


Use of sulfonamides for the treatment of bovine neonatal diarrhea: clinical and performance parameters

Utilização de sulfonamidas para o tratamento de diarreia neonatal bovina: parâmetros clínicos e zootécnicos

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ABSTRACT

This study was developed to evaluate the clinical, hematological, and performance parameters of calves affected by diarrhea caused by the bacterial enteric pathogen *Escherichia coli*, treated with three different protocols containing sulfonamides. Fourteen Holstein calves were monitored from birth to 60 days of age, and divided into 3 groups. Group 1 (n=5) animals were treated with 80 g of Kaopek[®], Ibasas, Brazil, administered orally (BM), dissolved in 160 ml of water every 24 h; Group 2 (n=4) received IM sulfadiazine and trimethoprim (Ibatrim[®], Ibasas, Brazil), and Group 3 (n=5) animals were treated with 80 g of Kaopek[®], Ibasas, Brazil, which is made up of 16 g of phthalylsulfathiazole, associated with 2.28 g neomycin sulfate, 1.6 g of pectin, and 80 g of kaolin, dissolved in 160 ml of water and administered every 24 h by mouth, in addition to sulfadiazine and trimethoprim (Ibatrim[®], Ibasas, Brazil), at a daily dose of 16 mg/kg live weight, through IM injection. During the study, the animals were clinically evaluated, and once they were diagnosed with diarrhea, feces samples were collected to identify the bacterial enteric pathogen, antibiogram, polymerase chain reaction (PCR), and coproparasitological exams. Blood samples were collected to evaluate the hematological profile, and the performance profile was monitored weekly. In the clinical examination, all calves presented a reduction in body temperature (<39.2°C) and some improvement in hydration after treatment (p=0,31). However, group 2 had a better concentration of lymphocytes and TP concerning the other animals, as well as better performance. Besides, *E. coli* was detected in 100% of feces samples. Thus, the therapeutic protocols with sulfonamides used to treat bovine neonatal diarrhea were effective in the clinical improvement of the animals. Nonetheless, the protocol with systemic therapy using sulfadiazine and trimethoprim (Ibatrim[®], Ibasas, Brazil) intramuscularly, provided better performance, with better weight gain, and body development of the animals.

Keywords: Antibiotic therapy. Calf. Disease. *E. coli*.

RESUMO

Este estudo foi desenvolvido com o objetivo de avaliar parâmetros clínicos, hematológicos e zootécnicos de bezerras acometidas por diarreia provocada pelo agente bacteriano *Escherichia coli*, tratadas com três diferentes protocolos contendo sulfonamidas. Quatorze bezerras da raça Holandês foram monitoradas do nascimento até os 60 dias de vida e divididas em 3 grupos: Grupo 1 (n=5), animais tratados com a dose de 80g de Kaopek[®], Ibasas, Brasil, por via oral (VO), dissolvido em 160ml de água a cada 24 horas; Grupo 2 (n=4) receberam sulfadiazina e trimetropim (Ibatrim[®], Ibasas, Brasil), na dose de 16mg/Kg de peso vivo, por dia, por via intramuscular (IM); Grupo 3 (n=5) 80g de Kaopek[®], Ibasas, Brasil, por VO dissolvido em 160ml de água, a cada 24 horas e com 16mg/Kg de peso vivo, por dia, de sulfadiazina e trimetropim (Ibatrim[®], Ibasas, Brasil), por via IM. Durante o estudo, os animais foram avaliados clinicamente e a partir do diagnóstico de diarreia foram coletadas amostras de fezes para a identificação do agente bacteriano, antibiograma, reação em cadeia da polimerase (PCR) e exames coproparasitológicos. Amostras de sangue foram coletadas para avaliação do perfil hematológico e o perfil zootécnico foi acompanhado semanalmente. No exame clínico, todas as bezerras apresentaram redução na temperatura corporal (<39,2°C) e melhora na hidratação após o período de tratamento (p=0,31), porém

o grupo 2 obteve maior concentração de linfócitos e PPT em relação aos demais, assim como melhor desempenho zootécnico, além disso em 100% das amostras de fezes foi detectado *E. coli*. Sendo assim, os protocolos terapêuticos com sulfonamidas utilizados para o tratamento da diarreia neonatal bovina foram eficazes na melhora clínica dos animais. Porém, o protocolo com terapia sistêmica com sulfadiazina e trimetoprim (Ibatrim®, Ibase, Brasil) por via intramuscular proporcionou um melhor desempenho zootécnico, com melhor ganho de peso e desenvolvimento corporal dos animais.

Palavras-chave: Antibioticoterapia. Bezerro. Doenças. *E. coli*.

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Introduction

Calf rearing is a crucial point in milk production because of the high neonatal mortality rates diagnosed in this period and its negative effects on the future lactating cow. This represents a major challenge to dairy farming systems (Aghakeshmiri et al., 2017). Respiratory and digestive diseases are more prevalent at this time, with diarrhea the one that most affects calves in the first days of life, causing important economic losses, impairing body development and the productive potential of these animals (Aghakeshmiri et al., 2017; Berge et al., 2009).

Neonatal diarrhea causes high mortality rates, ranging from 10 to 34%, being characterized as a multifactorial disease caused by the interaction between failures in the animal's immunity, nutritional status, and infectious pressure of the environment (Feitosa et al., 2001; Renaud et al., 2018). Infectious etiologies are more frequent, and the main enteric pathogens involved are *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens*, *Rotavirus*, *Coronavirus*, *Eimeria* spp., and *Cryptosporidium* spp. (Athira et al., 2018; Blanchard, 2012; Holschbach & Peek, 2018; Lorenzetti et al., 2014). Although some authors show high economic losses and difficulty in coping with this disease, fighting the etiological enteric pathogen properly can significantly reduce its incidence and, consequently, damage to production (Berge et al., 2009; Constable, 2009). Thus, the treatment of neonatal diarrhea aims to decrease the proliferation of

the causative pathogen, together with the reduction of fluid and electrolyte loss (Constable, 2009).

Drug misuse for many decades has resulted in a wide variety of resistant microorganisms, which challenge the effectiveness of antibacterial treatments, both in animals and humans (Chouchani et al., 2018; Constable, 2009). However, Constable (2009) states that the use of antibiotics in the treatment of bovine neonatal diarrhea is necessary due to the overgrowth of commensal bacteria in the small intestine, which can cause clinical complications associated with bacteremia. Nonetheless, the use of antimicrobial agents is not always beneficial. For example, viral and parasitic diarrhea can delay the improvement process in the clinical setting. Therefore, the choice of antibiotic therapy must be justified by the identification of etiological agents, changes in animal behavior, and severe clinical manifestations, such as fever and reduced food intake (Gomez et al., 2017).

Another factor that can influence the effectiveness of the treatment is the route of drug administration. Although there is a recommendation for the use of sulfonamides orally and intramuscularly, the pros and cons of administration via different routes and the speed at which the therapeutic effects are desired must be considered (Lopes et al., 2006).

Given the above, this study aims to evaluate the clinical, hematological, and performance parameters of calves affected by diarrhea caused by the bacterial enteric pathogen *Escherichia coli*, treated with three different protocols containing sulfonamides.

Material and Methods

This study was approved by the Ethics Committee for Animal Experimentation ECAC-UFPEL (number 23110.041629/2018-03).

Location and characterization of the calves rearing system

The experiment was carried out on a dairy farm of a complete cycle, located in the south of Rio Grande do Sul, Brazil (32° 16' S, 52° 32' E). The study evaluations took place in the calves sector, where the animals were kept in an intensive system of production, under the same management conditions, housed on an elevated shed with individual pens, daily sanitized, receiving six liters of milk per day (divided into two meals), with free access to water and concentrate.

Evaluated animals and experiment design

Fourteen Holstein calves were used from their first day of age. After birth, colostrum was carried out naturally, keeping the calf with the cow for 24 h. Afterward, total plasma protein concentration (TP) was evaluated to determine the proper passive transfer of maternal immunity, considering the minimum concentration of 5.5 g/dL (Blanchard, 2012).

The calves were monitored daily from birth to 60 days of age and divided randomly into 3 groups according to the diagnosis of neonatal diarrhea. The clinical case detection was performed based on the evaluation of the animals' behavior, clinical examination, and feces score according to the classification used by McGuirk (2008), with scores 2 and 3 characterized by diarrheic feces. Thus, according to the mentioned criteria, the groups were:

Group 1 (n=5): animals treated with 80 g of Kaopek®, Ibasá, Brazil, which is composed of 16 g of phthalylsulfathiazole associated with 2.28 g of neomycin sulfate, 1.6 g of pectin, and 80 g of kaolin, administered by mouth, dissolved in 160 ml of water, every 24 h;

Group 2 (n=4): animals treated with sulfadiazine and trimethoprim (Ibatrim®, Ibasá, Brazil), at a daily dose of 16 mg/kg live weight, through IM injection. There are 40 g of sulfa and 8 g of trimethoprim in 100 ml of this product;

Group 3 (n=5): animals treated with 80 g of Kaopek®, Ibasá, Brazil, which is made up of 16 g of phthalylsulfathiazole, associated with 2.28 g neomycin sulfate, 1.6 g of pectin, and 80 g of kaolin, dissolved in 160 ml of water and administered every 24 h by mouth, in addition to sulfadiazine and trimethoprim (Ibatrim®, Ibasá, Brazil), at a daily dose of 16 mg/kg live weight, through IM injection. In 100 ml of Ibatrim®, there are 40 g of sulfa and 8 g of trimethoprim.

All evaluated treatments lasted for 5 days. The animals received support therapy BM with an electrolytic and energetic solution twice a day, according to the farm protocols, besides 1.1 mg/kg of flunixin meglumine (Flumedin®, Jofadel, Brazil), intramuscularly for 3 days.

Calves with TP <5.5 g/dL in the first 24 h of age, as well as animals with diarrheic feces and fever (temperature >39.2°C) and/or leukocytosis were excluded from this study.

Evaluated clinical parameters

Given the diagnosis of diarrhea, where the scores found in Table 1 were considered, clinical evaluations were performed on the diagnosis day (day 0) and the following days (days 3, 5, and 7). The following parameters were evaluated: heart rate (HR), respiratory rate (RR), body temperature (T°C), mucosal color, and the level of dehydration (through skin turgor), and capillary refill time (CRT) (Feitosa, 2014).

Table 1 – Stool score classification (McGuirk, 2008)

Stool score	Characteristics
1	Pasty stools
2	Pasty stools with more water and feces adhered to the perineum and tail
3	Liquid stools with fecal content adhered to the perineum and tail

Feces samples

Samples were collected on days 0 and 7 from all evaluated animals (totaling 28 samples), for score evaluations, coproparasitological exam, microbiological exam, antibiogram, and polymerase chain reaction (PCR). The microbiological culture and the antibiogram were carried out in the Regional Diagnostic Laboratory (RDL), the coproparasitological exam in the Food Microbiology Laboratory (DCTA), all located at the Federal University of Pelotas (UFPEL, Pelotas, Brazil).

The feces were placed in Petri dishes containing blood agar and MacConkey agar.

The formed bacterial colonies were evaluated for Gram stain as negative or positive (Alterthum & Trabulsi, 2015). After that, a sequence of biochemical tests was carried out: Sulfide production (H₂S), indole production and motility, TSI (triple sugar and iron), sorbitol, and inositol, aiming to analyze the bacterial metabolism, more specifically, the ability to degrade certain substrates and/or produce gases (Alterthum & Trabulsi, 2015). After the pathogen identification and characterization, the *in vitro* sensibility test was performed, antibiogram following the methodology of Quinn et al. (2005). In the isolated ones confirmed as *E. coli*, virulence genes were screened by PCR (Crăciunaș et al., 2012; Paton & Paton, 1998).

Blood samples

Blood samples were collected on days 0, 3, and 7 (concerning the diarrhea diagnosis) to evaluate the hematological profile, fibrinogen, and TP. The hematological analysis was performed in an automated analyzer (BC 2800 Vet, Mindray®) and the leukocyte differential was made with stained blood smear, after counting 100 cells, in optical microscopy (González & Silva, 2018). The plasma fibrinogen content was obtained by the heat precipitation method and by a refractometer reading. The TP concentrations were determined through commercial reagents (Labtest, Belo Horizonte, Brazil) and the sample reading was performed in a visible light spectrophotometer (BioEspectro® SP 220, Bioespectro, Curitiba, Brazil) (González & Silva, 2018).

Performance monitoring

The measurements of the thoracic perimeter were carried out weekly, using a flexible tape, while the withers height and rump width were measured with a ruler graduated in centimeters, from day 7 to 30. Still, together with the mentioned evaluations, the animals were weighed using a weight tape. However, this analysis continued to be performed weekly until 60 days, aiming to obtain the weight gain mean from birth to weaning.

Statistical analysis

Residual distribution was assessed for normality by the Shapiro-Wilk test and homoscedasticity by graphic evaluation (residual vs predictive value). The data were analyzed using the SAS Institute statistical program (SAS Institute INC., 2009) using the MIXED procedure for repeated measures, with group, days and their interaction (group * days) evaluated as fixed effects and animal being treated as a random effect. The autoregressive variance-covariance matrix was used to analyze repeated measures in clinical examination data, stool score, hematological, and performance parameters. The comparison of means was performed using the Tukey test, considering the statistically significant difference $P < 0.05$ and trend $P = 0.063$.

Results and Discussion

Although there are several enteric pathogens responsible for causing neonatal diarrhea, in this study, in 100% of the analyzed feces samples the presence of *Escherichia coli* was detected, regardless of the collecting time. The culture and biochemical tests were negative for the presence of Salmonella, and the presence of Cryptosporidium was not detected in the centrifugal sedimentation test. The viral pathogens (Rotavirus and Coronavirus) were not detected either.

From the 28 collected samples the PCR examination detected the presence of genes of hemolysin virulence (hlyA), Shiga toxin (stx1), and intimin (eae) in 39.28%, 10.71%, and 10.71% of the stool samples, respectively, especially observed in the collections of day 7. The thermostable toxin A (stA) was present in 10.71% of the collected feces. Yet 28.59% of the samples were negative for these genes as shown by Klaus et al. (2020). The found genes are characteristic of enterohemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic (ETEC), and enteropathogenic *E. coli* (EPEC), respectively, with the STEC and EHEC present in 2.7% to 50% of neonatal diarrhea, while the occurrence of ETEC can be detected in up to 30% of samples, according to what was reported

by Andrade et al. (2012). In this study, the main virulence gene found was hlyA, in 28.59% of samples. Its presence is determined by the EHEC and its action is characterized by the ability to cause a lesion in the intestinal cells, due to the pro-inflammatory changes, damaging, among others, the absorption of nutrients (Badoue et al., 2016).

The calves affected by neonatal diarrhea can have gastrointestinal disorders, reducing the nutrients intake and absorption, consequently decreasing the weight gain and causing possible future negative effects for the animal (Aghakeshmiri et al., 2017). Although the disease can bring about several harms to the animal, the use of antibiotics needs to be carefully monitored. Gomez et al. (2017) applied an algorithm in two farms, in which only calves affected by diarrhea associated with systemic signs, such as fever, were treated, and after that, some reduction in the use of antibiotics in the studied farms was observed, and an increase in the concentration of beneficial bacteria to intestinal health, especially the *Lachnospiraceae* and *Ruminococcaceae* families.

Yet, recent studies have advocated not using antibiotics for diseases caused by *E. coli*, according to what is reported by Schukken et al. (2011). However, Bashahun & Amina (2017) recommend the therapy using antimicrobials, mainly due to dehydration and loss of electrolytes caused by the disease, which can change the acid-base balance leading to a high mortality rate in diarrheic animals.

The majority of studied calves were affected with diarrhea in the first 5 days and were monitored from the first to the seventh day of treatment concerning feces score. In these evaluations, all animals had a feces score of 3 at the onset of the disease, meaning there was a likelihood to cause dehydration and blood pH imbalance, which, according to what was mentioned above, justifies the treatment (Figure 1). After using medication for five days, the scores returned to physiological conditions, except for group 3 (treated with oral + injection sulfonamides), which presented score 2 (diarrheic feces) at day 5, differing from group 1 ($p < 0.05$), which was only treated with sulfonamides by mouth. This difference between groups 1 and 3 can be related to factors that interfered in motility and intestinal absorption, such as stress and food causes, or the ones related to management (Foster & Smith, 2009). However, in the evaluations carried out on day 7, all animals treated with the three different protocols had normal feces.

According to Foster & Smith (2009), calves affected with neonatal diarrhea constantly produce watery feces, clinically characterized by different levels of dehydration, apathy, inappetence, and changes in vital signs that would

probably cause some changes in the clinical examination. In this study, although there was no statistical difference in clinical parameters, all animals had tachypnea at the moment of the diagnosis, and the RR was characterized as an important parameter since its elevation is associated with a compensatory mechanism of metabolic acidosis. Such a condition (i.e., metabolic acidosis) is commonly observed in diarrheic calves (Blanchard, 2012).

Furthermore, all animals in this study had a body temperature equal to or above 39.2°C at the moment of the diarrhea diagnosis. This parameter is considered an important criterion to define the use of antibiotics (Constable, 2009). After treatment, both RR and the rectal temperature reduced to the values considered ideal for the animal category, demonstrating the efficiency of the protocols used.

The hematological profile plays an important role in the prognosis because it demonstrates the immune response

of the animal, as well as its contribution to choose the appropriate therapy (Benesi et al., 2012; Knowles et al., 2000). All studied animals presented a difference in the lymphocyte blood levels, although the concentrations kept within the reference values (Table 2; Figure 2), there was one of this parameter in group 2 (group 1: 4798.40 ± 247.03 , group 2: 5586.00 ± 184.21 , group 3: 4808.50 ± 290.23), from the third day of treatment. Possibly, this can be related to the animals' age, because the levels of lymphocytes gradually increase according to age range, achieving the neutrophil-lymphocyte ratio which is regarded as normal for adult animals at around 15 days of age (Knowles et al., 2000).

TP is a marker that increases in infectious or inflammatory processes, as well as in dehydration conditions (Rosa et al., 2018). In this context, the blood levels of TP were higher in groups 2 and 3, a result that is related to the inflammatory process as a response to the enteric pathogen, not the dehydration, as this change was not clinically observed (Figure 3) (Foster & Smith, 2009; Rosa et al., 2018). It is important to highlight that some bacteria involved in diarrhea have certain virulence factors that cause or stimulate an acute inflammatory response in the intestinal mucosa (Foster & Smith, 2009). The other parameters did not differ among groups.

The leukocyte profile, as well as temperature, were considered as criteria of inclusion in the study. Although there was no difference among groups ($p=0,83$), Figure 2 shows that all groups had leukocyte levels above the physiological one at moment zero. From the beginning of the treatment, there was a reduction to normal levels (collections performed at days 3 and 7 concerning diarrhea diagnosis), except for group 1, which presented leukocytosis at day 7, two days after the end of treatment. Furthermore, segmented neutrophil levels above the physiological one were also observed at the onset of diarrhea, reducing to normal levels after treatment.

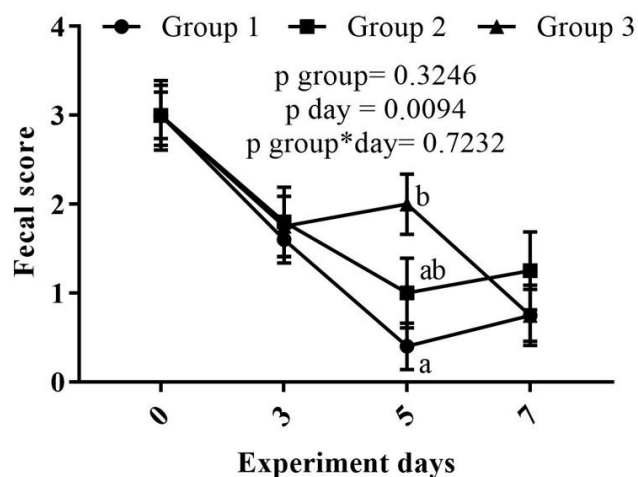


Figure 1 – Stool score of dairy heifers affected by neonatal diarrhea and submitted to different therapeutic protocols containing sulfonamides. Group 1: Animals submitted to oral administration of sulfonamides; Group 2: Animals submitted to injectable administration of sulfonamides; Group 3: Animals submitted to oral and injectable sulfonamide administration. ^{ab}Different letters indicate statistical difference between groups.

Table 2 – Blood parameters of dairy heifers affected by neonatal diarrhea submitted to different therapeutic protocols containing sulfonamides. Pelotas, Rio Grande do Sul, Brazil, 2020, at 10:00 am

Param.	Groups p value			G ⁴	D ⁵	G*D	Ref. value
	Group 1 ¹	Group 2 ²	Group 3 ³				
Leuko.	11701±2169.32	10768 ± 1068.94	11557 ± 934.55	0.83	0.09	0.90	4.000-12.000
NR	149.27 ± 45.69	151.27 ± 50.76	128.58 ± 17.09	0.85	0.07	0.86	0-120
NS	5496.60 ± 1798.36	4004.20 ± 821.42	5394.58 ± 964.65	0.49	0.06	0.87	600-4.000
Lymph.	4798.40 ± 247.03 ^a	5586.00 ± 184.21 ^b	4808.50 ± 290.23 ^a	0.02	0.01	0.98	2.500-7.500
TP	6.78 ± 0.12 ^a	7.73 ± 0.16 ^b	8.03 ± 0.10 ^b	<0001	0.27	0.70	>5.5d/dL
Fibrin.	700.00 ± 53.33	586.67 ± 38.87	716.67 ± 39.67	0.06	0.99	0.69	300-700

¹Animals submitted to oral administration of sulfonamides; ²Animals submitted to injectable administration of sulfonamides; ³Animals submitted to oral and injectable sulfonamide administration; ⁴Group; ⁵Day. Leuko.: Total leukocytes (/ mm³); NR: Neutrophil rods (/ mm³); NS: segmented neutrophils (/ mm³); Lymph.: Lymphocytes (/ mm³); TP: Total Proteins (g/dL); Fibrin.: Fibrinogen (g/dL). ^{ab}Different letters indicate statistical difference between groups. References value, Kaneko et al. (2008).

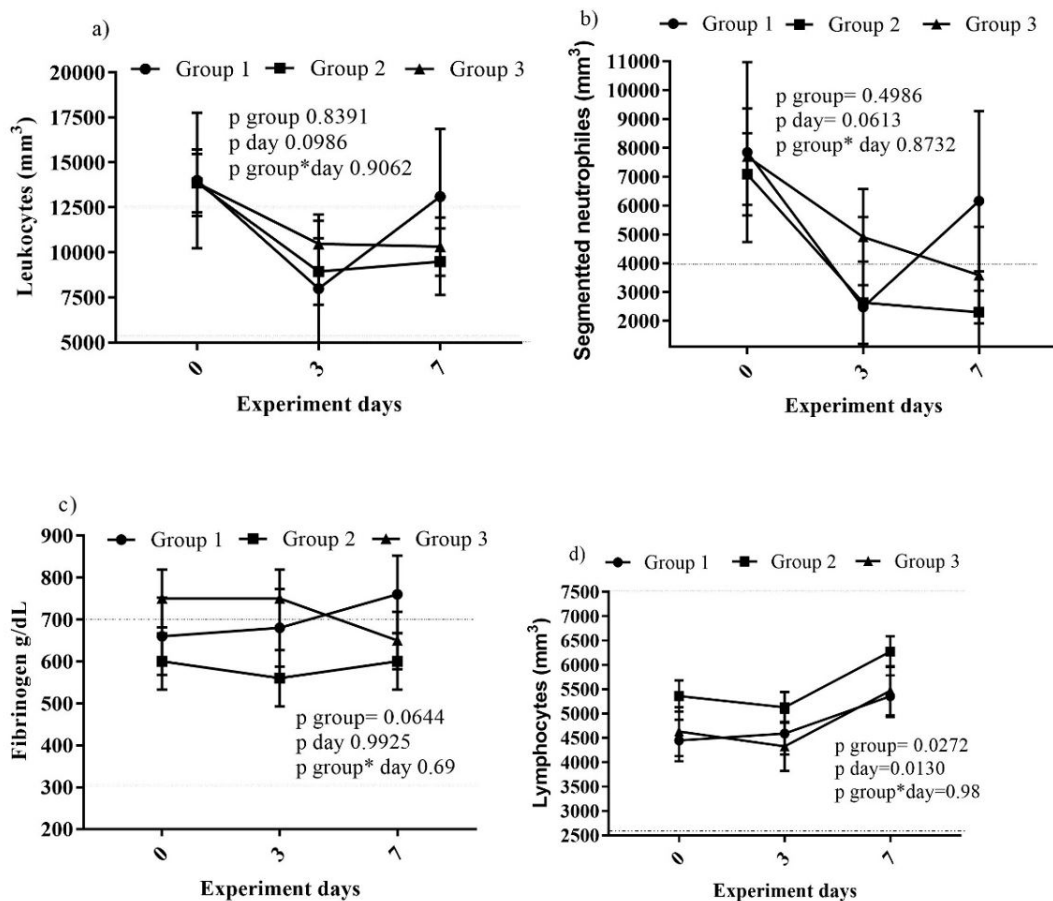


Figure 2 – Blood levels of total leukocytes, segmented neutrophils, fibrinogen, and lymphocytes from dairy calves affected by neonatal diarrhea and submitted to different protocols treatments containing sulfonamides. Group 1: Animals submitted to oral administration of sulfonamides; Group 2: Animals submitted to injectable administration of sulfonamides; Group 3: Animals submitted to oral and injectable sulfonamide administration. The dashed line indicates the physiological interval, according to Kaneko et al. (2008). ^{a,b,c,d}Different letters indicate a statistical difference between groups.

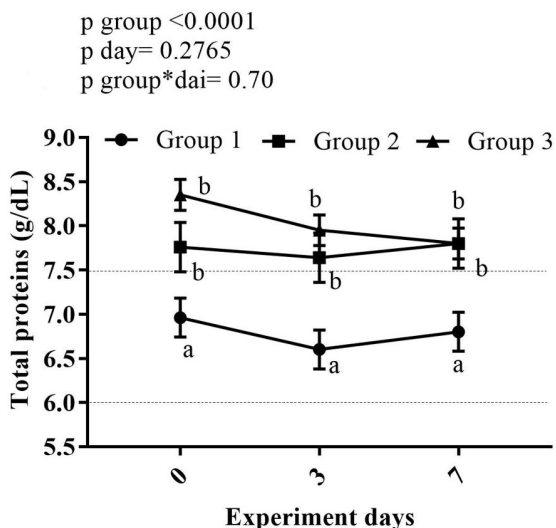


Figure 3 – Total plasma proteins of dairy heifers affected by neonatal diarrhea and submitted to different therapeutic protocols containing sulfonamides. Group 1: Animals submitted to oral administration of sulfonamides; Group 2: Animals submitted to injectable administration of sulfonamides; Group 3: Animals submitted to oral and injectable sulfonamide administration. ^{a,b}Different letters indicate a statistical difference between groups; Dashed line indicates the physiological interval, according to Kaneko et al. (2008).

However, group 1 had neutrophilia on day 7, two days after the end of treatment (Table 2, Figure 2).

Given the results, it is possible to observe that at first all the therapeutic protocols used in this study were effective to stop diarrhea caused by *E.coli*. However, at the end of treatment, calves treated with protocols 1 and 2 showed signs of relapse, with the appearance of leukocytosis with neutrophilia. In treatment 1, animals received phthalylsulfathiazole associated with neomycin orally, in protocol 2, besides the oral treatment, sulfadiazine and trimethoprim were administered IM. Protocol 3 only had sulfadiazine and trimethoprim administered IM. This way, it is possible to note that all animals were provided with therapies using broad-spectrum bactericidal substances. Sulfas act to inhibit the synthesis of folic acid, which is used in the process of bacterial cell growth (bacteriostatic). However, when associated with trimethoprim, it becomes bactericidal (Léo et al., 2009; Opal & Pop-Vicas, 2015). Neomycin, in turn, is an aminoglycoside that acts on the protein synthesis causing bacterial death (Almenara et al., 2008).

Therefore, the main difference among treatments is the routes of administration and the active principles, since sulfa was associated with other antimicrobials and these factors can be connected with the different animals' responses to treatment.

When drugs are administered orally, they can be partially inactivated by the abomasal pH, which can result in underdosing (Papich, 2016). Thus, the involved bacteria might have developed resistance to the orally administered antimicrobial, neomycin sulfate, which could have caused only the inhibitory effect on the bacteria growth, and this, due to intrinsic factors, started to grow again and cause injury. This may have been the reason why there was less effectiveness in group 1 (oral treatment, and only local action) when compared to the other groups (Trefz et al., 2017). Therefore, treatments only administered by mouth can culminate with bacterial resistance, turning the protocol more expensive and less costly when not associated with systemic therapy, as shown by Papich, (2016).

Animals treated with protocol 2 (sulfa with trimethoprim IM), had better performance with greater average weight when compared to the other groups (Figure 4). Also, they had better results in body development during the neonatal period. The result can be attributed to factors

such as reduction of stressful management with oral drug administration, together with the fact that sulfadiazine associated with trimethoprim IM provides a bactericidal effect faster than phthalylsulfathiazole, neomycin sulfate administered orally, since this route can suffer inactivation (Papich, 2016; Spinosa et al., 2002). The IM faster action happens because the vascularization of the tissue allows the drug to be absorbed quickly, minimizing systemic inflammations caused by bacterial colonization early in the calf's life (Araújo et al., 2015; Cassiani et al., 2003).

Results show that protocol 3 was the best to fight the disease, using active principles phthalylsulfathiazole, neomycin sulfate, sulfadiazine, and trimethoprim, besides pectin, and the latter can potentially protect the intestinal mucosa, reducing cases of diarrhea and/or speeding the recovery of this disease (Moreira et al., 2008). However, protocol 2 was more effective regarding the performance of treated animals, even though the clinical condition of diarrhea is responsible for causing changes in the intestinal mucosa that damage the absorptive capacity affecting weight gain and body development (Foster & Smith, 2009).

Studies point out that calves affected with diarrhea have reduced weight gain in 10.7 Kg at weaning when compared with healthy calves (Aghakeshmiri et al., 2017).

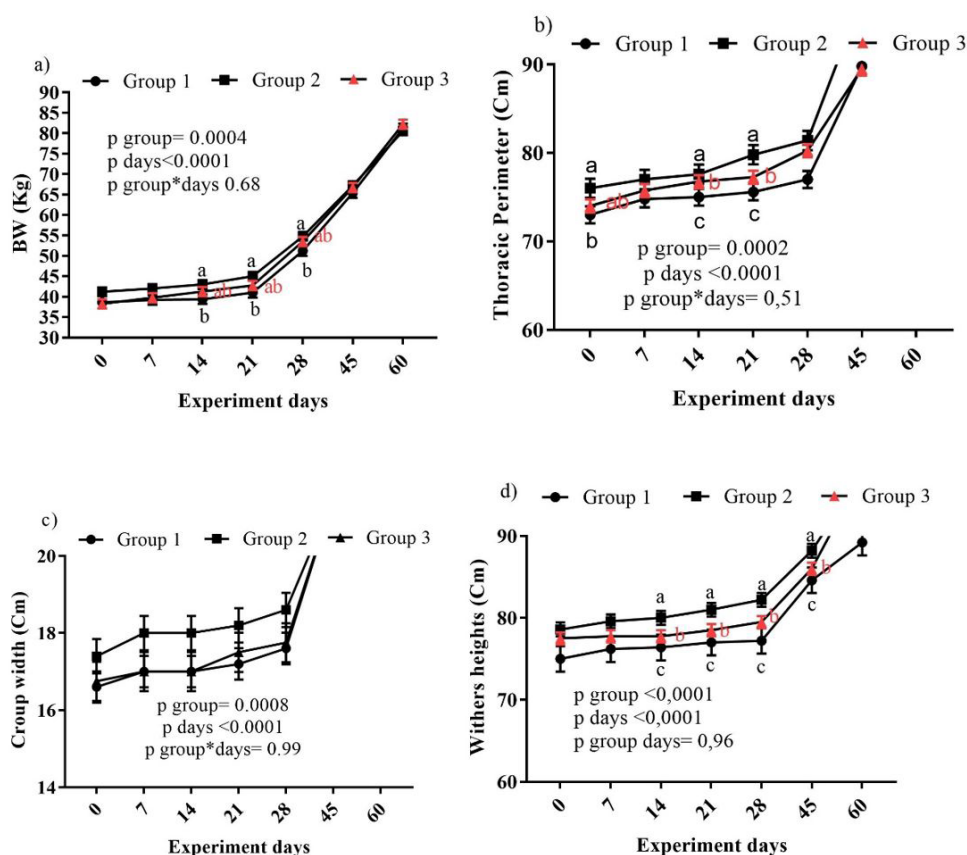


Figure 4 – Zootechnical parameters during the first 60 days of life in dairy heifers affected by neonatal diarrhea submitted to different protocol treatments containing sulfonamides. Group 1: Animals submitted to oral administration of sulfonamides; Group 2: Animals submitted to injectable administration of sulfonamides; Group 3: Animals submitted to oral and injectable sulfonamide administration. ^{a,b,c,d}Different letters indicate statistical difference between groups.

This developmental delay can damage the mammary gland that is developed within the first nine months of age, being highly influenced by the animal's weight gain. Then, it is estimated that animals presenting delayed development tend to become less efficient milk producers (Botteon et al., 2008). Corroborating this, a study by Aghakeshmiri et al. (2017) showed that calves having neonatal diarrhea increased age at first calving, due to a lower growth during the breeding period, reducing their productive performance. In this context, it is important to establish effective treatment alternatives, enabling feed conversion, and promoting animals' weight gain (Botteon et al., 2008).

Given the above, protocol 2 can be considered as more effective, because animals that received this treatment presented better performance, although they were exposed to fewer amounts of active principles when compared with group 3 animals. Besides, not using oral drug administration minimizes the stress caused by these animals' management. Although the animals of group 2 have presented leukocytosis with neutrophilia, their feces were normal, according to the assessment carried out on day 7 after treatment.

To carry out this study, all animals affected with diarrhea on the farm, during the assessment period were selected, totaling 14 calves. All tested protocols had sulfa, although it was administered through different routes, besides involving other active principles. Although an evaluation of a control group is recommended, the mortality risk turns it to be unfeasible to adopt this methodology on commercial farms. Regardless of these factors, the results seem to show that even considering these facts, all protocols

were effective, and the one associating oral and systemic therapies stood out.

Conclusion

The therapeutic protocols with sulfonamides used for the treatment of neonatal calves with diarrhea were effective in their clinical improvement. However, the protocol with systemic therapy with sulfadiazine and trimethoprim (Ibatrium®, Ibasá, Brazil) administered intramuscularly, provided better performance, with better weight gain and body development.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Ethics Statement

This study was approved by the Ethics Committee for Animal Experimentation ECAE-UFPEL (number 23110.041629/2018-03).

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