Predictive biomarkers of bronchopneumonia in bovine neonates

Biomarcadores preditivos de broncopneumonia em neonatos bovinos

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ABSTRACT
The aim of this study was to determine predictive markers of bronchopneumonia in bovine neonates. A total of 70 Holstein calves were monitored from birth to the sixth week of life. Eighteen of the calves were selected and divided into two groups: bronchopneumonia group (n=12) and healthy group (n=6). For gene expression analysis, on the first day of life all animals were submitted to transcutaneous hepatic biopsy. A clinical examination was performed twice a week, where respiratory rate and nasal secretion were monitored to establish the diagnosis of bronchopneumonia. In addition, serum total plasma protein (TPP), gamma glutamyl transferase (GGT), albumin, paraoxonase-1 (PON1) and haptoglobin levels were determined on days -28, -21, -14 and -7, in relation to the day of diagnosis of bronchopneumonia. PON1 (P<0.0001) and TPP (P=0.01) were lower in animals that presented bronchopneumonia, while GGT tended to be lower in the healthy group (P=0.07). Furthermore, there were no differences in the expression of genes linked to the metabolism and inflammatory profile of calves at birth, between healthy animals and those that later presented bronchopneumonia. Therefore, this study suggests that PON1 and TPP are important early markers in the diagnosis of bronchopneumonia in calves.

Keywords: biomarkers, bronchopneumonia, paraoxonase, predictors, total plasma protein

RESUMO
O objetivo desse estudo foi determinar marcadores preditivos de broncopneumonia em neonatos bovinos. Foram monitoradas 70 bezerras da raça Holandês, desde o nascimento até a sexta semana de vida. Destas, foram selecionadas 18 e divididas em dois grupos: grupo broncopneumonia (n=12) e grupo sadias (n=6). Para analises de expressão gênica, no primeiro dia de vida todos os animais foram submetidos a biópsia hepática transcutânea. Foi realizado exame clínico duas vezes por semana, onde a frequência respiratória e a presença de secreção nasal foram monitoradas para estabelecer o diagnóstico de broncopneumonia. Além disso, foram determinados os níveis séricos de proteínas plasmáticas totais (PPT), gama glutamil transferase (GGT), albumina, paraoxonase-1 (PON1) e haptoglobina, nos dias -28, -21, -14 e -7 em relação ao dia do diagnóstico de broncopneumonia. A PON1 (P<0,0001) e PPT (P=0,01) foram menores nos animais que apresentaram broncopneumonia, enquanto a GGT apresentou uma tendência de ser menor no grupo sadio (P=0,07). Além disso, não houve diferenças na expressão de genes ligados ao metabolismo e perfil inflamatório das bezerras ao

nascimento, entre os animais sadios e aqueles que posteriormente apresentaram broncopneumonia. Portanto, este estudo sugere que a PON1 e as PPT são importantes marcadores precoces no diagnóstico de broncopneumonia em bezerras.

Palavras-chave: biomarcadores, broncopneumonia, paraoxonase, preditores, proteína plasmática total

1 INTRODUCTION

The first weeks are the most challenging period of calf life when the health is directly influenced by the efficient passive immunity transfer and environmental hygiene (Al-Alo *et al.*, 2018; Lora *et al.*, 2018) This period accounts for 50% of deaths during the first year of life, being respiratory tract disorders the most incidence diseases (Gonçalves *et al.*, 2011; Lora *et al.*, 2018). One of these disorders is bronchopneumonia, a multifactorial disease that requires an active combination between one or more infectious agents and the host environment for its development (Poulsen and McGuirk, 2009)

This disease is reported to be one of the main causes of death in animals with failure in the transfer of passive (Feitosa *et al.*, 2010) immunity resulting in large economic losses in calf rearing system due to the treatment costs, veterinary medical care and, mainly, the delay in weight gain (Poulsen and McGuirk, 2009; Gonçalves *et al.*, 2011) as well as negative effects on reproduction efficiency in the future heifers (Aghakeshmiri *et al.*, 2017). According to the Agriculture Department of the United States, the incidence of respiratory diseases for this animal category has been around 20% (USDA, 2007), being responsible for 24% of the deaths during the pre-weaning and 58.9% during the post-weaning (USDA, 2014). In an attempt to reduce this impact, it is extremely important to have a fast diagnosis, and the quantification of acute phase proteins can be a good alternative to help this diagnosis (Krause *et al.*, 2014).

Immune mediators are released in the acute phase response, such as cytokines, which cause, among other effects, variation in the concentration of proteins present in plasma, such as haptoglobin and paraoxonase-1 (PON1) (Schrödl *et al.*, 2016). These proteins, when quantified in plasma, may be useful in the diagnosis, prognosis, and monitoring of the evolution of diseases (Cray, 2011; Jain *et al.*, 2011). In addition, the study of genes expression linked to metabolism and inflammatory response provide information about characteristics of higher resistance to diseases and body development, which is influenced by the condition that the animal is exposed during the prenatal period (Osorio *et al.*, 2014; Jacometo *et al.*, 2018). Thus, knowledge of the metabolic changes

and the inflammatory profile of neonatal calves can be an effective tool for early diagnosis in morbidity situations, allowing the monitoring of organism function (Russell and Roussel, 2007). Therefore, the objective of this study was to establish predictive markers of bronchopneumonia in bovine neonates.

2 MATERIALS AND METHODS

The experiment was carried out in a commercial farm located in the south of Rio Grande do Sul – RS (32.8º16' S; 52.8º32' E). Seventy Holstein calves were monitored and evaluated from birth to the sixth week of age. Of these, 18 who remained healthy or had bronchopneumonia continued in the study. Animals that presented other diseases were excluded from the study. All procedures were approved by the Animal Experimentation Ethics Committee of the Federal University of Pelotas (UFPel), under code 2827.

After calving, the animals remained with their mother for approximately 12 h in order to receive the colostrum naturally and in accordance with the farm management practice. After this period, they were housed in individual pens with an area of 1 m², covered and suspended 1.5 m above the ground. The diet provided was composed of 4 liters of milk per day, divided into two meals provided at 7:00 and 17:00 h, with free access to the initial pelleted concentrate (Supra Terneira Laminado, Supra®, São Leopoldo, Brazil), according to the National Research Council (NRC, 2001). Water was provided *ad libitum*.

Text On the first day of life, transcutaneous hepatic biopsies were performed on all calves according to methodology (Radcliff *et al.*, 2003). The hepatic tissue samples were frozen in liquid nitrogen until analysis.

Total RNA was extracted using the Trizol method, treated with DNase and purified using the RNeasy® kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. The samples were quantified in NanoDrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA) and the integrity verified using agarose gel electrophoresis.

The cDNA synthesis was performed using the iScript™ - Synthesis kit (Bio-Rad, Hercules, California, USA) with 1 µg of total RNA in a final volume of 20 µL. The reactions were conducted in a thermocycler (MyCycler™ ThermalCycler, Bio-Rad,

Hercules, Florida, USA) according to the following protocol: 5 min at 25 ºC, 30 min at 42 ºC, and 5 min at 85 ºC. The final product was diluted to 5 ng/µL.

To evaluate the expression of genes of the bovine growth hormone receptor 1A (*GHR1A*), the insulin-like growth factor 1 (*IGF1*), the superoxide dismutase 2 (*SOD2*), the nuclear factor Kappa B subunit 1 (*NFKB1*), and the signal transducer and activator of transcription 3 (*STAT3*), a real-time polymerase chain reaction (Real-Time PCR) was conducted using the SYBR Green PCR Master Mix kit (Applied Biosystems™, Foster, California, USA). Aubiquitously expressed transcript gene (protein) (*UXT*) was used as an endogenous control. The primers are described in the Tab. 1, the PCR reactions were performed in duplicate using a 10 µL volume, containing 5 µL from SYBR Green kit, 0.4 µL of each primer (10 µM), 4 µL of cDNA and 0.2 µL of ultrapure water. The reactions were performed using an ECO™ Real-Time PCR system (iLLumina®, San Diego, California, USA). For each test, 40 cycles were carried out (95 ºC for 3 s and 60 ºC for 30 s) and a dissociation curve was included at the end of the reaction to verify the amplification of just one product from the PCR. Each test plate included one sample of ultrapure water as the negative control.

The variation coefficient was less than 5% for all the initiator pairs used. The relative expression was calculated using the equation $2^{A-B}/2^{C-D}$, in which A is the cycle threshold value (Ct) for the gene of interest in the control sample; B is the Ct value for the gene of interest in the analyzed sample; C is the Ct value of the *UXT* gene in the first control sample; and D is the Ct value of the *UXT* in the analyzed sample (Masternak *et al.*, 2005). The first control sample was expressed as 1.00 in this equation and all the other samples were calculated relative to this value. Thereafter, the results from the control group (healthy animals) were weighted and all the other results were divided by the mean value of the control group relative expression to obtain changes in gene expression compared to the control group (*fold change*).

Table 1. Sequence and size of *primers* (pb) for *Bos taurus* used in the gene expression.

Gene	Access #	Primers sequence (5'–3')	Fragment length (Pb)
GHR1A	AC_000177.1	F: TCCAGCCTCTGTTTCAGGAG R: GCTGCCAGAGATCCATACCT	64
IGF1	AC_000162.1	F: CCAATTCATTTCCAGACTTTGCA R: CACCTGCTTCAAGAAATCACAAAA	103
STAT3	AC_000176.1	F: GGTAGCATGTGGGATGGTCTCT R: GCATCCCTAGAAACTCTGGTCAA	95
NFKB1	AC_000163.1	F: TTCAACCGGAGATGCCACTAC R: ACACACGTAACGGAAACGAAATC	110
SOD2	AC_000166.1	F: TGTGGGAGCATGCTTATTACCTT R: TGCAGTTACATTCTCCCAGTTGA	95
UXT	AC_000187.1	F: TGTGGCCCTTGGATATGGTT R: GGTTGTCGCTGAGCTCTGTG	101

GHR1A= bovine growth hormone receptor 1A; *IGF1*= insulin-like growth factor 1; *NFKB1*= nuclear factor Kappa B subunit 1; *SOD2* =superoxide dismutase 2; *STAT3* =signal transducer and activator of transcription 3; *UXT* = ubiquitously expressed transcript gene.

Clinical examinations were performed twice a week in all animals during the experimental period, while animal behavior and clinical condition were observed daily. When any change was observed, the clinical examination was performed once more to identify signs of respiratory tract impairment. In the specific clinical evaluation of the respiratory system, the respiratory type and presence of nasal discharge were monitored, as well as auscultation of the lung projection area in the chest to determine the presence of crackling rales, and the presence of these signs, plus fever (body temperature above 39.5 ºC), were used to determine the diagnosis of bronchopneumonia (Gonçalves *et al.*, 2011).

Whenever the animals were diagnosed with bronchopneumonia evaluation of heart rate (HR), respiratory rate (RR), body temperature and capillary perfusion time (CPT) were performed, as proposed by Raiser (2003) at 0, 24, 72 and 120 hours from the diagnosis moment. Animals that did not show changes in parameters at clinical examination at up to 42 days of age were considered healthy; animals that presented any other diseases were excluded from the study.

After clinical evaluations, the animals were divided into two groups. The bronchopneumonia group (n = 12) was composed of animals diagnosed with bronchopneumonia, the diagnosis being based on the presence of signs of respiratory tract involvement and fever (body temperature above 39.5 °C). The healthy group (n = 6) was composed of animals that did not present with fever or any clinical involvement during the first 42 days of life.

Samples of blood (10 mL) were collected from all animals once a week (Vacuplast®, Shandong, China) from birth to the appearance of clinical signs of bronchopneumonia, and/or up to the sixth week of life in the case of animals that did not present with bronchopneumonia. Blood samples were collected into tubes without anticoagulant and centrifuged at 1800 *g* to obtain serum samples; these were labelled and frozen at -70 °C.

For evaluation of the metabolic profile prior to clinical involvement, blood samples collected on days -28, -21, -14 and -7 in relation to the day of bronchopneumonia diagnosis were used. TPP, GGT, albumin, PON1 and haptoglobin were determined during the pre-involvement period for bronchopneumonia.

Commercial reagent kits of the Labtest brand (Labtest, Belo Horizonte, Brazil) were used for the determination of TPP, GGT and albumin. The samples were analyzed using a visible light spectrophotometer (BioEspectro® SP 220, Bioespectro, Curitiba, Brazil) with a wavelength appropriate for each test.

Activity of PON1 was determined using a previously described protocol (Browne *et al.*, 2007). The working solution was a 20 mM Tris/HCl buffer containing 1 mM calcium chloride and 4 mM phenylacetate. Samples were diluted (1:3) in 20 mM Tris/HCl buffer. Readings were performed using a spectrophotometer, adding 3.3 µL of the diluted sample to 500 µL of the working solution. The wavelength used was 270 nm and a reading time of 1 min. The activity of the enzyme was determined using the following formula: Δ absorbance × 115 × 3, expressed as U/mL.

The haptoglobin was measured using the colorimetric method, based on the reaction of hydrogen peroxide with hemoglobin that oxidizes the guaiacol and releases light, according to the protocol of Connell and Smithies (1959). In all analyses, the coefficient of variation was less than 10%.

Data were analyzed using the SAS® statistical program (see 9.1, SAS Institute Inc., Cary, USA). The variables were submitted to the Shapiro-Wilk's normality test ($P>0.9$) and were later analyzed using the MIXED MODELS method, considering the group, day and their interactions as fixed effects, and the animals within the group as a random effect (Littell *et al.*, 1998). Variables that did not present normal distribution of the residues were transformed to log P. The comparison of means was conducted using the Tukey-Kramer test. Values of $P<0.05$ were considered significant, and, as a trend, values between 0.05 and 0.10.

3 RESULTS

During the evaluation period of the 70 heifers, six remained healthy and 12 presented with bronchopneumonia without manifestation of other diseases. PON1 was lower in animals that presented with bronchopneumonia (Fig. 1; P<0.0001). Similar results were observed in the TPP analysis (Fig. 1; P=0.01). In contrast, GGT showed a tendency to be lower in the healthy group (Tab. 2; P=0.07). However, with regard to haptoglobin and albumin levels in pre-bronchopneumonia animals, there were differences between the animals that presented with or without the disease (Tab. 2; P>0.05).

With regard to the expression of genes linked to metabolism (*GHR1A* and *IGF1*) and inflammatory profile (*NFKB1*, *SOD2* and *STAT3*) of calves at birth, there were no differences between healthy animals and animals that developed bronchopneumonia at a later date (Tab. 3; P>0.05).

Figure 1. Metabolic parameters in heifers evaluated prior to bronchopneumonia. A. Total plasma protein concentrations (TPP). B. Paraoxonase-1 activity (PON1).

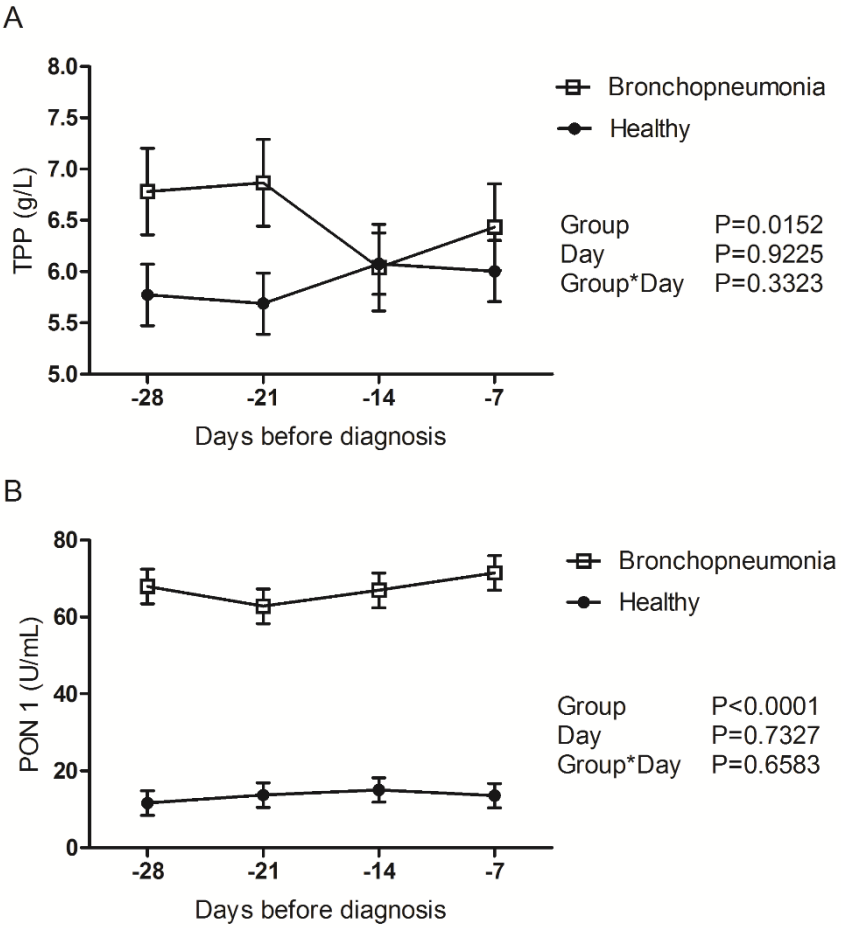

Table 2. Mean values and standard error (SE) of metabolic parameters in calves evaluated before clinical involvement of bronchopneumonia.

Parameters	Groups		Significance (P value)		
	Healthy	Bronchopneumonia	Group	Day	Group*day
Albumin (g/dL)	2.36 (0.07)	2.46 (0.05)	0.26	0.66	0.10
TPP (g/dL)	6.53 (0.21)[b]	5.88 (0.15)[a]	0.01	0.92	0.33
Haptoglobin (g/L)	1.23 (0.15)	1.09 (0.10)	0.42	0.12	0.65
PON1 (U/mL)	67.29 (2.25)[b]	13.47 (1.60)[a]	<0.0001	0.73	0.66
GGT (U/L)	92.56 (35.65)	172.58 (25.21)	0.07	0.73	0.71

[ab] Mean values followed by different lowercase letters for the same parameter differ between groups using the Tukey test (P<0.05). TPP = total plasma protein; PON1 = paraoxonase 1; GGT = gamma-glutamyl transferase.

Table 3. Mean values and standard error (SE) of the gene expression in heifers at birth and who subsequently had clinical involvement of bronchopneumonia.

Gene	Groups		Significance
	Healthy	Bronchopneumonia	(P value)
GHR1A	1.00 (0.63)	1.08 (0.45)	0.80
STAT3	1.00 (0.24)	0.95 (0.17)	0.90
SOD2	1.00 (0.19)	0.88 (0.14)	0.77
NFKB1	1.00 (0.37)	1.10 (0.26)	0.56
IGF1	1.00 (0.30)	1.08 (0.22)	0.49

GHR1A =bovine growth hormone receptor 1A; *IGF1* =insulin-like growth factor 1; NFKB1 =nuclear factor Kappa B subunit 1; SOD2 =superoxide dismutase 2STAT3 =signal transducer and activator of transcription 3.

4 DISCUSSION

In the present study was observed a reduction of PON1 levels at 28 days before bronchopneumonia clinical affection, showing that PON1 can be an important early marker in the diagnosis of bronchopneumonia in calves. Similarly, Schneider *et al.* (2013) also related low levels of PON1 in the peripartum of cows with a higher incidence of postpartum uterine diseases. PON1 is a negative acute phase protein (APP) (Feingold *et al.*, 1998) which a reduced activity may indicate an inflammatory condition, as observed by Bionaz *et al.* (2007), where during the first 30 days of lactation in cattle, it was correlated with increased cases of metritis, mastitis and laminitis; while higher levels at this stage were related to better uterine health (Bionaz *et al.*, 2007). Thus, a reduction of PON1 in this period may be due to a lower rate of synthesis in the liver, as occurs with negative APPs (Bionaz *et al.*, 2007).

However, haptoglobin was not effective in the early diagnosis of bronchopneumonia, since in this study there were no differences between healthy and diseased animals for this APP. The lack of response observed for this APP are in agreement with related by (Nielsen *et al.*, 2004; Rubio and Schmidt, 2014), who reported that the levels of this protein remain low in physiological situations with an increase in

concentration during the acute phase of inflammation and a decrease at the end of the inflammatory process. The same was demonstrated by Seppa-Lassila *et al.* (2015), who found an increase in haptoglobin in calves with diarrhea, similar to Idoate *et al.* (2015) who observed an increase in calves confined with a respiratory infection.

The lower TPP value observed during the period of 28 to 21 days before the onset of the disease, that is in the first weeks of neonate life, suggest that the animals that later developed bronchopneumonia could be less immunocompetent since TPP is a transfer indicator of passive immunity via colostrum (Borges *et al.*, 2001). However, albumin, which represents a fraction of TPPs and is an important indicator for other diseases, was not altered in the present experiment and neither to Leal *et al.* (2003) nevertheless the same was not reported by Schneider *et al.* (2013), which observed that the albumin may be an important predictive biomarker to uterine diseases during the peripartum period of dairy cows. The same was observed in prior studies of Burke *et al.* (2010) and Huzzey *et al.* (2009) which reported that albumin was already lower 14 days before calving in cows with postpartum endometritis, they also reported that the DMI, fatty liver and liver function may also be involved in this metabolic process.

Furthermore, the tendency for GGT to increase prior to bronchopneumonia may be due to higher hepatic protein synthesis, indicating an inflammatory process that would occur (Ganheim *et al.*, 2003), since most of the acute phase proteins are produced in the liver (Eckersall *et al.*, 2001; Chemonges *et al.*, 2014).

According to the results of gene expression, bronchopneumonia was due to management failures after birth and was not related to the metabolic and inflammatory condition of these animals in the prenatal period. In the liver, the greater expression of *IGF1* and *GHR1A* demonstrates the maturation of metabolic pathways, such as the gluconeogenesis pathway, which are fundamental for the development of calves (Hammon *et al.*, 2012). According to Butler *et al.* (2003), when there is a decrease of plasma insulin, such as in periods of negative energy balance (BEN), the expression of *GHR* and *IGF1* is decreased. Jacometo *et al.* (2016) found increased *GHR* and *IGF1* expression in the liver of calves over time, both in the offspring of cows who received supplementation with amino acids and energy in the diet and those who received the control diet prepartum, there was no observed effect of diet on these genes.

Inflammatory markers, *NFKB1* and *SOD2*, have a higher expression when the immune response is activated and supplementation with protected amino acids in the

rumen of cows during the transition period increased the expression of these markers (Osorio *et al.*, 2014; Jacometo *et al.*, 2016). found a greater expression of *SOD2* at the liver level and a tendency for greater expression of *NFKB1* in calves whose mothers had received methionine supplementation in the prepartum period. However, a lower expression of *STAT3* was reported to signal high susceptibility to endotoxic shock and increased production of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1) and interferon gamma (IFN-γ) (Takeda *et al.*, 1999). As there were no differences between the gene expression of animals that subsequently developed bronchopneumonia and animals that did not, we can verify that the occurrence of the disease was not due to metabolic factors related to fetal development, but due to environmental and management issues.

5 CONCLUSION

Calves with bronchopneumonia had reduced serum concentrations of PON1 and total plasma proteins at 28 days before the diagnosis of the disease compared to healthy calves. These results suggest that PON1 is the most sensitive indicator for early diagnosis of this disease between the APPs evaluated in the present study, which can be a useful tool to identify calves at risk of developing respiratory diseases.

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REFERENCES

AGHAKESHMIRI, F.; AZIZZADEH, M.; FARZANEH, N. et al. Effects of neonatal diarrhea and other conditions on subsequent productive and reproductive performance of heifer calves. *Vet. Res. Commun.*, v.41, p.107-112, 2017.

AL-ALO, K.Z.K.; BRUJENI, G.N.; LOTFOLLAHZADEH, S. et al. Correlation between neonatal calf diarrhea and the level of maternally derived antibodies. *Iran. J. Vet. Res.*, v.19, p.3, 2018.

BIONAZ, M.; TREVISI, E.; CALAMARI, L. et al. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. *J. Dairy Sci.*, v.90, p.1740-1750, 2007.

BORGES, A.S.; FEITOSA, F.L.F.; BENESI, F.J. et al. Influência da forma de administração e da quantidade fornecida de colostro sobre a concentração de proteína total e de suas frações eletroforéticas no soro sangüíneo de bezerros da raça Holandesa. *Arq. Bras. Med. Vet. Zootec.*, v.5, p.629-634, 2001

BROWNE, R.W.; KOURY, S.T.; MARION, S. et al. Accuracy and biological variation of human serum paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay. *Clin. Chem.*, v.53, p.310-317, 2007.

BURKE, C.R.; MEIER, S.; MCDOUGALL, S. et al. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. *J. Dairy Sci.*, v.93, p.5363-5373, 2010.

BUTLER, S.T.; MARR, A.L.; PELTON, S.H. et al. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. *J. Endocrinol.*, v.176, p.205-217, 2003.

CHEMONGES, S.; TUNG, J.P.; FRASER, J.F. Proteogenomics of selective susceptibility to endotoxin using circulating acute phase biomarkers and bioassay development in sheep: a review. *Proteome Sci.*, v.12, p.12, 2014.

CONNELL, G.E.; SMITHIES, O. Human haptoglobins: estimation and purification. *Biochem. J.*, v.72, p.115-121, 1959.

CRAY, C. Acute phase proteins in animals. *Prog. Mol. Biol. Transl.*, v.105, p.113-150, 2011.

ECKERSALL, P.D.; YOUNG, F.J.; MCCOMB, C. et al. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet. Rec.*, v.148, p.35-41, 2001.

FEINGOLD, K.R.; MEMON, R.A.; MOSER, A.H. et al. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. *Atherosclerosis.* v.139, p.307-315, 1998.

FEITOSA, F.L.F.; CAMARGO, D.G.; YANAKA, R. et al. Índices de falha de transferência de imunidade passiva (FTIP) em bezerros holandeses e nelores, às 24 e 48 horas de vida: valores de proteína total, de gamaglobulina, de imunoglobulina G e da

atividade sérica de gamaglutamiltransferase, para o diagnóstico de FTIP. *Pesqui. Vet. Bras.*, v.30, p.696-704, 2010.

GANHEIM, C.; HULTEN, C.; CARLSSON, U. et al. The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or Mannheimia haemolytica. *J. Vet. Med. B. Infect. Dis. Vet. Public Health.* v.50, p.183-190, 2003.

GONÇALVES, R.C.; ROCHA, A.E.A.; SILVA, A.A.D. et al. Influence of vitamin E on prophylaxis and treatment of moderate and severe bronchopneumonia in calves. *Pesq. Vet. Bras.*, v.31, p.127-135, 2011.

HAMMON, H.M.; STEINHOFF-WAGNER, J.; SCHONHUSEN, U. et al. Energy metabolism in the newborn farm animal with emphasis on the calf: endocrine changes and responses to milk-born and systemic hormones. *Domest. Anim. Endocrinol.*, v.43, p.171-185, 2012.

HUZZEY, J.M.; DUFFIELD, T.F.; LEBLANC, S.J. et al. Haptoglobin as an early indicator of metritis. *J. Dairy Sci.*, v.92, p.621-625, 2009.

IDOATE, I.; VANDER LEY, B.; SCHULTZ, L. et al. Acute phase proteins in naturally occurring respiratory disease of feedlot cattle. *Vet. Immunol. Immunopathol.*, v.163, p.221-226, 2015.

JACOMETO, C.B.; ALHARTHI, A.S.; ZHOU, Z. et al. Maternal supply of methionine during late pregnancy is associated with changes in immune function and abundance of microRNA and mRNA in Holstein calf polymorphonuclear leukocytes. *J. Dairy Sci.*, v.101, p.8146-8158, 2018.

JACOMETO, C.B.; ZHOU, Z.; LUCHINI, D. et al. Maternal rumen-protected methionine supplementation and its effect on blood and liver biomarkers of energy metabolism, inflammation, and oxidative stress in neonatal Holstein calves. *J. Dairy Sci.*, v.99, p.6753-6763, 2016.

JAIN, S.; GAUTAM, V.; NASEEM, S. Acute-phase proteins: As diagnostic tool. *J. Pharm. Bioallied Sci.*, v.3, p.118-127, 2011.

KRAUSE, A.R.; PFEIFER, L.F.; MONTAGNER, P. et al. Associations between resumption of postpartum ovarian activity, uterine health and concentrations of metabolites and acute phase proteins during the transition period in Holstein cows. *Anim. Reprod. Sci.*, v.145, p.8-14, 2014.

LEAL, M.L.D.R.; BENESI, F.J.; LISBÔA, J.A.N. et al. Proteinograma sérico de bezerras sadias, da raça holandesa, no primeiro mês pós-nascimento. *Braz. J. Vet. Res. Anim. Sci.* v.40, p.138-145, 2003.

LITTELL, R.C.; HENRY, P.R.; AMMERMAN, C.B. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.*, v.76, p.1216-1231, 1998.

LORA, I.; GOTTARDO, F.; CONTIERO, B. et al. Association between passive immunity and health status of dairy calves under 30 days of age. *Prev. Vet. Med.*, v.152, p.12-15, 2018.

MASTERNAK, M.M.; AL-REGAIEY, K.A.; DEL ROSARIO LIM, M.M. et al. Caloric restriction results in decreased expression of peroxisome proliferator-activated receptor superfamily in muscle of normal and long-lived growth hormone receptor/binding protein knockout mice. *J. Gerontol. A. Biol. Sci. Med. Sci.*, v.60, p.1238-1245, 2005.

NIELSEN, B.; JACOBSEN, S.; ANDERSEN, P. et al. Acute phase protein concentrations in serum and milk from healthy cows, cows with clinical mastitis and cows with extramammary inflammatory conditions. *Vet. Rec.*, v.154, p.361-365, 2004.

NRC. 7.ed. Nutrient requirements of dairy cattle. Washington, USA: Academies Press, 2001. 405p.

OSORIO, J.S.; JI, P.; DRACKLEY, J.K. et al. Smartamine M and MetaSmart supplementation during the peripartal period alter hepatic expression of gene networks in 1-carbon metabolism, inflammation, oxidative stress, and the growth hormone-insulin-like growth factor 1 axis pathways. *J. Dairy Sci.*, v.97, p.7451-7464, 2014.

POULSEN, K.P.; MCGUIRK, S.M. Respiratory disease of the bovine neonate. *Vet. Clin. N. Am-Food A.*, v.25, p.121-137, 2009.

RADCLIFF, R.P.; MCCORMACK, B.L.; CROOKER, B.A. et al. Growth hormone (GH) binding and expression of GH receptor 1A mRNA in hepatic tissue of periparturient dairy cows. *J. Dairy Sci.*, v.86, p.3933-3940, 2003.

RAISER, A.G. Alterações no equilíbrio hidroeletrolítico. In: Raiser, A.G. (Ed). Patologia cirúrgica veterinária. Santa Maria: 2003. p.1-21.

RUBIO, C.P.; SCHMIDT, E.M.S. Proteínas de fase aguda em cães: possíveis aplicações em cirurgia. *Vet. e Zootec.*, v.21, p.492-502, 2014.

RUSSELL, K.E.; ROUSSEL, A.J. Evaluation of the ruminant serum chemistry profile. *Vet. Clin. North Am. Food Anim. Pract.*, v.23, p.403-426, 2007.

SCHNEIDER, A.; CORREA, M.N.; BUTLER, W.R. Short communication: acute phase proteins in Holstein cows diagnosed with uterine infection. *Res. Vet. Sci.*, v.95, p.269-271, 2013.

SCHRÖDL, W.; BÜCHLER, R.; WENDLER, S. et al. Acute phase proteins as promising biomarkers: Perspectives and limitations for human and veterinary medicine. *Proteom. Clin. Appl.*, v.10, p.1077-1092, 2016.

SEPPA-LASSILA, L.; ORRO, T.; LASSEN, B. et al. Intestinal pathogens, diarrhoea and acute phase proteins in naturally infected dairy calves. *Comp. Immunol. Microbiol. Infect. Dis.*, v.41, p.10-16, 2015.

TAKEDA, K.; CLAUSEN, B.E.; KAISHO, T. et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity*. v.10, p.39-49, 1999.

USDA. Heifer calf health and management practices on US dairy operations, 2007; No. 550.0110; USDA–Animal and Plant Health Inspection Service (APHIS)–Veterinary Services (VS)–Center for Epidemiology and Animal Health (CEAH)–National Animal

Health Monitoring System (NAHMS): Fort Collins, CO, USA, 2018. *USDA-APHIS-VS, CEAH.*2007.

USDA. Health and Management Practices on US Dairy Operations, 2014; No. 696.0218; USDA–Animal and Plant Health Inspection Service (APHIS)–Veterinary Services (VS)–Center for Epidemiology and Animal Health (CEAH)–National Animal Health Monitoring System (NAHMS): Fort Collins, CO, USA, 2018. *USDA-APHIS-VS, CEAH.*2014.