A study of lipid and protein profiles and liver enzyme levels in neonatal diarrheic calves based on clinical severity of the disease

Research Article

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ABSTRACT

The purpose of this study was to investigate the serum lipid and protein profile as well as liver enzyme levels in neonatal calves with diarrhea. The study included 40 calves, 30 with diarrhea and 10 healthy (control). Calves with diarrhea were divided into three groups mild, moderate, and severe based on clinical findings. Blood samples were taken from the *vena jugularis* after routine clinical examinations of the calves to determine the serum lipid and protein profile, liver enzymes and glucose levels. Serum total cholesterol (TC) ($P<0.01$), high-density lipoprotein (HDL) ($P<0.01$), and low-density lipoprotein (LDL) ($P<0.05$) levels were found to be lower in the mild, moderate, and severe groups compared to the control group. No significant difference in total protein (TP) and albumin (ALB) values was found between the groups. Additionally, serum aspartate aminotransferase (AST) ($P<0.01$) and alkaline phosphatase (ALP) ($P<0.05$) levels were higher in calves with diarrhea than in the control group, conversely glucose levels ($P<0.05$) were lower. The current study concluded that there was no change in the protein profile, but the lipid profile was negatively affected, and liver function was impaired in calves with neonatal diarrhea. Furthermore, as the clinical severity of the disease increased the impairment in liver function raised.

Keywords: Calf diarrhea, liver enzymes, lipid profile, protein profile

INTRODUCTION

Neonatal calf diarrhea is a significant problem in cattle breeding that is caused by both infectious and non-infectious causes, is widespread, and causes significant economic loss (Izzo et al., 2011). Bacteria, viruses, and protozoa are among the infectious agents that contribute to the disease's progression (Cho and Yoon, 2014). Clinical findings in neonatal calf diarrhea vary greatly depending on the severity of the diarrhea and the level of inflammation. It progresses from mild watery diarrhea to lethargy and coma in dehydrated and acidotic animals (Grünberk, 2022). Therefore, various clinical scoring methods are used to evaluate the clinical situation (Walker et al., 1998; Sayers et al., 2016).

Lipids serve as a form of energy storage (triglycerides), a component of cell membranes, and the precursor to all steroid hormones (cholesterol) (Arfuso et al., 2017). Therefore, changes in plasma lipid levels affect other organ or tissue functions (Nassaji and Ghorbani, 2012).

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Lipids are insoluble in plasma and are transported bound to carrier proteins called lipoproteins, including high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) (Arfuso et al., 2017). Infections and inflammations are known to interfere with lipid and lipoprotein metabolism, such as lipid oxidation and cholesterol transport, to stimulate the anti-inflammatory response (Tall and Yvan-Charvet, 2015).

Proteins are important components of biological processes; some are involved in the structural support of connective tissues, while others are involved in biochemical reactions (Anderson and Anderson, 2002). Diseases can be caused by any dysfunction or imbalance in their concentration (Pieper et al., 2003). Blood proteins are likely to change during the course of diseases and measuring them is an important part of routine biochemistry and clinical laboratory practice (Tóthová et al., 2016).

The systemic inflammatory response that may develop during infection or endotoxemia, if severe enough, can damage and impair various organ or system functions such as the liver, kidney, respiratory and circulatory system (Ramachandran, 2014).

Digestive system diseases in calves can cause irregularities in the homeostasis of animals by causing changes in blood biochemical parameters including serum lipid and protein levels (Nagyová et al., 2015). The most accurate approach for determining the prognosis of the disease and the treatment protocol in neonatal calf diarrhea is to evaluate the changes in biochemical components in conjunction with the clinical severity of the disease. Few studies have examined the serum lipid and protein profile and liver enzyme levels in calves with diarrhea (Bozukluhan et al., 2017; Al-Alo et al., 2017; Athanasiou et al., 2019). However, no study was found in which the relevant parameters were evaluated according to the clinical severity of the disease. Therefore, the objective of this study was to assess the liver enzyme levels and serum lipid and protein profiles in neonatal calves with diarrhea in accordance with the clinical course of the disease.

MATERIAL and METHOD

Animal Material

The animal material of the study consisted of a total of 40 calves, aged 0-15 days, of different breeds and genders, 30 of them with diarrhea and 10 of which were healthy.

Groups

The study included four groups, one healthy and three diseased groups, each with ten calves. Calves with diarrhea were classified as mild, moderate, or severe based on dehydration and depression criteria (Walker et al., 1998) (Table 1).

Table 1. Clinical scoring in calves with diarrhea (Walker et al, 1998).

Eyeball recession into the orbit	**0: normal position**
	1: mild
	2: severe
Thoracic skin tent duration (s)	0: recovery after 1 seconds
	1: recovery after 1-3 seconds
	2: recovery after >4 seconds
Sucking Reflex	0: strong regular sucking reflex
	1: poor ineffective sucking reflex
	2: no sucking reflex
Fecal consistency	1: watery
	2: pastose
	3: solid

Control group (n=10): This group consisted of healthy calves with no health problems with a score of eyeball appearance: 0, skin tent duration: 0, sucking reflex: 0, and fecal consistency: 3.

Mild group (n=10): This group consisted of calves with mild diarrhea with a score of eyeball recession into the orbit: 0, skin tent duration: 0, sucking reflex: 0, and fecal consistency: 1.

Moderate group (n=10): This group consisted of calves with moderate diarrhea with a score of eyeball recession into the orbit: 1, skin tent duration: 1, sucking reflex: 1, and fecal consistency: 1.

Severe group (n=10): This group consisted of calves with severe diarrhea with a score of eyeball recession into the orbit: 2, skin tent duration: 2, sucking reflex: 2, and fecal consistency: 1.

Blood Sampling

For biochemical analysis, 8 ml blood samples were taken from the *vena jugularis* of all calves into gel serum tubes (Vacutainer®, BD, UK). Blood samples were allowed to clot for 30 minutes at room temperature and centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge (Beckman Coulter®, USA). The obtained sera were taken into eppendorf tubes and stored at -80 °C until the day of analysis.

Determination of Lipid Profile

Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) levels in serum samples were determined with commercial kits using an autoanalyzer (Mindray BS-300 Chemistry Analyzer®, China).

Determination of Protein Profile

Total protein (TP) and albumin (ALB) levels in serum samples were determined with commercial kits using an autoanalyzer (Mindray BS-300 Chemistry Analyzer®, China).

Determination of Liver Enzymes, Direct Bilirubin and Glucose Levels

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin (DBIL) and glucose levels in serum samples were determined with commercial kits using an autoanalyzer (Mindray BS-300 Chemistry Analyzer®, China).

Statistical Analysis

The data obtained from the study were analysed using the Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, IL). Normality of data distribution for each parameter was evaluated using a Kolmogorov–Smirnov test. In order to compare the groups, parametric variables (TC, HDL) were evaluated using one-way ANOVA test, while non-parametric variables (AST, ALP, TG, LDL, TP, ALB, DBIL, Glucose) were evaluated using Kruskal-Wallis test. P values equal or less than 0.05 were considered statistically significant.

RESULTS

Lipid Profile Findings

Table 2 displays the results of the lipid profile analysis. The TC and HDL levels of calves with diarrhea in the mild, moderate, and severe groups were found to be lower than the control group (P=0.000). LDL levels were found to be lower in calves with mild and moderate diarrhea compared to the control group, with the moderate group having the lowest LDL levels (P=0.013). There was no statistically significant difference in the TG levels of the groups.

Table 2. Lipid profile findings in groups.

Parameters	Control (n=10)	Mild (n=10)	Moderate (n=10)	Severe (n=10)	P
TC	71.20±23.98[a]	42.90±15.30[b]	32.50±16.19[b]	42.30±19.92[b]	**0.000**
TG	38.00±16.37	26.60±22.18	40.40±48.56	41.00±49.17	0.326
HDL	44.50±12.87[a]	27.70±9.25[b]	23.40±9.40[b]	26.00±8.95[b]	**0.000**
LDL	36.60±14.80[a]	21.60±7.15[b]	16.20±6.67[c]	23.10±10.12[abc]	**0.013**

TC = total cholesterol, TG = triglyceride, HDL= high-density lipoprotein, LDL = low-density lipoprotein. Data are presented as the mean ± SD, SD: standard deviation. Different letters in the same line are statistically significant (P<0.05)

Protein Profile Findings

Table 3 displays the protein profile findings. The TP levels of the calves with diarrhea in the mild, moderate, and severe groups were found to be higher than the control group, but this difference was not statistically significant. Similarly, ALB levels in calves with diarrhea in the moderate and severe groups were higher than in the control group, but this difference was not statistically significant.

Table 3. Total protein, albumin, liver enzymes, direct bilirubin and glucose levels in the groups.

Parameters	Control (n=10)	Mild (n=10)	Moderate (n=10)	Severe (n=10)	P
TP	6.12±1.03	6.70±1.22	7.02±1.78	7.78±1.82	0.242
ALB	2.66±0.15	2.60±0.26	2.75±0.28	2.96±0.64	0.266
AST	39.70±15.80[a]	48.50±18.52[ab]	63.80±27.04[b]	159.70±164.33[c]	**0.002**
ALP	231.10±79.65[a]	313.10±175.17[ab]	294.40±93.74[ab]	496.90±207.80[b]	**0.017**
DBIL	0.12±0.12	0.15±0.14	0.21±0.20	0.11±0.12	0.515
Glucose	114.20±31.31[a]	83.30±22.17[b]	81.30±27.96[b]	87.60±67.20[b]	**0.040**

TP = total protein, ALB = albumin, AST = aspartate aminotransferase, ALP = alkaline phosphatase, DBIL = direct bilirubin Data are presented as the mean ± SD, SD: standard deviation. Different letters in the same line are statistically significant (P<0.05)

Liver Enzymes, Direct Bilirubin and Glucose Levels

Table 3 displays the levels of liver enzymes, DBIL, and glucose. The AST levels of the calves with diarrhea in the moderate and severe groups were higher than the control group, with the severe group having the highest AST levels (P=0.002). Calves in the severe group had the highest ALP levels (P=0.017). It was determined that there was no statistical difference among the groups' DBIL levels. All diarrheic calves had lower glucose levels than the control group (P=0.040).

DISCUSSION

The purpose of this study was to evaluate the serum lipid and protein profile, as well as liver enzyme levels, in neonatal diarrheic calves based on the clinical course of the disease. When compared to the control group, TC, HDL, LDL, and glucose levels decreased while AST and ALP levels increased in calves with diarrhea. It was found that as the clinical severity of the disease increased, so did the level of liver enzymes.

Calf diarrhea affects not only the gastrointestinal system but also other system or organ functions such as the lung, kidney, and liver (Sobiech et al., 2013). Calf diarrhea, like infectious and inflammatory diseases, can also cause changes in serum lipid and protein profiles (Tall and Yvan-Charvet, 2015; Tóthová et al., 2016). In the present study, a significant decrease was determined in the TC, HDL and LDL levels of the calves with diarrhea compared to the control group. However, no statistical significance was determined in the intergroup comparison of these reductions among calves with diarrhea. There was no difference in TG levels between the groups. It was thought that these data could be used as an indicator of infection in calves. Lipopolysaccharides have been shown to be neutralized by lipoproteins (Barati et al., 2011; Morin et al., 2015) and thus changes in lipid levels may be an important indicator of acute bacterial infections (Nassaji and Ghorbani 2012). Furthermore, significant changes in plasma lipid and lipoprotein concentration, composition, and function have been observed in humans (Alvarez and Ramos 1986; Wendel et al., 2007; Barati et al., 2011; Cirstea et al., 2017), calves (Civelek et al., 2007; Joshi et al., 2015; Bozukluhan et al., 2017; Aydogdu et al., 2018) and dogs (Yilmaz and Sentürk, 2007) during inflammation and infections, as a decrease in TC, LDL, and HDL levels and an increase in TG levels. The changes in the lipid profile observed in our study were thought to be caused by cytokine release (Hardaróttir et al., 1994; Fraunberger et al., 1999; Khovidhunkit et al., 2000; Murch et al., 2007; Lekkou et al., 2014; Morin et al., 2015; Albayrak and Kabu, 2016). In support of this view, El-Bahr and El-Deep (2013) reported that the cytokine levels in buffalo calves with bronchopneumonia were significantly higher than in healthy calves, while serum TC, HDL and LDL levels were significantly lower. Similarly, in cases of inflammation, it has been reported that while TC levels decrease, cytokine levels such as TNF-α and IL-6 increase (Akgün et al., 1998; Gordon et al., 2001; Lekkou et al., 2014). Sepsis has also been linked to an increase in TG levels in humans and animals, which may be due to the induction of hepatic and adipose tissue lipolysis as well as an increase in VLDL production (Alvarez and Ramos, 1986; Civelek et al., 2007).

Changes in protein profile often occur as secondary manifestations in many diseases. Determining their concentrations can provide important information for differential diagnosis to the clinician (Bartosz and Katarzyna, 2016). High TP levels may indicate higher colostral protein levels in young animals, as a favourable indicator (Marcato et al., 2018), however high TP and ALB levels may indicate dehydration, as an unfavourable indicator (Knowles et al., 1999; Swanson and Morrow-Tesch, 2001). ALB can be used as a prognostic marker or to evaluate the severity of diseases, in addition to measuring dehydration (Humblet et al., 2004; Schneider et al., 2013). Low ALB concentrations in dairy cattle, for example, have been linked to uterine infections (Schneider et al., 2013) and inflammation (Jacobsen et al., 2004). However, it is necessary to investigate whether the increase or decrease in ALB levels in young animals is related to the disease or to future health problems (Marcato et al., 2018). In this study, although there was no significant difference between TP and ALB levels of calves with diarrhea and healthy calves, it was determined that these values were higher in calves with diarrhea and this level was directly proportional to the clinical severity of diarrhea. Dehydration was suspected as the cause of this situation.

A systemic inflammatory response that may develop as a result of infection or endotoxemia can damage and impair the functions of the circulatory system and vital organs. The liver is one of these essential organs (Ramachandran, 2014). On the other hand, it has been reported that new-born calf diarrhea can cause liver function damage, as well as severe necrotic and dystrophic lesions. (Grodzki et al., 1991). Increases in serum creatinine, total bilirubin, DBIL, gamma glutamyl transferase (GGT), AST, and ALT levels have been reported in calves with diarrhea (Irmak and Güzelbekteş, 2003; Russel and Roussel, 2007; Kaneko et al., 2008; Başer and Civelek, 2013; Merhan et al., 2016). In our study, there was no difference between DBIL levels of calves with diarrhea and healthy calves, but AST and ALP levels of calves with diarrhea were found to be significantly higher than those of healthy calves. It was found that the clinical severity of the condition correlated with an increase in this elevation. In this case, it was concluded that liver damage occurred in calves with new-born diarrhea, and that the severity of liver damage correlated with the severity of the disease. Similarly, Grodzki et al. (1991) reported that there were significant increases in ALP levels in calves aged 1-10 days with diarrhea and liver damage could occur in calves with diarrhea. Bozukluhan et al. (2017) also found significant increases in ALP, total bilirubin and DBIL levels in calves with diarrhea compared to the control group, and they suggested that these findings indicate liver and biliary tract damage in calves with diarrhea. The relationship between hypoglycemia and septicemia/endotoxemia in neonatal calves has been demonstrated in experimental studies (Ballou et al., 2011; Constable et al., 2017). In line with these reports, the current study revealed a significant decrease in glucose levels in calves with diarrhea compared to healthy calves.

CONCLUSION

In the present study, it was concluded that there was no change in the protein profile in calves with neonatal diarrhea, the lipid profile was adversely affected and liver function was impaired, and as the clinical severity of the disease increased, the impairment in liver function also increased.

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Ethical approval:

This study was carried out per Atatürk University's approved ethical rules (protocol no. 2018/50 date: 06/04/2018), and written informed consent was obtained from the owner for each calf.

Conflict of interest: The authors declare no conflict of interest.

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