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Carnitine concentrations in healthy and septicaemia suspected neonatal calves and its relation to passive immunty

Erhan GÖKÇE[1], Mahmut SÖZMEN[2], Canan GÜLMEZ[3], Kadir BOZUKLUHAN[4], Gürbüz GÖKÇE[1], Emine ATAKIŞI[5], Mehmet ÇITIL[6], Hidayet Metin ERDOĞAN[7,*]

[1]Department of Internal Medicine, Faculty of Medicine, Kafkas University, Kars, Turkey
[2]Department of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey
[3]Department of Pharmacy, Tuzluca Vocational School, Iğdır University, Iğdır, Turkey
[4]Kars Vocational High School, Kafkas University, Kars, Turkey
[5]Department of Biochemistry, Faculty of Medicine, Kafkas University, Kars, Turkey
[6]Department of Internal Medicine, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey
[7]Departmet of Internal Medicine, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

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Abstract: This study was designed to determine carnitine concentrations in newborn calves with suspected septicaemia, healthy calves, and colostrum samples as well as to detect alterations in blood carnitine concentrations after colostrum intake. Carnitine concentrations were detected in both neonatal calves with suspected septicaemia (n = 195) and healthy calves (n =10) as well as in colostrum/milk samples from their dams (n = 20). The mean carnitine concentration on the 2nd day after colostrum intake (32.61 µmol/L) was significantly higher than that measured before colostrum intake (17.61 µmol/L) ($P < 0.05$). The striking result was significantly lower mean carnitine concentration detected in calves with suspected septicaemia (17.11 µmol/L), compared to healthy calves (24.92 µmol/L) ($P < 0.001$). Apart from a sudden increase on day 7, no significant alteration was observed in colostrum/milk carnitine concentrations throughout the postpartum period. The results indicated that colostrum contained carnitine, which passed into the bloodstream of the calf by passive colostral transfer, and that a dramatic reduction in blood carnitine concentrations of calves with suspected septicaemia existed. The results might be of help in dealing with septicaemia suspected neonatal calves through both measurement of blood carnitine and, in response, to supplement such cases with carnitine.

Key words: Carnitine, septicaemia, neonatal calves, passive immunity, colostrum/milk

1. Introduction

Septicaemia, defined as the existence of microorganism or microbial toxins in the bloodstream due to a systemic disease [1], is closely linked to systemic inflammatory response syndrome (SIRS) triggered by the activation of inflammatory mediators [2], which might eventually lead to multiple organ dysfunction and high mortality if left untreated [3]. Septicaemia is manifested by several clinical findings, including abnormal fever, bradycardia or tachycardia, tachypnoea, and abnormal leukocyte count. Although extensively studied in human, there exists no widely eccepted model for the diagnosis and prognosis of septicaemia or sepsis [4].

In large animal medicine, a few study involving septicaemia exist and the majority of these studies lacked bacterial diagnoses [5] and had diagnosis mostly relied on clinical examination [6,7] and postmortem findings in euthanized or dead calves suspected of septicaemia [1,8]. In a recent study [9], confirmation of sepsis was based on SIRS, clinical signs, and bacterial culture results performed after death of cases in which a local infection such as omphalitis or swollen joints, was noted. Furthermore, only a few study has used clinical and laboratory models, and there is no internationally accepted definition of septicaemia [1,5,7,10]. In veterinary medicine, a complete blood cell count and scores combined with clinical findings aid in the diagnosis of sepsis/septicaemia. Haematological analyses increase diagnostic accuracy [7] as related haematological abnormalities are known to vary with the severity of the septicaemia. Commonly observed findings include an increased number of immature cell forms (band, myelocyte, metamyelocyte), toxic neutrophil and abnormal neutrophil counts (neutrophilia or neutropenia) [6,11,12].

In the neonatal period, particularly during the first week of life, high morbidity and mortality rates are

* Correspondence: hmerdogan@hotmail.com

observed in calves [13,14]. The risk of mortality associated with infection range between 15-30% but poor herd management practices may increase the mortality rate up to 50%. Concurrent inadequate passive colostral immunity (PCI) may increase the prevalence of neonatal septicaemia up to 30%, and may decline the survival rate to 12% in calves [7,15]. The only defence mechanism of newborn calves against neonatal septicaemia is PCI. The level of PCI is determined by the direct measurement of the blood IgG concentration. Studies have mainly focused on the role of IgG in preventing infections of neonates [14,16]. However, in practice some newborns with adequate transfer of colostral IgG acquire infection and some with insufficient transfer of IgG resist infection. This fact adds credential to the idea that significant components other than IgG in colostrum may ptotect neonates against microbial disease. Some of these companents may include cytokine, acute phase protein, and lactoferrin [17,18]. One of these protective companents may also be carnitine. The detection of significant alterations in any parameter after colostrum intake suggests a possible association of this parameter with PCI [19].

Septicaemia/sepsis progresses rapidly in newborn calves, unfurtunately usually results in death. In such cases, it is essential to initiate antimicrobial and supplementary treatment as early as possible. Because, each hour of delay increases the mortality rate by 6% [1]. In this respect, recent studies have focused on the use of novel biomarkers for the early diagnosis of septicaemia and the development of an optimum treatment strategy. One of these novel biomarkers could be carnitine. While L-carnitine concentrations have been investigated in septic human neonates [20], no study has been performed in neonatal calves with regard to septicaemia.

L-carnitine (gamma-triethylamine-beta-hydroxybutyric acid, vitamin $B_{T)}$ is synthesized from amino acids in the liver, brain and kidneys, and has a major role in the generation of energy from fatty acids in the mitochondria [21–23]. In blood, 80% of carnitine is found in free form and the remaining is in ester form and is delivered to tissues by blood [24]. Carnitine serves two main functions: i) a catalytical function in the transport of fatty acids into the mitochondria, ii) a metabolic function by buffering excessive esters. Carnitine is also reported to affect the immune system by increasing the cellular antioxidant capacity, inducing phagocytic activity in granulocytes, activating monocytes/macrophages, increasing antibody concentrations (i.e. IgA and IgG) through the stimulation of T and B lymphocytes, accelerating phagocytosis, increasing the production of cytokines such as TNF-α, IL-2ß and IL-6, and increasing or regulating the acute phase reaction [25–28]. The determination of free carnitine and carnitine ester in the blood provides insight on carnitine metabolism, which aids in the prevention of metabolic diseases and the development of treatment protocols for clinical practice [23,29]. Septicaemia and microbial agents can lead to impaired lipid metabolism and hepatic energy generation due to fatty acid oxidation [20,27]. This, in result, may cause a risk of carnitine deficiency in critically ill newborn calves.

Available information suggests that carnitine may provide a protective effect against neonatal diseases in calves, yet to date, this topic has not been studied in the veterinary field. In view of the paucity of information, this study was aimed at the determination of carnitine levels in newborn calves with suspected neonatal septicaemia, healthy calves, and colostrum samples. Furthermore, this study targeted, for the first time, the detection of alterations in neonatal blood carnitine concentrations after colostrum intake. Thereby, the significance of carnitine in terms of the pathogenesis and prognosis of neonatal calf diseases was ascertained.

2. Material and methods

Suspected septicaemia group: The study included 195 septicaemia suspected neonatal calves admitted or referred to the Teaching Hospital of Faculty of Veterinary Medicine, Kafkas University by owners or veterinary practitioners in the Kars region. A modified version of the previously described model was used for the selection of cases [5-7,30]. The inclusion criteria for suspected septicaemia were: (1) the presence of local or generalized infection (enteritis, pneumonia, swollen joints or omphalitis etc.), (2) existence of at least two findings related to SIRS, and (3) manifestation of at least two clinical signs related to septicaemia.

SIRS related inclusison criteria comprised of abnormal rectal temperature [hypothermia or hyperthermia, (reference interval 36-39.5 ˚C), heart rate (tachycardia: > 160 beats/min or bradycardia: < 90 beats/min), respiratory rate (tachypnoea: > 45 breaths/min), and leukocyte count, i.e. leukopenia or leukocytosis (reference intervall, 4–12 10^9/L) as reported previously [1,5].

Septicaemia related clinical signs used for inclusion were poor or absence of suckling reflex, calf posture or attitude (lethargy, fatigue, lateral recumbency), observation of any of the following: full congestion, marked hyperaemia, cyanosis, anaemia, petechiae, haemorrhage or hypopyon of mucosal or scleral vessels/membranes, severe dehydration, prolonged capillary refill time or low urine output, and weakness or depression [1,9,30,31]. Calves were grouped according to age on admission as 1, 2, 3–4, 5–7, 8–14 and 15–28 days old.

Treatment protocol: These calves did not receive any treatment before admission to the hospital. Ill calves were subjected to a standardized treatment protocol

according to presenting clinical problem, which included intravenous fluid therapy (performed according to the patients' requirements), administration of appropriate antimicrobials, nonsteroidal antiinflammatory drugs, and supportive care on admission [31]. Clinical examinations, selection of the cases, and treatment were all performed by the first author.

Healthy group: This group involved ten calves, raised on the dairy farm of the Faculty of Veterinary Medicine, Kafkas University, and were confirmed to be healthy based on laboratory and clinical examinations. A total of twenty healthy cows, ten of which were dam of the healthy group, from the same farm was also used for colostrum and milk sampling.

Haematological evaluation: Blood samples were collected into 2-mL EDTA coated tubes and analysed in an automated haematology cell counter (Hemocell Counter MS4e, Melet Schloesing Laborators, France) within 5 min of blood collection. Following leukocyte count, three blood smears were prepared for each calf from the fresh blood samples collected into EDTA coated tubes for leukocyte analysis. The smears were air-dried, methanol fixed, Giemsa stained and examined under a light microscope (Nikon Eclipse E600, Japan) at ×100 magnification. Thereby, degenerative changes in WBCs were assessed semiquantitatively. The percentage (%) of metamyelocytes or myelocytes in the calves with suspected septicaemia was evaluated as follows: ≤ 2% (normal or slight elevation) and > 2% (mild/marked increase). The band neutrophil percentages were categorized as: ≤ 2%, 3%–10%, 11%–20%, and > 20%. Toxic changes in neutrophils were determined based on the percentages of cytoplasmic basophilia, toxic granules, Dohle bodies, and dark blue grey foamy cytoplasm. The percentage (%) of toxic neutrophils were categorized as: ≤ 1% and 2%.

Collection of blood samples: Blood samples were collected from healthy and sick calves before treatment from all calves by jugular vein puncture into 5-mL clot activated tubes and EDTA coated tubes (BD Vacutainer, BD, Franklin Lakes, NJ, USA). Serum and plasma were harvested by centrifugation at 4000 rpm and 2000 rpm, respectively, for 10 min and stored at −20 °C until analyses. In order to monitor carnitine and IgG concentrations, 10 healthy neonatal calves were also blood sampled before (precolostral time = 0 h) and after colostrum intake (on days 1, 2, 3–4, 5–7, 8–14, and 15–28) as planned beforehand. The healthy calves were bottle-fed with colostrum in an amount equivalent to 10% of their body weight.

Serum IgG and glucose analysis: Serum IgG concentrations were measured using a commercial ELISA kit (Bio-X Elisa Kit for Bovine Immunoglobulin Assays-BIO K 165, Bio-X Diagnostics, Rochefort, Belgium). Serum glucose levels were measured spectrophotometrically using a commercial kit (Tanı Medical, Ankara, Turkey, TML) on an autoanalyser (Roche Cobas C501, Roche Diagnostic, Germany).

Plasma carnitine measurement: The method described by Galan et al. [32] for the measurement of plasma L-carnitine concentrations is based on a reaction catalyzed by carnitine acetyltransferase (CAT) (EC.2.3.1.7) acting on L-carnitine. The acyl group of acyl-CoA is transferred to carnitine by CAT. Coenzyme A reacts with 5,5-dithiobis (2-nitrobenzoic acid) to form the phenolate ion. The formation of phenolate is proportional to the amount of L-carnitine and measured by a spectrophotometer at 405 nm. This method was later modified by Prieto et al. [33]. The methods described by Galan et al. [32] and Prieto et al. [33] were used to measure the plasma concentrations of free carnitine in calves with suspected septicaemia and healthy calves.

Preparation of colostrum samples: Colostrum samples were taken from healthy cows (n = 20) into 10 mL glass tubes (Venoject, Code: VT-100SU, Terumo) within 0–4 h of parturition. Colostrum with IgG > 50 g/L was considered as good quality. Of the 20 cows, 10 were the dam of those healthy calves used as control. Milk samples were taken from the same cows on postpartum days 1, 3, and 7. Milk and colostrum samples in tubes were centrifuged upside down at 4000 rpm for 30 min to remove fat and sediments. The samples were stored in 1.5 mL eppendorf or microcentrifuge tubes at −20 °C until analysis. Prior to analysis, the colostrum and milk samples were diluted in physiological saline (10-fold for milk and 100-fold for colostrum analysis) and filtered twice through a nylon-66 syringe filter (0.45-µl MILLIPORE, USA) to obtain transparent samples [18,34]. Transparent samples were used for the analyses.

Colostral/Milk IgG and free carnitine analyses: Colostrum and milk IgG concentrations were tested using the same kit as serum IgG measurements. Colostrum and milk carnitine concentrations were measured spectrophotometrically as described by Prieto et al. [33] and Galan et al. [32]. Carnitine and IgG concentrations were expressed as µmol/L and mg/dL, respectively.

Statistical analysis: The statistical comparison of the data was performed using the SPSS software program (SPSS 18.0, Chicago, IL, USA). The distribution of the data was tested for conformity to a normal distribution by the Shapiro–Wilk test. Afterwards, the comparison of two groups was performed with the independent sample T-test. One-way ANOVA and Tukey's HSD test were used to compare multiple groups. The statistical differences between the different days were determined with repeated-measures ANOVA and the Bonferroni method. Pearson correlation was used to determine correlation between carnitine and IgG concentrations. Data are expressed as mean ± SE, and the statistical significance was set at $P < 0.05$.

3. Results

A total of 195 cases were admitted or referred to the hospital. Number of cases determined on days 1, 2, 3–4, 5–7, 8–14, and 15–28 were 26 (13.3%), 40 (20.6%), 47 (24.1%), 36 (18.5%), 17 (8.7%), and 29 (14.9%), respecticely. The majority of cases (76.4%, 149/195) were in their first week of life. The case fatality rate was 31% (60/195) and 61.7% (37/60) of the deaths occurred within their first week of life. Of these cases, 56.9% (111/195) were diagnosed with diarrhoe, 16.4% (32/195) with pneumonia, 6.1% (12/195) with omphalitis, 6.1% (12/195) with pneumonia-enteritis, 4.1% (8/195) with omphalitis-enteritis, 3.6% (12/195) with pneumonia-omphalitis and 6.6% (13/195) with unkonw reason (Table 1). Case fatality rate was 26.1% (29/111) for diarrhoea, 40.6% (13/32) for pneumonia, 16.7% (2/12) for omphalitis, 50% (6/12) for pneumonia-enteritis, 37.5% (3/8) for omphalitis-enteritis, 14.3% (1/7) for pneumonia-omphalitis and 46.2% (6/13) for unknown reason (Table 1).

Table 1. The frequency of clinical diagnosis and case fatality rate in neonatal calves.

Clinical diagnosis	Frequency (%)	Case fatality (%)
Diarrhoe	56.9 (111/195)	26.1 (29/111)
Pneumonia	16.4 (32/195)	40.6 (13/32)
Omphalitis	6.1 (12/195)	16.7 (2/12)
Pneumonia-enteritis	6.1 (12/195)	50 (6/12)
Omphalitis-enteritis	4.1 (8/195)	37.5 (3/8)
Pneumonia-omphalitis	3.6 (7/195)	14.3 (1/7)
Unknown	6.6 (13/195)	46.2 (6/13)

The difference between serum IgG concentrations determined before colostrum intake and after colostrum intake was statistically significant ($P < 0.001$) (Table 2). Following colostrum intake, a significant increase was observed in the plasma carnitine concentrations on day 2 (31.56 ± 0.79 μmol/L) compared to day 0 (17.04 ± 0.24 μmol/L). However, it did not differ in samples collected on the other days or periods (Table 2).

A negative correlation was determined between IgG and carnitine concentrations measured before colostrum intake (day 0) and after colostrum intake (day 1, day 2, days, 3–4, days 5–7, days 8–14, days 15–28), but this correlation was not statistically significant (Table 3).

Comparison of the carnitine concentrations measured in the ill and healthy calves: The plasma carnitine concentrations of ill calves admitted on day 2, between days 3–4 and between days 8–14, were found to be significantly lower than those of healthy calves ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively). Similarly, carnitine concentrations of sick calves of day 1 were lower than the concentrations measured in healthy calves. However, this difference was statistically insignificant ($P = 0.059$). On the other days and during the sampling, the carnitine cocncentrations of the ill and healthy animals were observed to be similar (Table 4).

Failure of passive colostral transfer (IgG < 800 mg/dL) was determined to have developed in 57.89% of the calves with suspected septicaemia. Mean serum glucose level of the ill calves (71 ± 2.1 mg/dL) was significantly ($P < 0.001$) lower than that of healthy calves (105 ± 2.4 mg/dL). Similarly, plasma carnitine concentrations of ill calves (17.12 ± 0.10 μmol/L) were significantly lower than those of healthy calves (24.94 ± 0.26 μmol/L) ($P < 0.001$, Figure 1). In addition, the plasma carnitine concentrations of calves died (16.68 ± 0.14 μmol/L) and recovered (16.02 ± 0.08 μmol/L) were determined to be significantly lower than that of healthy calves (24.12 ± 0.24 μmol/L) ($P < 0.001$, Figure 1).

The assessment of the degenerative changes in the leukocytes (WBC) showed that carnitine concentration was 16.86 ± 0.09 μmol/L in the group with a peripheral blood myelocyte/metamylocyte rate of ≤ 2%, and was 14.64 ± 0.12 μmol/L in those of > 2%. These two groups were found to significantly differ in terms of carnitine concentrations ($P = 0.034$). The carnitine concentrations determined for different percentages of the groups of calves with band neutrophils detected in the peripheral blood [≤ 2%, 3%–10%, 11%–20% and > 20%, respectively] did not statistically differ ($P > 0.05$). The carnitine concentrations in calves with a toxic neutrophil incidence of ≤ 1% and of ≥ 2% were 16.8 ± 0.09 μmol/L and 14.46 ± 0.16 μmol/L, respectively. These two values were significantly different ($P = 0.049$) (Table 5).

Colostrum and milk analyses of carnitine: Carnitine concentrations measured in the first colostrum (14.48 ± 0.56 μmol/L) and in the samples collected on postpartum day 1 (14.08 ± 1.11 μmol/L) and day 3 (13.42 ± 1.04 μmol/L) (transition to milk period) were found to be significantly ($P < 0.001$) lower than that measured in milk, on postpartum day 7 (20.85 ± 3.12 μmol/L) ($P < 0.001$) (Figure 2).

4. Discussion

To the best of our knowledge, this is the first investigation on the monitorization of blood carnitine concentrations after colostrum intake during the neonatal period in healthy calves and the alterations and prognosis of blood carnitine concentrations in calves with suspected septicaemia.

The present study also disclosed critical importance of the first week of neonatal period as approximately 76% of calves developed diseases and 62% of them died in the first

Table 2. Plasma carnitine (µmol/L) and serum IgG (mg/dL) concentrations (Mean±SE) in healthy calves during the neonatal period (n = 10).

Days							
	0*	1	2	3–4	5–7	8–14	15–28
Carnitine	17.04 ± 0.2	23.22 ± 0.03	31.56 ± 0.77*	26.76 ± 0.85	21.24 ± 0.51	19.5 ± 0.48	23.22 ± 0.61
IgG	17 ± 2.2	1797 ± 193**	1953 ± 307**	1709 ± 230**	1236 ± 88**	1216 ± 51**	1094 ± 87**

day 0*= Precolostral time, significantly different from day 0 (*P < 0.05, **P < 0.001).

Table 3. Correlations between carnitine and IgG concentrations in healthy neonatal calves.

Days							
	0	1	2	3-4	5-7	8-14	15-28
Pearson correlation	–0.067	0.083	–0.477	–0.464	–0.060	–0.343	–0.73
Significance (2-tailed)	0.863	0.821	0.163	0.149	0.888	0.333	0.841

Table 4. Plasma activity of carnitine (µmol/L) concentrations (Mean ± SE) in healthy and ill calves during the neonatal period.

Carnitine	Neonatal days												
	N	1	N	2	N	3–4	N	5–7	N	8–14	N	15–28	N	Total
Healthy	10	23.22 ± 0.03	10	31.56 ± 0.07	10	26.76±0.85	10	21.24 ± 0.51	10	19.5 ± 0.48	10	23.22 ± 0.61	60	24.12 ± 2.72
İll	27	16.56 ± 0.25	40	14.82 ± 0.14***	45	16.38 ± 0.22**	37	20.4 ± 0.19	17	15.54 ± 0.34*	29	15 ± 0.17	195	16.56 ±0 .10***

Significantly different between the groups *=P < 0.05, **=P < 0.01, ***=P < 0.001.

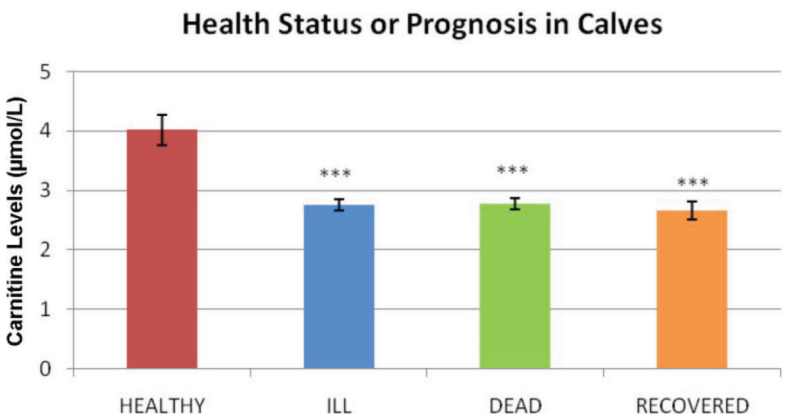

Figure 1. Carnitine concentrations according to the health status of the calves. *** P < 0.001.

week of life. This finding once again implies the paramount importance of the first week in the life of neonatal calves [19, 35, 36].

Blood carnitine levels measured before and after colostrum intake in this study suggested that colostrum contains a high concentration of carnitine and carnitine was readily absorbed from the intestines; however, we had no confirmation that carnitine could be used as a marker of passive immunity as it required demonstrating the distribution of carnitine levels in tissue fluids, intestinal

Table 5. Changes in carnitine concentrations according to degenerative leukocytes classification.

Leukocyte analysis (%)	Carnitine concentrations (µmol/L)								
Band neutrophisl	n	≤%2	n	%3-10	n	%11-20	n	%>20	P value
	38	15.12 ± 0.17	36	18.84 ± 0.29	45	16.86 ± 0.17	49	15.06 ± 0.13	
Myelocyte/metamelocytes	n	≤%2	n	>2%	NA	NA	NA	NA	
	121	16.86 ± 0.09	45	14.64 ± 0.12					0.034*
Toxic neutrophils	n	≤1%	n	≥2%	NA	NA	NA	NA	
	117	16.8 ± 0.09	31	14.46 ± 0.17					0.049*

*Statistically different, NA not applicable.

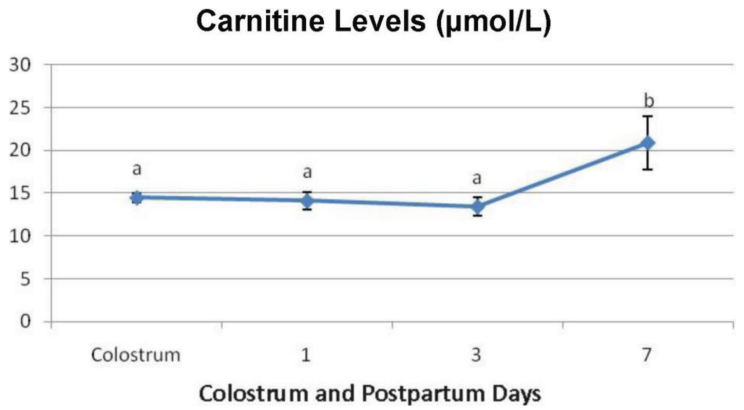

Figure 2. Carnitine concentrations in colostrum and transition milk (Postpartum days 1, 3, 7) a,b: $P < 0.001$.

receptors mediating the absorption of carnitine, and absorption kinetics of carnitine after colostrum intake [37], which was not studied. Nonetheless, available information indicates that carnitine, which has an important role in neonatal immunity, is synthesized at very low levels in newborn animals, and thus, is required to be supplied from colostrum or milk in the form of carnitine ester, which is converted to free carnitine in mitocondria [21, 38, 39]. This may explain the increases detected in blood carnitine concentrations after colostrum intake in our study as a significant rise was noted in blood carnitine concentrations of the healthy newborn calves after the second day of birth that might indicate higher level of maternal carnitine ester in colostrum. In spite of an increase in carnitine concentrations of milk on day 7, a parallel rise was not noted in blood carnitine concentrations. This divergence might be attributed to substantial reduction in intestinal absorption of colostral component, including carnitine, after 48 h of life [16, 18] or to carnitine metabolism, where milk content might not influence the blood concentration as it is mainly synthesized from amino acides in liver, kidney, and brain as calves age [21, 23]. However, it should be stated that carnitine absorbtion dynamics reqires further study as there exists inadequate research addressing to this issue.

The most remarkable result was a decline in blood carnitine levels of newborn calves with suspected septicaemia. Similarly, carnitine was lower in calves died and recovered of a suspected septicaemia when compared to healthy calves in this study. This decrease may be explained in three ways. The first of these may be inadequate amount of carnitine absorption along with colostrum as 57% of ill calves developed failure of passive transfer causing the colostrum to be either insufficient in quantity or poor in quality (containing low IgG, immuncomponents including carnitine, etc). It is well documented that following intestinal absorption colostral carnitine ester is converted to free carnitine in the liver of healthy calves [20, 21, 40], but those with suspected septicaemia, which mainly result in multiple organ failure, might not had the ability to synthesize enough carnitine as liver is usually affected [21,41,42]. The second explanation may be the loss of carnitine due to enteropathy as 44% of calves developed enteritis in our study. In the condition of entropaties, constituents of colostrum or milk were not sufficiently utilised due to disruption of glucose

and sodium absorption by the immature cells replacing damaged intestinal cells, which eventually results in impairments of colostrum absorption [43–46]. This finding may also indicate the likely role of carnitine deficiency in the pathogenesis of neonatal calf diseases, including septicaemia as it is reported to be associated with growth retardation and increased risk of infection [26,47,48] The third way may be the role of carnitine in fight against microorganisms, where carnitine is used up so the blood level decreases [20,26,41,49,50].

Newborn calves have a very limited hepatic glucose or energy reserve. Therefore, a poor maternal instinct, remaining wet, and unfavourable environmental conditions lead to rapid exhaustion of glucose reserve of the newborn, which eventually results in hypoglycaemia if calf does not receive sufficient quality and quantity of colostrum on time [5,51] as colostrum contains plenty of fats and glucose [52]. Carnitine has a major role in energy production. Carnitine deficiency may also have a significant role in the development of hypoglycaemia, which poses a high risk of mortality [5,53]. In calves with suspected neonatal septicaemia, the decrease in glucose level in parallel with carnitine could be a reflection inadequate colostrum intake or poor intestinal absorption as high passive colostral immune deficiency was detected in our study.

Use or discovery of novel biomarkers for clinical monitorization of diseases can significantly contribute efficiency to prophylactic and therapeutic strategies [20]. In the present study, the low blood carnitine levels detected in calves with suspected septicaemia may suggest that carnitine could be used as a novel biomarker. A similar previous study carried out in septic neonates in human reported that carnitine concentrations were lower than normal values and the detection of carnitine concentrations would aid in diagnosis, and therefore, carnitine supplementation would support treatment [20].

The assessment of the degenerative changes in the neutrophils showed that the > 2% rate of myelocytes/metamyelocytes and ≥ 2% rate of toxic neutrophils, both of which pointed out to septicaemia, had occurred in parallel with decreased carnitine concentrations. An increased rate of myelocytes/metamyelocytes and toxic neutrophils in the peripheral blood can be a direct indicator of septicaemia and is observed in cases with poor prognosis, where the bone marrow fails to respond to infections [6,12,54]. In our study, significantly decreased blood carnitine concentration observed in association with increased myelocyte/metamyelocyte and toxic neutrophils rates in the peripheral blood suggests that carnitine is related to septicaemia or the clinical severity of the disease. However, further studies are required to support this hypothesis.

The milk carnitine concentration on postpartum day 7 was found significantly higher than the levels detected in colostrum and postpartum day 1 and day 3. This finding contradict to that of Sato et al., [21] where high level of carnitine in colostrum is reported to decline during the transition to milk. However, Sato et al. [21] also reported that sudden increases may develop on postpartum day 6 where they measured carnitine on postpartum days of 2, 6, and 30. On the other hand, colostrum/milk carnitine levels were measured during the first week of the postpartum period in the present study.

Overall, this study demonstrated, for the first time, blood carnitine concentrations in newborn calves, which were healthy or suspected of having septicaemia, and the correlation of blood carnitine concentrations with passive colostral immunity. In calves, immunity is of utmost significance in protection against infections. The present study showed that, as an indicator of the active intestinal absorption of carnitine in newborn calves, the blood carnitine concentration on postpartum day 2 was higher than the level measured before colostrum intake. This study also demonstrated, for the first time, almost 50% decrease in the carnitine concentrations of calves with suspected septicaemia. Thus, it is suggested that the measurement of carnitine concentrations could aid in the diagnosis/prognosis of neonatal calf septicaemia and that dietary carnitine supplementation or parenteral administration would be of benefit in the treatment of these diseases.

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