.001$) at day 20. Instead MCV in CG remained constant throughout the experiment ($P=0.105$) (Figure 2A). No differences between groups for MCH and MCHC parameters were found throughout the study. The MCH in CG remained unchanged from the beginning of the experiment until day 20 and decreased significantly ($P=0.036$) at the end of the experiment. In contrast, the PG values varied over time, but differently to those of GC. On day 20, MCH values for PG were significantly higher than those at the beginning of the experiment, and then decreased again at the end of the experiment ($P=0.008$) (Figure 2B). The time factor showed differences for MCHC which levels behaved similarly in both groups, decreasing significantly at the end of the experiment, with $P=0.01$ for PG and $P=0.05$ for GC (Figure 2C).

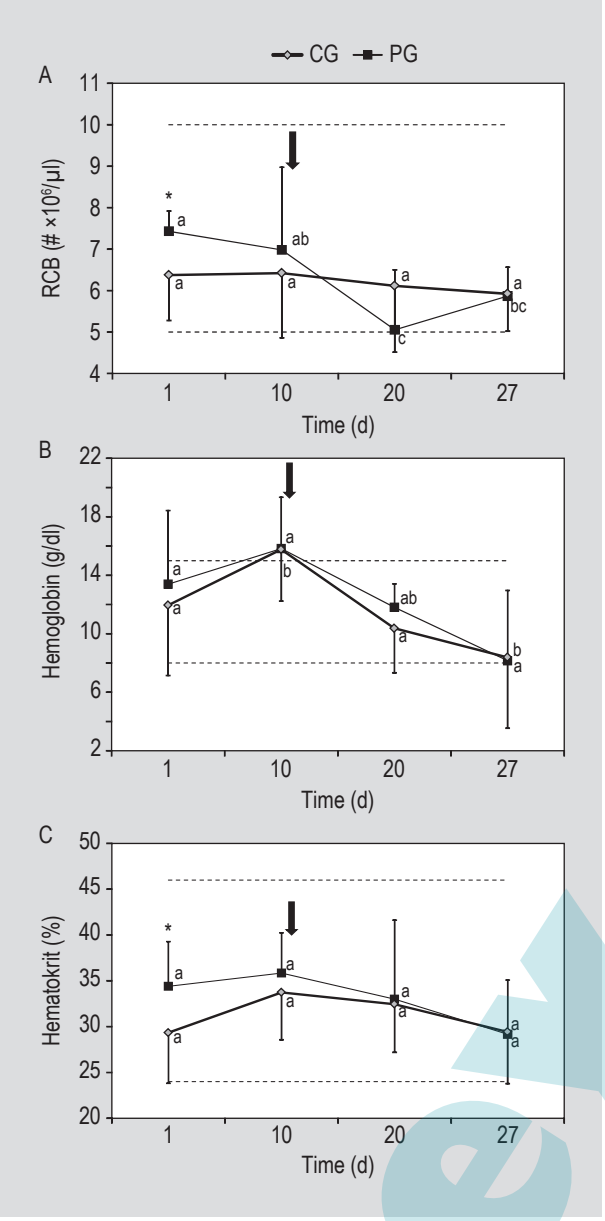

Figure 1. Determination of (A) red blood cell count (RBC), (B) haemoglobin and (C) haematocrit through time for the probiotic (PG, n=13) and control group (CG, n=15). The arrow indicates the time of inoculation with *Salmonella* Dublin DSPV 595T. Different letters mean that there are significant differences through time within each group for one way ANOVA. * Means that there are significant differences between the control group (CG) and probiotic group (PG) for the same time. Dotted lines indicate the range of normal values for each of the parameters in bovines (Meyer and Harvey, 2007).

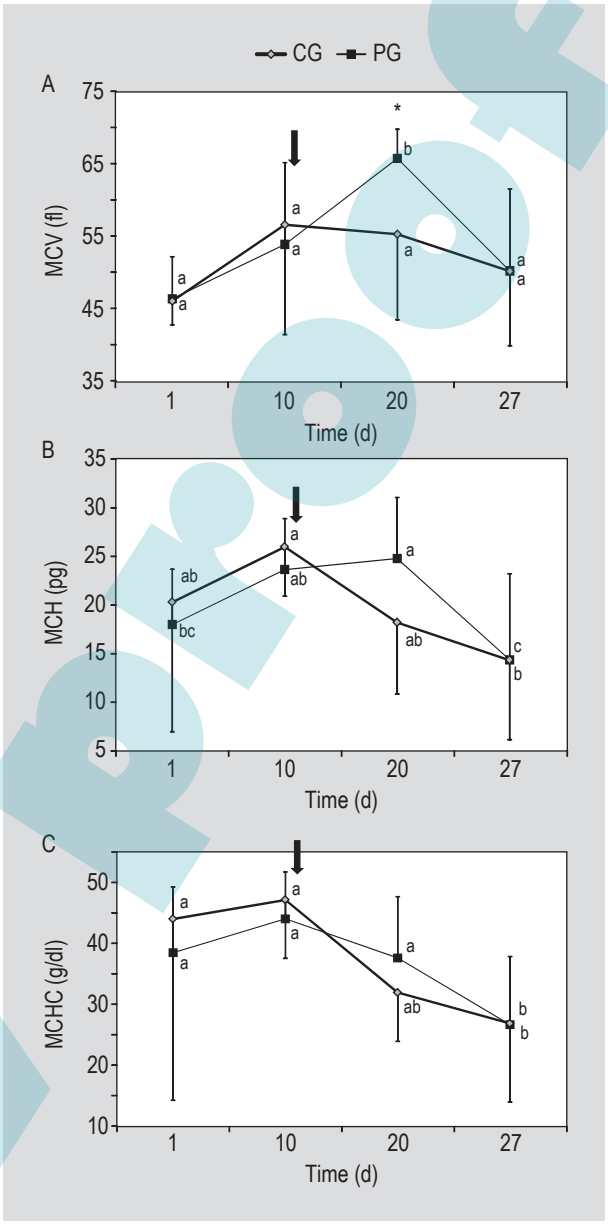

Figure 2. Red blood cells: (A) mean corpuscular volume (MCV), (B) mean corpuscular haemoglobin (MCH) and (C) mean corpuscular haemoglobin concentration (MCHC) through time for the probiotic (PG, n=13) and control group (CG, n=15). The arrow indicates the time of inoculation with *Salmonella* Dublin DSPV 595T. Different letters mean that there are significant differences through time within each group for one way ANOVA. * Means that there are significant differences between the control group (CG) and probiotic group (PG) for the same time.

Blood biochemical profile

Figure 3 shows the results of the evaluation of the blood biochemical profile. No differences in AST, GGT, and urea values were found between groups. Also, the three blood biochemical parameters showed a similar behaviour in both groups for time factor. At the beginning of the experiment, AST values were lower (P<0.001) than after the infection. Instead, at beginning of the experiment, GGT concentrations exceeded normal values in both groups. In the following days, the concentrations decreased to values that were within the normal range. Urea showed an increase in concentration (P=0.01) on day 20 (time factor), corresponding to the critical moment of the disease when both groups were analysed together. At the end of the experiment, it decreased again (Figure 3C).

White blood cell count

Differences were found for WBC counts between the groups (P=0.032) on day 10 (P=0.005) of the treatment, before infection with *Salmonella*. Despite these differences, the WBC counts remained within normal limits throughout the experiment, even after the infection. Both groups showed different behaviours over time. The WBC count of PG decreased significantly (P=0.005) at the end of the experiment while CG WBC did not change over time (P=0.640) (Figure 4A). Lymphocyte counts was higher for PG at day 1 (P=0.012) and day 20 (P=0.004), but no differences were found through time for both groups (Figure 4B). Regarding the neutrophil count no differences between groups were observed, but the groups showed different behaviours over time. In CG, the neutrophil count remained constant throughout the experiment (P=0.792), while in PG the counts decreased (P=0.012) after infection. These different behaviours showed a time×treatment interaction effect (P=0.036) (Figure 4C).

These discrepancies between PG and CG in terms of counts of lymphocytes and neutrophils, also caused differences between groups (P=0.001) for the neutrophils/lymphocytes ratio on day 20 of the experiment (P=0.028). This ratio remained constant over time in CG (P=0.072), whereas in PG the ratio decreased from day 10 until the end of the experiment (P=0.001) (Figure 5).

There were no differences between groups in band neutrophils, eosinophils or monocytes. There were no changes in the counts of these cell populations over time (Figure 4D-F). No basophils were found in any of the samples evaluated.

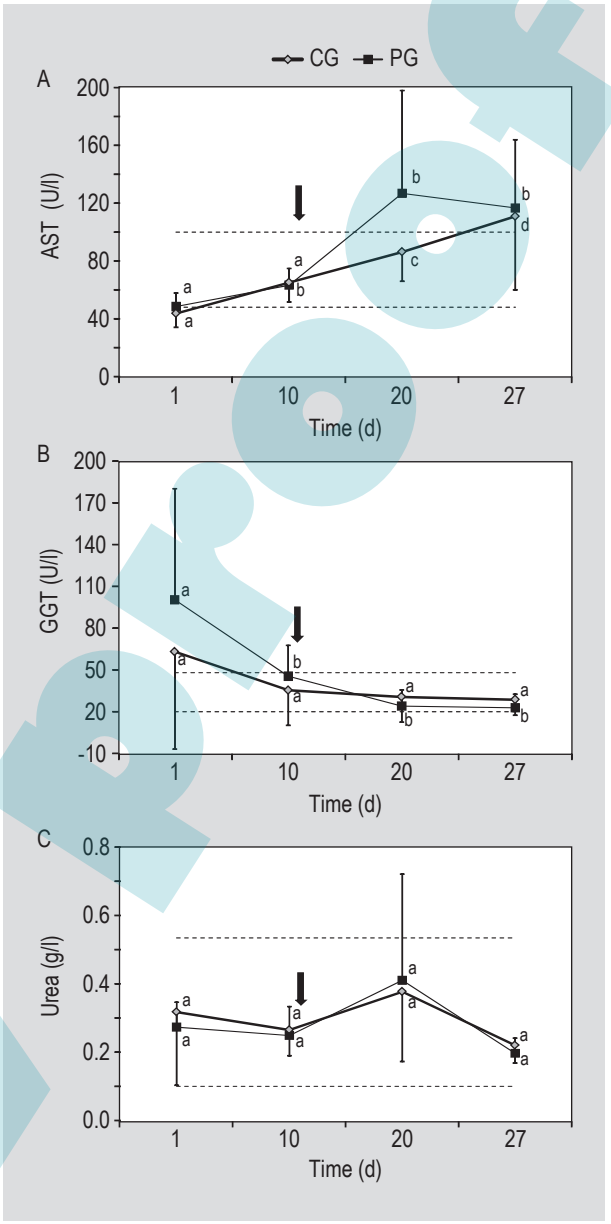

Figure 3. Concentration of (A) aspartate aminotransferase (AST), (B) gamma glutamyl transpeptidase (GGT), and (C) urea through time for the probiotic (PG, n=13) and control group (CG, n=15). The arrow indicates the time of inoculation with *Salmonella* Dublin DSPV 595T. Dotted lines indicate the range of normal values for each of the parameters in bovines (Meyer and Harvey, 2007). Different letters mean that there are significant differences through time within each group for one way ANOVA.

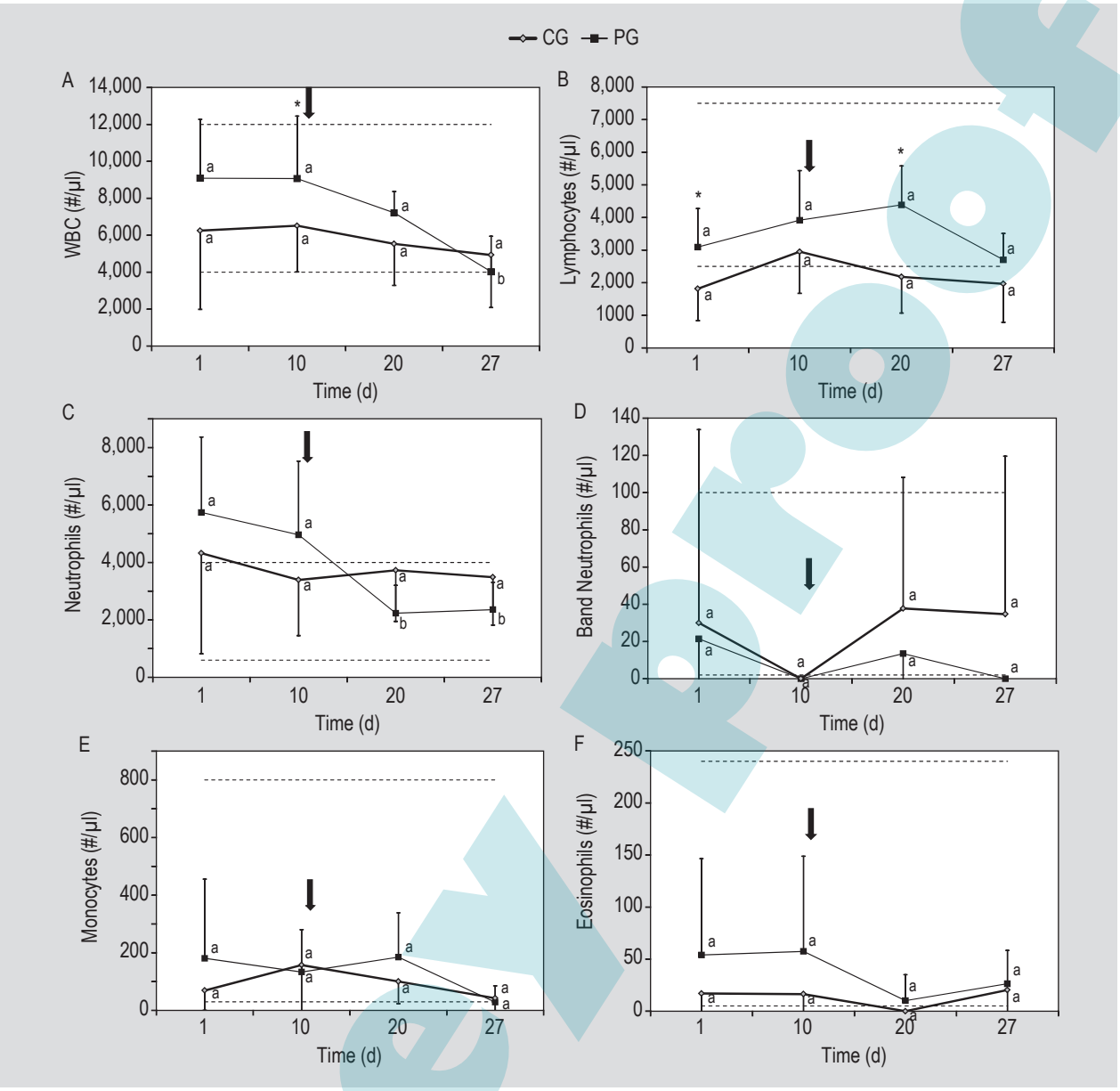

Figure 4. White blood cells: (A) white blood cell count (WBC), (B) lymphocytes, (C) neutrophils, (D) band neutrophils, (E) monocytes, and (F) eosinophils through time for the probiotic (PG, n=13) and control group (CG, n=15). The arrow indicates the time of inoculation with *Salmonella* Dublin DSPV 595T. * Means that there are significant differences between the CG (control group) and PG (probiotic group) for the same time. Dotted lines indicate the range of normal values for each of the parameters in bovines (Meyer and Harvey, 2007). Different letters mean that there are significant differences through time within each group for one way ANOVA.

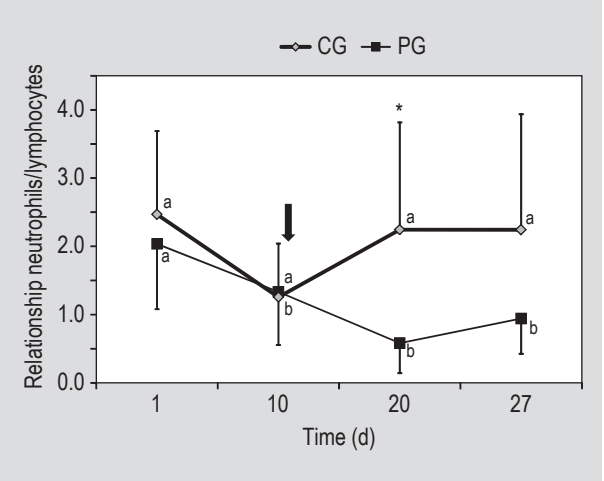

Figure 5. Neutrophils/lymphocytes ratio through time for the probiotic (PG, n=13) and control group (CG, n=15). The arrow indicates the time of inoculation with *Salmonella* Dublin DSPV 595T. * Means that there are significant differences between the CG (control group) and PG (probiotic group) for the same time. Different letters mean that there are significant differences through time within each group for one way ANOVA.

Immunostaining of immunoglobulin A in the jejunum

The cells of the intestinal mucosa and submucosa showed intense cytoplasmic staining. A specific positive reaction was found in cells and mucus. Immunostaining was also observed in the secretion of mucous glands. No differences in percentage staining was found between both groups (Figure 6).

4. Discussion

In this study, we evaluated renal, hepatic and serum biochemical parameters of calves challenged with *S.* Dublin and supplemented with a probiotic/lactose inoculum. Lymphocytes, erythrocytes and haematocrit levels showed differences between groups at the beginning of the experiment, suggesting that these differences are not related to the effect of the probiotic/lactose inoculum. According to some authors, the animals show marked changes in haematological traits due to various reasons, such as the time of first colostrum feeding, the duration and amount of ingested colostrum, whether or not the calves are born with assisted calving, and stress conditions (Coppo *et al.*, 2000; Egle and Blum, 1998; Jezek *et al.*, 2006; Tizard, 2009). Animals may also show differences in these parameters during the first days of life due to the typical changes that calves suffer in this stage of life (Knowles *et al.*, 2000).

No differences were found between the two groups of calves at day 10 in those parameters that showed differences at the beginning of the experiment. This is consistent with the proposal by Knowles *et al.* (2000) that differences in the

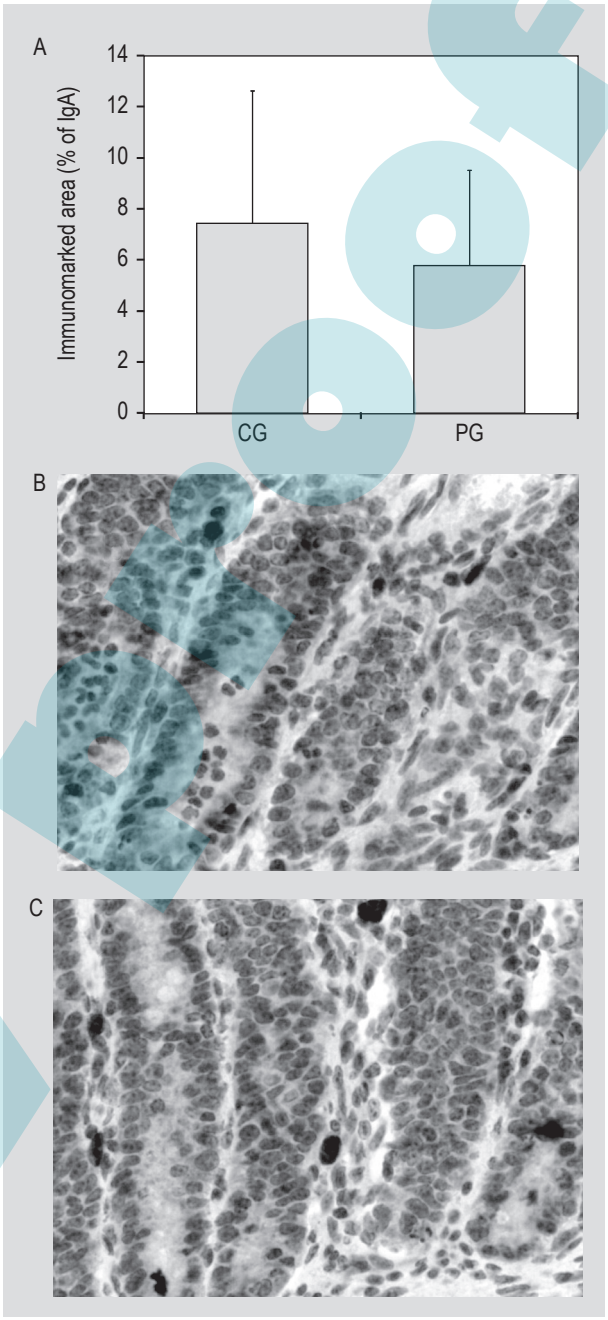

Figure 6. Immunostaining for immunoglobulin A (IgA) in the jejunum of calves affected by salmonellosis. (A) Immunomarked area (% of IgA) for the probiotic (PG) and control group (CG). Bars represent standard deviation. (B) Immunostaining for IgA in the jejunum after *Salmonella* infection for CG. (C) Immunostaining for IgA in the jejunum after *Salmonella* infection for PG.

counts of leukocyte subpopulations disappear as the animals grow. However, significant differences were observed in total leukocyte count after 10 days of treatment with the inoculum. This parameter may have been influenced by the probiotic/lactose inoculum, since other authors have observed this effect in animals supplemented with probiotic bacteria (Shu *et al.*, 2001; Strompfová *et al.*, 2006).

As regards to erythrocytes, haemoglobin and haematocrit, no differences between groups were found, just differences in behaviour over time between both experimental for erythrocytes. A modification of the MCV index of the PG calves during the acute phase of infection may be a mechanism to compensate haemolysis by increasing the size of RBC (Sharma *et al.*, 2000). Despite the differences in the behaviour of some red cell parameters, all values were within normal ranges indicating that none of the two groups suffered anaemia still during infection with *Salmonella.*

The enzyme AST showed the liver damage caused by infection, yielding higher values after the administration of the pathogen, in both groups. In contrast, GGT showed normal behaviour, characteristic of young calves. This enzyme was above the normal range at the beginning of the experiment, because it is supplied through the colostrum (Kurz and Willett, 1991; Weaver, 2000). Later, the enzyme values were normalised and not affected by the infection. Urea remained within normal ranges at all times, even after infection. The probiotic/lactose inoculum had no detectable effect on these enzymes and urea.

Neutrophils counts were decreased, in relation to that observed before *Salmonella* challenge in PG calves. In contrast, these parameters were unchanged after the infection in the CG. Moreover, differences between groups in lymphocyte counts were observed. These discrepancies seem to show a distinct immune response manifested in a difference in the neutrophils/lymphocytes rate during the acute phase of *Salmonella* infection between groups. This difference between groups during the acute phase showed a different behaviour in terms of the specific response respect of innate response. This relationship allowed standardisation of groups, cancelling the differences at the start of experiment (in lymphocytes) and allowing clear analysis of the specific and innate immune response.

It has been reported that LAB are able to activate the systemic immune response in animals (Flore *et al.*, 2010; Mizumachi *et al.*, 2009) and humans (Link-Amster *et al.*, 1994; Mañé *et al.*, 2011). The type of response generated varies and depends on many factors, such as strain, dose and timing of administration. Perdigón *et al.* (2001) observed that the strain *L. casei* administered simultaneously with the pathogen does not exert an inhibitory effect on the pathogen, and that administered during infection with *Salmonella* is beneficial for the host, in a dose-dependent manner. Mañé *et al.* (2011) observed that lower doses of probiotics 'might be useful as coadjuvant therapy to vaccinations as far as they promote acquired humoral immune responses, while higher doses might be useful to prevent infections, as they promote more immediate and unspecific cellular responses'. The probiotic/lactose inoculum did not lead to the stimulation of the mucosal immune system in the jejunum. Previous studies reflected that LAB strains produce a protective effect on the host, but not through the activation mechanism of induction of secretory IgA (Link-Amster *et al.*, 1994; Perdigón *et al.*, 2001). The immunoadjuvant capacity would be a property of the strain assayed (Vitiñi *et al.*, 2000). In addition to the selected LAB strains, as mentioned above, also the dose, timing of administration, and the time at which the amount of IgA in the mucosa is measured significantly influence levels of secretory IgA (Alvarez *et al.*, 1998; Perdigón *et al.*, 2001; Vitiñi *et al.*, 2000).

The results of this study showed that some haematological parameters in calves responded to the administration of the probiotic/lactose inoculum, especially during the acute phase of infection with *Salmonella* Dublin DSPV 595T, suggesting that this probiotic/lactose inoculum increases the calf's ability to respond to the disease. However, to determine with greater accuracy the effect of probiotics on the specific immunity, studies in humoral and cellular response should be conducted.

5. Conclusions

Daily administration of a probiotic/lactose inoculum before and after infection with *Salmonella* Dublin caused a decrease in the neutrophils/lymphocytes relationship showing a predominance of the specific systemic immune response with respect to innate response during the acute phase of infection. Further studies must be conducted to evaluate different probiotic/pathogens doses and different sampling times, to achieve a greater understanding of the effects of this inoculum on intestinal infections in young calves and of its mechanism of action.

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