

Non-invasive assessment of renal haemodynamics and tubular injury in diarrhoeic calves: the role of renal resistive index and novel biomarkers

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Abstract

Neonatal diarrhoea is a critical health problem threatening productivity, leading to growth retardation and high mortality rates in the cattle industry. While diarrhoea-induced fluid loss and dehydration can lead to acute kidney injury over time, traditional indicators such as serum creatinine may be slow to detect tubular damage. This study aimed to investigate the diagnostic utility of novel biomarkers such as renal resistive index (RRI) and kidney injury molecule 1 (KIM-1) for early detection of kidney injury in calves with diarrhoea. The study included 30 calves with diarrhoea and 20 healthy calves. Samples were taken before treatment and biochemical indices in blood and urine analysis and KIM-1, neutrophil gelatinase-associated lipocalin (NGAL) and interleukins were analysed using ELISA kits. In addition, non-invasive RRI measurements were performed on the right kidney by Doppler ultrasonography and the data were analysed by *t*-tests. Urea, creatinine, phosphorus and gamma-glutamyl transferase (GGT) values were significantly higher in the diarrhoea group compared to the control group. Especially in calves with diarrhoea, RRI values were measured as 0.84 on average and showed a significant increase with KIM-1 values ($P < 0.001$). In addition, positive correlations were also found between RRI values and albumin and IL-8. In conclusion, renal haemodynamic changes in the early stages of diarrhoea can be observed by increasing RRI. In contrast to traditional methods, the combination of KIM-1 and GGT with RRI enables the detection of subclinical tubular damage. This non-invasive and cost-effective approach contributes significantly to the early diagnosis of acute renal failure in calves.

Cattle, calf, diarrhoea, kidney, Doppler ultrasonography

Neonatal calf diarrhoea (NCD) is one of the most critical health issues threatening productivity and animal welfare in the cattle industry worldwide. Studies show that this disease accounts for more than 50% of total calf mortality and leads to both short and long-term production losses (Wilson et al. 2023). The disease is characterized not only by high mortality rates, but also by lasting economic damage such as reduced live weight gain and reduced first lactation milk yield. The aetiology of NCD is multifactorial, involving viruses, bacteria, and protozoa (Jessop et al. 2024).

Acute kidney injury (AKI) is defined as the sudden onset of renal parenchymal damage resulting in inadequate excretory, metabolic, and endocrine functions produced by the kidneys and is also known as acute renal failure. The conditions leading to this disease have different classifications including prerenal, intrinsic, and postrenal causes (Kellum et al. 2015; Langston 2017). Infectious agents in calf diarrhoea cause intestinal damage and significant fluid loss in the intestine which leads to rapid dehydration in the calf (Kasa et al. 2020). Due to this dehydration, electrolyte imbalance, metabolic acidosis, hypovolaemia and renal failure may occur over time (Shehta et al. 2022; Yanar et al. 2024). Human studies have reported that one of the most important causes of AKI in children is diarrhoea due to infectious agents (Girişgen and Yüksel 2019). However, traditional indicators such as serum creatinine tend to overlook tubular damage and only increase when a significant portion of renal function has already been lost, which may

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delay necessary treatment (Brooks et al. 1997; Ulutaş and Şahal 2005; Dratwa-Chałupnik et al. 2012). Furthermore, traditional indicators are heavily influenced by neonatal physiological changes during the first month of life. Physiologically, creatinine crosses the placenta and reflects maternal renal function within the first 24–48 h, and its baseline levels fluctuate due to the ongoing maturation of renal functions, low creatinine clearance, and immature tubules in the early neonatal period (İder et al. 2023). At the same time, early identification of AKI in diarrhoeic calves is not only essential for diagnosis but also serves as a critical prognostic indicator for treatment success. When initial pre-renal azotaemia progresses to established structural tubular damage, the risk of septicaemia and mortality increases significantly (Lofstedt et al. 1999; İder et al. 2023). Furthermore, the presence of AKI severely complicates standard rehydration protocols. For instance, failure to urinate due to acute renal failure during intravenous fluid administration can lead to unfavourable clinical responses, such as the development of life-threatening pulmonary oedema (Constable et al. 2021). Therefore, novel diagnostic tools and accurate prognostic biomarkers are urgently required to reliably detect subclinical kidney injury despite rapid age-related physiological shifts, predict clinical outcomes, and guide fluid therapy to avoid ineffective treatment strategies (Makdam and Başbuğan 2020; Tüfekçi and Vehbi 2025).

This study aimed to determine renal resistive index (RRI) values in calves as a noninvasive method and to evaluate the diagnostic utility of novel biomarkers such as RRI and kidney injury molecule 1 (KIM-1) as early indicators for renal injury in calves with diarrhoea.

Materials and Methods

This study was conducted with the approval of the Local Ethics Committee for Animal Experiments at Afyon Kocatepe University (Ref. No: 49533702-310).

Animals

The research involved neonatal calves aged between 2 and 30 days that were presented to the Afyon Kocatepe University Animal Hospital with clinical signs of diarrhoea. Clinical examinations were performed to exclude other possible disease agents, and no treatment was administered before sampling. Only calves exhibiting diarrhoea and clinically evaluated within a maximum of two days were included in the study. Faecal samples from the 30 diarrhoeic calves were tested for pathogens using the commercial *in vitro* Anigen® Rapid BoviD-5 Ag Test Kit (Bionote, Inc., Gyeonggi, Korea). The control group consisted of 20 healthy calves, aged between 1 and 30 days, that exhibited no clinical signs of illness. In total, 50 calves were enrolled in the study, with 30 assigned to the experimental group and 20 to the control group.

Sample collection

Blood samples were obtained from the experimental and control group animals via the jugular vein into gel and activator tubes. These samples were centrifuged at $3,000 \times g$ for 5 min to obtain the separated sera. These sera samples were transferred into Eppendorf tubes (Eppendorf AG, Hamburg, Germany). These tubes were marked with their respective identification numbers, along with the date each sample was obtained. These samples were preserved in frozen condition at -80°C until biochemical analysis could be carried out. Urine samples from both the experimental and control groups of calf animals were collected through cystocentesis via syringes with assisted abdominal ultrasound.

Biochemistry measurements

Measurements for urea, albumin, calcium (Ca), creatinine, gamma-glutamyl transferase (GGT), magnesium (Mg), and phosphorus (P) from blood and urine samples were conducted using an Abbott® Architect ci8200 (Illinois, USA) spectrophotometer.

ELISA measurements

The serum samples were assessed for β -hydroxybutyric acid (BHBA), cystatin C (CYS-C), interleukin-8 (IL-8), interleukin-18 (IL-18), interleukin-6 (IL-6), kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and retinol binding protein (RBP) using ELISA kits from Bioassay Technology Laboratory® (Shanghai, China, Cat. No: E0267Bo, E2053Bo, E0002Bo, E0318Bo, E0001Bo, E2315Bo, E0407Bo, E0297Bo respectively). The ELISA absorbant measurements were conducted using ELISA reader devices (BioTek Elx800 and ELx50 BioTek Instruments Inc. Highland Park, Winooski, VT, USA).

Renal resistive index measurements

The renal resistive index (RRI) measurements were performed on calves from both the experimental and control groups by placing the animals in lateral recumbency and conducting abdominal ultrasonography focused on the right renal fossa. To optimise image quality, the region at the junction of the last rib and vertebrae was shaved before imaging. The right kidney was visualised using a Doppler ultrasound device (Esaote® Mylab5, Genoa, Italy). The interlobar arteries were identified using Doppler ultrasonography with a 5–8 MHz microconvex probe, enabling the acquisition of pulse wave (PW) measurements. It has been reported that angular correction is generally negligible in RRI calculations when vessel diameter is considered, as RRI is derived from the ratio of peak systolic velocity to the difference between peak systolic and end-diastolic velocities (Rivers et al. 1996; Di Nicolò and Granata 2017). In the present study, all measurements were acquired without artefacts. The ultrasound system automatically calculated the RRI values after manually marking the peak systolic and end-diastolic velocity points.

Statistical analysis

In the study, independent *t*-tests and Pearson's correlation analysis were employed to identify the differences between the healthy and working groups. The data obtained from this research were analysed using the Windows SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA). A significance level of $P < 0.05$ was established for the analyses.

Results

Biochemistry

These results, obtained from the urine samples provided by both groups, showed a statistically significant difference in the levels of urea, creatinine, magnesium, phosphorus, and GGT, with higher levels in the study group than in the control group. However, no statistically significant difference was found in urinary albumin and Ca levels. According to the spectrophotometric measurements made on the blood serum samples in the study, a statistically significant difference was observed in the levels of urea, albumin, Ca, creatine, GGT, Mg and P and lower levels were detected in the control group compared to the study group (Table 1).

Table 1. Serum and urine indicators in the study and control groups (data are expressed as means $\pm$ SEM).

Indicator	Control	Study	<i>P</i>
Serum urea (mmol/l)	1.47 $\pm$ 0.60	9.66 $\pm$ 8.64	0.001
Urine urea (mmol/l)	31.73 $\pm$ 10.17	67.73 $\pm$ 16.59	0.001
Serum albumin (g/l)	9.60 $\pm$ 4.30	16.70 $\pm$ 5.50	0.001
Urine albumin (g/l)	2.50 $\pm$ 0.00	2.60 $\pm$ 0.40	0.412
Serum Ca (mmol/l)	0.96 $\pm$ 0.35	1.65 $\pm$ 0.45	0.001
Urine Ca (mmol/l)	0.50 $\pm$ 0.00	0.68 $\pm$ 0.49	0.128
Serum CRE (mmol/l)	0.025 $\pm$ 0.013	0.133 $\pm$ 0.102	0.001
Urine CRE (mmol/l)	0.896 $\pm$ 0.300	2.771 $\pm$ 1.120	0.001
Serum GGT (U/l)	61.50 $\pm$ 64.40	185.82 $\pm$ 206.21	0.017
Urine GGT (U/l)	3.00 $\pm$ 0.00	23.31 $\pm$ 36.55	0.024
Serum Mg (mmol/l)	0.30 $\pm$ 0.09	0.73 $\pm$ 0.35	0.001
Urine Mg (mmol/l)	0.21 $\pm$ 0.02	3.06 $\pm$ 1.23	0.001
Serum P (mmol/l)	1.06 $\pm$ 0.41	2.23 $\pm$ 1.11	0.001
Urine P (mmol/l)	5.51 $\pm$ 1.40	7.14 $\pm$ 2.30	0.011

CRE: creatinine; Ca: calcium; GGT: gamma-glutamyl transferase; Mg: magnesium; P: phosphorus

ELISA

Regarding ELISA results, KIM-1 levels showed a statistically significant elevation in the diarrhoeic group compared to the control group (Table 2). However, no statistically significant differences were found for Cys-C, IL-8, IL-18, IL-6, NGAL, or RBP. Notably, there was a highly significant difference in RRI levels between the diarrhoeic group (mean: 0.84) and the control group ($P < 0.001$).

Table 2. ELISA-based measurements in the study and control groups.

Indicator	Control	Study	<i>P</i>
BHBA (mmol/l)	0.305 ± 0.065	0.276 ± 0.054	0.111
CYS-C (mg/l)	1.33 ± 0.42	1.43 ± 0.38	0.426
IL-8 (ng/l)	106.16 ± 24.85	118.18 ± 30.90	0.153
IL-18 (ng/l)	241.53 ± 55.18	285.82 ± 85.66	0.059
IL-6 (ng/l)	403.48 ± 89.35	432.23 ± 85.63	0.280
KIM-1 (ng/l)	212.91 ± 48.82	247.39 ± 42.07	0.021
NGAL (ng/l)	34.76 ± 8.67	36.46 ± 16.91	0.696
RBP (mg/l)	3.78 ± 1.22	4.43 ± 1.36	0.102
RRI	0.73 ± 0.07	0.84 ± 0.09	0.001

BHBA: β -hydroxybutyric acid; CYS-C: cystatin C; IL-8: interleukin-8; IL-18: interleukin-18; IL-6: interleukin-6; KIM-1: kidney injury molecule 1; NGAL: neutrophil gelatinase-associated lipocalin; RBP: retinol binding protein; RRI: renal resistive index

Renal resistive index

In the correlation analysis carried out between the study group and the control group concerning urinary findings, it was noticed that, with increasing RRI values, there was a marked increase in albumin contents. A significant relationship exists between these two variables, with a 95% confidence level (Plate I, Fig. 1). Positive correlations were found between RRI values in the study group and the control group, as well as between RRI values and IL-8 and KIM-1 values, ($P < 0.05$) (Plate II, Fig. 2). The correlation coefficients between the values in RRI for both the study group and the control group, regarding the levels of the biomarkers, showed a statistically significant relation in serum biochemical indices, namely, Ca, CRE, GGT, Mg, and P, ($P < 0.05$) (Plate III, Fig. 3).

Discussion

Albumin is recognized as a negative acute phase protein in most species. It can also be affected by factors such as inflammation and starvation (Cecilian et al. 2002; Jain et al. 2011). Albuminuria is a risk factor in acute kidney injury. Retrospective clinical studies have highlighted the importance of albuminuria in diagnosing acute kidney injury/insufficiency (Clark et al. 2011; Neyra et al. 2016; Levey et al. 2020).

On analysing the calf samples of the study group, it could be perceived that the contents of creatinine in the urine were increasing due to its increased density resulting from dehydration, in addition to decreased glomerular filtration rate (GFR). Upon analysing our own research, differences were identified between groups in terms of albumin, creatinine, blood, and protein contents (Table 1). These have contributed to higher albumin contents in the study group, which are considered to represent glomerular injury in the kidneys. Previous studies have indicated the presence of albuminuria in septicemic acute kidneys (Neyra et al. 2016). Its presence in the groups suggests its association with AKI. However, considering that diarrhoea in calves is of pathogenic origin, this condition may also be of purely inflammatory origin.

Recent developments in ultrasonography have enhanced the role of Doppler studies in diagnosing renal pathologies. To the best of our knowledge, this is the first study to investigate the combined use of Doppler-based RRI and novel biomarkers for the non-invasive assessment of renal haemodynamics in this species. It has also been observed in some studies that measurement values from only one kidney are sufficient to generate appropriate information about renal pathologies, thereby eliminating the need for

measurements from both kidneys. Studies have also revealed that there are no differences in measurement values between the right and left kidneys, with the upper limit being almost identical to that in humans at 0.63 (Barreiro-Vázquez et al. 2020). Observations from retrospective studies have highlighted a comparative increase in RRI measurement values with rises in serum creatinine and urea contents (Darabont et al. 2023). The importance of measurement in RRI values is especially emphasized in identifying AKI associated with septic shock (sepsis) due to its non-invasive property, which is not at all difficult to apply. Though there is an opinion about its importance in distinguishing between permanent renal injury in kidneys, its high value of more than 0.795 is more sensitive for identifying renal injury from analysis tests in urine. It is also proven to be exceedingly essential in determining whether AKI caused in these animals could return to normal or not (Di Nicolò and Granata 2019). In our research, RRI values in diarrhoeic calves (avg. 0.84) were significantly higher than in healthy ones. The RRI values obtained in our healthy control group provide a preliminary reference range that can be utilized by clinicians to distinguish physiological variations from pathological renal vasoconstriction in calves.

Kidney injury molecule-1 has been established as a critical biomarker for the diagnosis of AKI in humans and mice. In physiological conditions, KIM-1 is nearly undetectable and is enhanced threefold in AKI (Baillly et al. 2002; Ichimura et al. 2004). Studies have demonstrated a similar level of diagnostic potential in small animal veterinary practices, such as felines and canines, where enhanced KIM-1 concentrations indicate tubular damage (Bland et al. 2019). However, there is little information about KIM-1 expression in cattle. Our findings, therefore, add to the existing information about KIM-1, suggesting a considerable increase in its concentrations among diarrhoeic compared to normal calves. Moreover, there is a positive correlation between KIM-1 concentrations and RRI values. In contrast with serum creatinine, where filtration ability is mainly affected and presentation is often delayed, apparently, a cumulative increase of KIM-1 and RRI values acts as an indicator of subclinical tubular injury. This suggests that these indices can help detect kidney injury at an early, potentially reversible stage.

Although IL-6, IL-8, and IL-18 are systemic inflammatory mediators, their concentrations measured in the urine matrix play specific roles in diagnosing AKI. However, IL-18 is identified as a pro-inflammatory cytokine, causing inflammation, tissue damage, and ischaemia in organs (Lisowska-Myjak 2010). It is essential to state here that the expression levels of IL-6 and IL-8 are upregulated in both neonatal and adults suffering from sepsis. Likewise, in neonatal subjects, high expression has been noticed (Ercan et al. 2014). Previous studies on subjects with AKI have collectively evaluated inflammatory biomarkers in these subjects in general. In contrast, our research marks a significant step forward in its effort to specifically examine IL-6, IL-8, and IL-18 for assessing renal functionality in these subjects. On our investigation, we found an upregulation in IL-6 and IL-18 in the study group; however, there was no significance noticed compared to the control group. The absence of significant correlation with RRI confirmed the fact that this marker is not useful in diagnosing AKI or analysing kidney function in these subjects. Earlier investigations reported significance with subjects with AKI in respect to IL-8. In our study, it was determined that there was no statistically significant difference between the groups in IL-8 measurements. However, based on the significant correlation of IL-8 with RRI, it can be hypothesized that IL-8 levels in these patients can be used for the diagnosis of AKI. Anti-inflammatory cytokines, such as interleukins (IL-1, IL-6 and IL-8), are proinflammatory (Cray et al. 2009). Cytokine release varies depending on the type of pathogen (bacteria, virus) (Ganheim et al. 2007). In acute and chronic inflammatory events, an increase in cytokines, especially IL-1, IL-6 and TNF- α 1, causes the release of acute phase proteins in the liver (Akgül et al. 2019). In our study, there was no

significant difference in cytokines between the groups. However, when the results in calves with diarrhoea were examined, it was determined that cytokine contents were higher. In this case, although it cannot be directly associated with AKI, the data obtained are consistent with previous studies due to the increase in cytokines in inflammatory events due to diarrhoea.

Studies in humans have shown that NGAL is an important biomarker for the diagnosis of acute kidney injury, especially in ischaemic and toxic kidneys. Although there is no published data on the relationship between NGAL contents and changes in renal function in calves, studies in dogs have found that urinary NGAL contents are more specific than blood contents. However, it has also been found to increase in other types of bacterial infections (Schmidt-Ott et al. 2007; Clerico et al. 2012; Langston 2017). Our study has not revealed NGAL to be more specific than other biomarkers. Although there was an increase in NGAL contents in diarrhoea, no significant difference was observed compared to the control group. Compared to other studies, we can conclude that although there was an increase in NGAL contents in our study, the increase was not significant compared to other studies showing different results. However, the secretion of NGAL from neutrophils and its association with systemic inflammation (Paragas et al. 2012) should be taken into consideration in the evaluation of this increase. Considering that the calves in the study group had pathogen-induced diarrhoea, this may explain why NGAL was higher in this group than in the control group. Furthermore, no significant difference was found in relation to RRI, suggesting that NGAL is not to be considered an important biomarker for kidney function in calves.

Increased concentrations of cystatin C in the urine are considered indicators of tubular dysfunction. Cystatin C is regarded as a more accurate biomarker for estimating GFR levels compared to creatinine in human health. Despite the availability of literature pertaining to the utilization capacity of cystatin C, the literature mentioned contains some anomalies (Langston 2017). Experiments conducted on dog subjects showed that cystatin C proved to be an important marker to quantitate renal function and damage to kidneys, as the elevated concentrations were reflected in the damaged subjects (Jacob et al. 2002; Ko et al. 2021). In spite of its importance, the accuracy is affected by certain aspects, including weight and treatment with prednisolone (Miyagawa et al. 2020). An experiment conducted on diarrhoeic calf subjects showed that the contents were high, noting an important difference in the contents between various groups (Makdam and Başbuğan 2020). We observed that concentrations of cystatin C were high in diarrhoeic calf subjects compared to those in the control group. Although these studies share similarities with other research, statistically significant differences have also been identified. These studies indicated tubular injury in calf subjects experiencing diarrhoea.

Produced in high amounts in the liver and metabolized in the kidneys, RBP is considered a sensitive biomarker for the diagnosis of renal tubular damage. In human medicine, Yuan et al. (2016) reported elevated contents of RBP in patients with AKI compared to healthy participants and noted that contents decreased with clinical improvement. Similarly, veterinary studies on cats and dogs have shown that RBP contents increase with creatinine, especially in chronic conditions (Lisowska-Myjak 2010; Langston 2017). In our study, RBP contents were numerically higher in the diarrhoea group compared to the control group, reflecting a similar trend observed in other species. However, in contrast to previous studies, this elevation was not significant ($P > 0.05$). This indicates the presence of tubular damage, but suggests that the damage was not severe enough to cause a significant increase in RBP. Furthermore, the lack of a significant correlation between RRI and RBP may mean that RBP may represent a different pathophysiological pathway independent of the haemodynamic changes detected by RRI. Our findings suggest that RBP is ineffective when used alone as a diagnostic tool for renal function in calves, but its use in combination with other markers warrants further investigation.

It is thought that creatinine concentrations are affected by different variables such as age, sex, body mass index, hydration and GFR, and therefore, its evaluation alone is insufficient for the diagnosis of AKI (Lisowska-Myjak 2010). On the other hand, some studies have reported that serum creatinine concentration is an important consideration in the analysis of AKI in addition to staging (Kellum et al. 2015). On the other hand, another retrospective study emphasized the importance of serum creatinine concentration as an important criterion for the diagnosis of AKI. However, it was also pointed out that the accuracy of the diagnosis of AKI based on creatinine alone is questionable (Siew and Matheny 2015). This is because creatinine may vary depending on hydration status and muscle mass. When the data obtained in our study were evaluated, creatinine results in the diarrhoea group were higher than in the control group. These results are consistent with previous studies and support the relationship between AKI and creatinine concentration. In addition, a positive correlation was found between creatinine and RRI, emphasizing the importance of creatinine for AKI. However, relying solely on creatinine value may cause delays in diagnosis. Because, as reported in previous studies, the reason for the elevation of creatinine in the study group may be dehydration due to diarrhoea. Therefore, this study emphasizes that it is much more effective to evaluate RRI and KIM-1 instead of traditional biomarkers alone for the diagnosis of AKI.

The ability of RRI and KIM-1 to detect subclinical tubular injury has major prognostic implications for predicting treatment success in diarrhoeic calves. Traditional markers like creatinine often increase only after significant filtration capacity is lost. At this point, endotoxin-induced tubular necrosis or hypoxic-ischaemic damage may already have been established, increasing the risk of mortality and septicaemia by up to 8-fold (Lofstedt et al. 1999; Ider et al. 2023). In such advanced stages of AKI, conventional aggressive intravenous fluid therapies can result in treatment failure and complications such as pulmonary oedema due to anuria (Constable et al. 2021). Similar to the prognostic utility of other novel biomarkers in assessing disease severity and preventing ineffective treatment costs (Makdam and Başbuğan 2020; Tüfekçi and Vehbi 2025), the combined use of RRI and KIM-1 provides a critical window of opportunity. The early prediction of treatment failure risk enables clinicians to either discontinue intravenous fluid replacement therapy or individualize the treatment regimen prior to the development of irreversible structural damage and fatal complications.

In retrospective research, it has been recorded that urinary GGT can diagnose acute kidney injuries within a period of 4 to 12 h. They strongly felt that, according to the half-life of GGT, its principal characteristic or quality in chronic diseases is relative. Nevertheless, they maintained the critical analysis or examination of GGT is a primary biomarker or indicator for diagnosing acute kidney injuries, especially for acute diseases (Ralib et al. 2014; Pickering and Endre 2014). One more study showed that the analysis of GGT excreted in the urine is an optimal, economical, and feasible way to diagnose acute kidney injuries, acting as an alternative way for diagnosing such injuries (Masaroğulları et al. 2013). The study findings also authenticate similar perceptions, indicating the enhancement of GGT values within the experimental calves. The conformity or positive relation between GGT values and RRI, along with similar values for KIM-1 within the experimental group, authenticate its primary role for diagnosing acute kidney injuries within cattle.

From a buiatric perspective, the routine feasibility of measuring the investigated biomarkers under field conditions presents both challenges and practical opportunities. Currently, the measurement of novel biochemical markers such as KIM-1, NGAL, and interleukins relies heavily on laboratory infrastructure, requiring sample centrifugation, deep-freeze storage (-80°C), and ELISA readers (Ider et al. 2023; Tüfekçi and Vehbi 2025). Given that veterinary practitioners frequently manage neonatal calf diarrhoea on farms without immediate access to advanced laboratory analyses (Lofstedt et al. 1999;

Constable et al. 2021), the routine use of these biochemical markers for real-time clinical decision-making is presently limited. However, RRI assessment via Doppler ultrasonography offers a highly feasible alternative. As portable ultrasound devices become increasingly standard in large-animal practice, RRI provides a non-invasive, rapid, and highly practical method for evaluating renal haemodynamics directly at the calf side. For biochemical markers like KIM-1 to achieve similar routine applicability in bovine production systems, future research must focus on translating these laboratory-based assays into rapid, cow-side point-of-care test kits.

A notable limitation of this study is the relatively wide age range of enrolled calves (2 to 30 days), without further age stratification. The first month of life is a highly dynamic period for renal maturation, which can significantly affect the physiological baseline concentrations of kidney indicators. As reported in recent studies, certain biomarkers are initially present at higher concentrations due to ongoing renal maturation and progressively decrease with advancing age, while others increase in correlation with the growing number of functional nephrons and kidney mass (Ider et al. 2023). Although the lack of strict age stratification limits the establishment of age-specific baseline intervals, it is highly noteworthy that KIM-1 and RRI values showed profound statistical significance between the diarrhoeic and healthy groups despite these inherent age-related physiological variations. This underscores their robust diagnostic capability for identifying AKI in calves. Future large-scale studies incorporating age stratification are warranted to establish precise age-specific reference intervals for RRI and KIM-1 in the neonatal period.

This study aimed to determine RRI values as a noninvasive method for neonatal calves and to be one of the first references to determine the importance of its role in the diagnosis of AKI caused by diarrhoea. Within the scope of this study, histopathological evaluation of kidney damage in neonatal diarrhoeal calves could not be performed. Furthermore, the inability to conduct long-term follow-up and assessment of the severity of clinical findings in the calves were identified as limitations of our study. Our results show that in the early stages of diarrhoea cases, renal haemodynamic changes and AKI are observed in the kidneys and there is a significant increase in RRI. Furthermore, KIM-1 and GGT were significantly increased, suggesting that these markers, evaluated together with RRI, can be used to diagnose renal failure compared to traditional markers such as urea and creatinine. In contrast to these two markers, which can cause a delay in diagnosis, the use of KIM-1 and GGT in combination with RRI can identify subclinical tubular damage. Considering the non-invasive and cost-effective techniques of this method, using Doppler-based RRI measurement contributes significantly to the early diagnosis of AKI in the assessment of renal function in calves.

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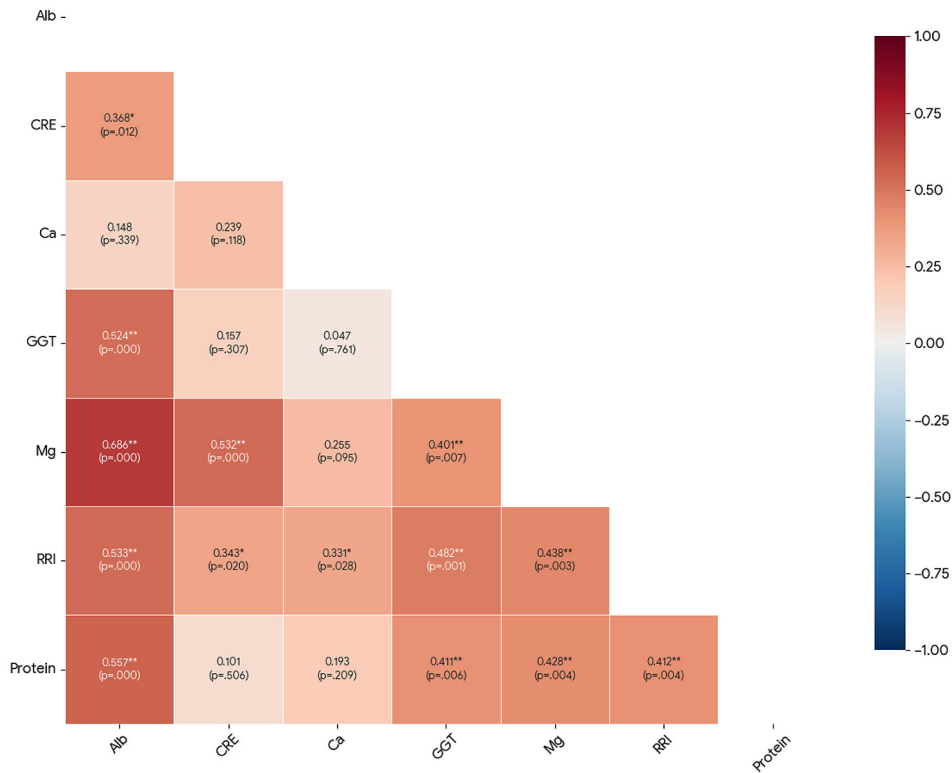


Fig. 1. Correlation heatmap showing the relationship between RRI (renal resistive index) and urinary indices in the study and control groups. Indicators in the matrix include Alb (albumin), CRE (creatinine), Ca (calcium), GGT (gamma-glutamyl transferase), Mg (magnesium), and protein. The upper value in each cell indicates the correlation coefficient (r_s), and the lower value in parentheses indicates the statistical P value. The colour scale represents direction (red: positive, blue: negative) and intensity of the correlation. * Indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.01$.

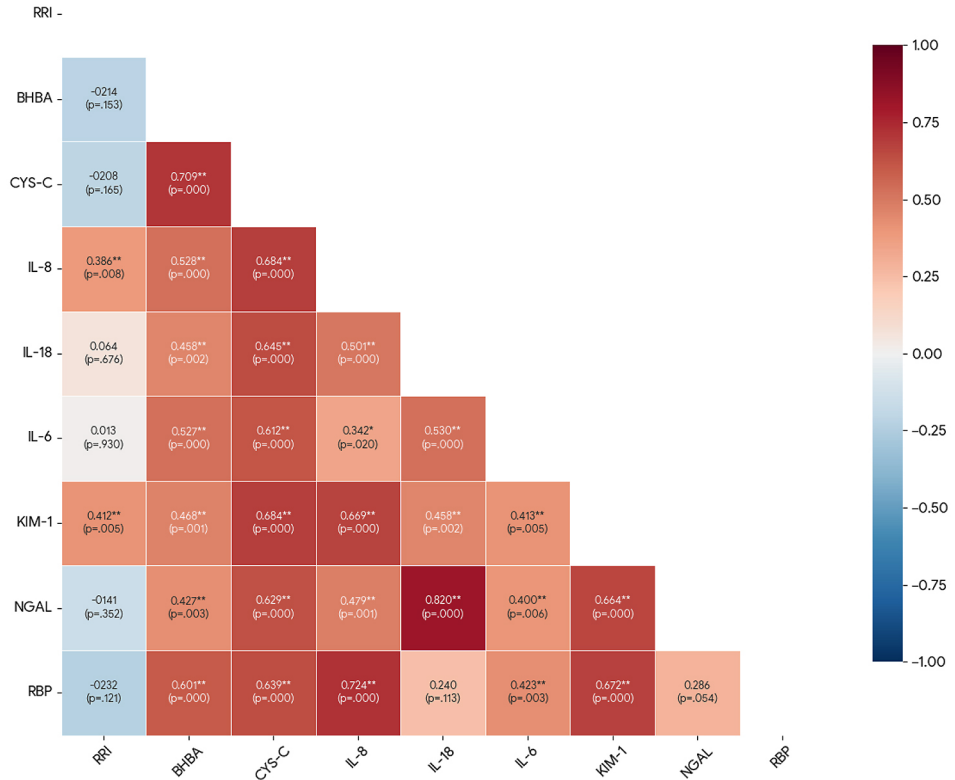


Fig. 2. Correlation analysis results between RRI (renal resistive index) and ELISA-based biomarkers. Analysed biomarkers include BHBA (β -hydroxybutyric acid), CYS-C (cystatin C), IL-8 (interleukin-8), IL-18 (interleukin-18), IL-6 (interleukin-6), KIM-1 (kidney injury molecule 1), NGAL (neutrophil gelatinase-associated lipocalin), RBP (retinol binding protein). Values on the heatmap include the correlation coefficient and P value. Dark red colours indicate strong positive correlation, while dark blue colors indicate strong negative correlation. * Indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.01$.

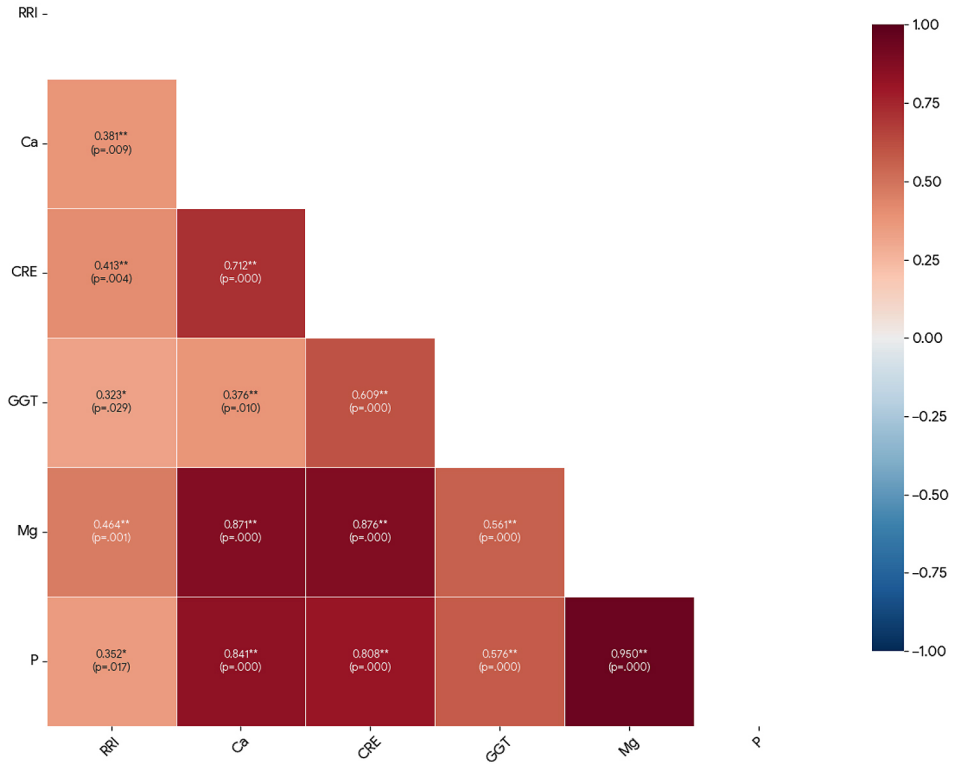


Fig. 3. Correlation matrix showing the relationships between RRI (renal resistive index) and serum indicators in the study and control groups. The variables include Ca (calcium), CRE (creatinine), GGT (gamma glutamyl transferase), Mg (magnesium), and P (phosphorus). Numerical data in the cells show the correlation coefficient (r_s) and the calculated P value. * Indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.01$.