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Early postnatal dynamics of nitrogen metabolism and renal biomarkers in summer-and winter-born Tharparkar and Vrindavani calves

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Abstract

The neonatal period is characterized by rapid physiological and metabolic adaptations, with renal function biomarkers providing valuable insights into kidney maturation and nitrogen metabolism. This study evaluated the early postnatal dynamics of serum creatinine, urea, and blood urea nitrogen (BUN) in summer-and winter-born Tharparkar and Vrindavani calves. Twelve healthy calves (six Tharparkar and six Vrindavani) were monitored from birth to 56 days of age at 14-day intervals. Blood samples were collected on days 0, 14, 28, 42, and 56 for estimation of serum creatinine and urea using commercial diagnostic kits, while BUN was calculated from serum urea values. Data were analyzed using multivariate analysis of variance to assess the effects of season, breed, postnatal age, and their interactions. Serum creatinine was significantly influenced by season ($p < 0.001$), breed ($p = 0.044$), and postnatal age ($p = 0.013$), with a significant breed $\times$ age interaction ($p < 0.001$). Summer-born calves, particularly Vrindavani, exhibited higher creatinine concentrations than winter-born calves. Serum urea and BUN were significantly affected by season ($p = 0.004$), breed ($p = 0.002$), and age ($p = 0.023$), whereas interaction effects were not significant. Overall, summer-born and Vrindavani calves recorded higher urea and BUN concentrations than winter-born and Tharparkar calves, and both biomarkers increased with advancing age, reflecting maturation of protein metabolism and renal function. The findings demonstrate that Tharparkar calves showed greater metabolic stability than Vrindavani calves under summer conditions, indicating superior adaptation to thermal stress.

Keywords: Neonatal calves, birth season, nitrogen metabolism, renal biomarkers, creatinine, urea, blood urea nitrogen (BUN), heat stress

1. Introduction

The neonatal period is a critical phase in the life of dairy calves, during which rapid physiological, metabolic, and renal adaptations are required to support the transition from intrauterine to extrauterine life. During the first few weeks after birth, the kidneys undergo functional maturation, enabling efficient regulation of water and electrolyte balance, nitrogen excretion, and maintenance of acid-base homeostasis. Consequently, circulating concentrations of creatinine, urea, and blood urea nitrogen (BUN) change markedly during early postnatal development and are widely recognized as reliable indicators of renal function, protein metabolism, and nutritional status in neonatal ruminants (Kaneko *et al.*, 2008; Radostits *et al.*, 2007) [11, 14].

Serum creatinine primarily reflects glomerular filtration rate and muscle metabolism. Neonatal calves generally exhibit relatively high creatinine concentrations immediately after birth due to placental transfer and fetal muscle metabolism, followed by a gradual decline as renal clearance improves and kidney function matures. In contrast, serum urea and BUN are influenced by dietary protein intake, hepatic urea synthesis, renal excretion, hydration status, and tissue protein turnover. As calves transition from dependence on colostrum to milk feeding and progressive rumen development, these metabolites undergo dynamic changes that reflect both metabolic adaptation and renal maturation (Mohri *et al.*, 2007; Jezek *et al.*, Kaneko *et al.*, 2008) [3, 11]. Increasing evidence indicates that prenatal environmental conditions can influence postnatal physiological development through fetal programming.

Maternal heat stress during gestation has emerged as an important challenge to dairy production because elevated ambient temperature alters placental function, fetal growth, endocrine regulation, nutrient partitioning, and organ development. These prenatal alterations may persist after birth, affecting growth performance, metabolic efficiency, immune competence, and organ function in offspring (Tao and Dahl, 2013; Dado-Senn *et al.*, 2020; Laporta *et al.*, 2020) [16, 8, 12]. Calves born during hot summer months are therefore more likely to experience altered metabolic and physiological responses than calves born under thermoneutral winter conditions. Nitrogen metabolism is particularly sensitive to both nutritional and environmental factors. Heat stress modifies feed intake, protein turnover, and nitrogen utilization, which may influence circulating concentrations of urea and BUN. Furthermore, dehydration and altered renal perfusion associated with thermal stress can affect serum creatinine concentrations. Evaluation of these biomarkers during the neonatal period therefore provides valuable information regarding renal maturation, metabolic adaptation, and the overall health status of calves exposed to contrasting prenatal thermal environments (Baumgard and Rhoads, 2013; Bernabucci *et al.*, 2010) [6, 7]. Breed-related differences also contribute substantially to the physiological response to environmental stress. Indigenous cattle breeds such as Tharparkar possess superior thermoregulatory capacity and greater resilience to tropical climatic conditions, whereas crossbred cattle such as Vrindavani generally exhibit higher susceptibility to heat stress because of their greater metabolic heat production and lower heat tolerance. Consequently, calves derived from these breeds may differ in renal function, nitrogen metabolism, and postnatal adaptation when exposed to different seasonal conditions during fetal development (Hansen, 2004; Sejian *et al.*, 2018; Pragna *et al.*, 2018) [9, 15, 13].

Despite extensive research on heat stress physiology in adult cattle, limited information is available regarding the combined influence of breed, birth season, and early postnatal age on renal function biomarkers in neonatal calves. Understanding the temporal patterns of serum creatinine, urea, and BUN during the first two months of life is essential for establishing physiological reference values and assessing the effects of prenatal environmental stress on neonatal adaptation. Therefore, the present study was undertaken to investigate the early postnatal dynamics of nitrogen metabolism and renal biomarkers in summer- and winter-born Tharparkar and Vrindavani calves from birth to 56 days of age.

2. Materials and Methods

2.1 Location of the experimental area and climatic condition

The experimental studies were conducted at the Cattle and Buffalo Farm, LPM Division, Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India. The region has a mean annual temperature of approximately 25 °C. June is the warmest month, with a mean monthly temperature of 32.8 °C, whereas January is the coolest month, recording an average temperature of 15 °C.

2.2 Experimental animals

The study involved one indigenous breed, Tharparkar, and one crossbred breed, Vrindavani. Vrindavani is a crossbred

and a synthetic strain of cattle with Haryana inheritance varying between 25 to 50% and exotic (Holstein Friesian, Brown Swiss, and Jersey) level of inheritance between 50 to 75% as given by Singh *et al.* (2011) [5]. Calves were classified as summer-born (June-July) and winter-born (January-February) according to the season in which they were born in which. These calves (n = 12; 6 Tharparkar and 6 Vrindavani) were monitored from birth through the neonatal period at an interval of 14 days for up to 56 days. Loose housing with a group management system with BIS standard housing space was followed with the provision of separate calf pens with open paddocks. The indigenous calves were allowed to suckle for 5 min until their dams were in milk, while crossbred calves were allowed to suckle for 5 min for the first three days to ensure adequate colostrum intake. Subsequently, they were provided milk for three months, which was hand-fed, followed by the provision of concentrate and ad libitum succulent fodder from the second week of age.

2.3 Blood Collection and Serum Separation

Blood samples were aseptically collected from the jugular vein of each calf into sterile plain vacutainer tubes on days 0, 14, 28, 42, and 56 after birth. The samples were allowed to clot at room temperature for 30-45 min and subsequently centrifuged at 3,000 rpm for 15 min to obtain serum. The separated serum was carefully transferred into sterile, labelled microcentrifuge tubes, examined for haemolysis, and stored at -20 °C until analysis. Serum creatinine and urea concentrations were estimated using commercial diagnostic kits, while blood urea nitrogen (BUN) was calculated from the measured serum urea values.

2.4 Analysis of the samples

2.4.1 Creatinine: Serum creatinine concentration was determined using a Creatinine Kit (Coral Clinical Systems) based on the Modified Jaffe's kinetic method. In an alkaline medium, creatinine reacts with picric acid to form an orange complex, and the absorbance was measured at 520 nm.

$$\text{Creatinine (mg/dl)} = \frac{\text{Change in Test Absorbance}}{\text{Change in Standard Absorbance}} \times 2$$

2.4.2 Urea: Serum urea concentration was estimated using a Urea Kit (Coral Clinical Systems) based on the Modified Berthelot method. Urea is hydrolyzed by urease to produce ammonia, which reacts with phenolic chromogen and hypochlorite to form a green-colored complex that is measured at 570 nm.

$$\text{Urea (mg/dl)} = \frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times 40$$

2.4.3 Blood Urea Nitrogen (BUN): Blood Urea Nitrogen (BUN) was calculated by dividing 2.14 by the urea value (mg/dl) obtained.

2.5 Statistical analysis

All experimental data were analyzed using multivariate analysis of variance (ANOVA) in RStudio Version (2025.09.2 + 418) to determine the effects of season, breed, days, and their interactions in calves. Results are expressed as mean $\pm$ standard error of the mean (SEM), and

differences were considered significant at the prescribed probability level.

3. Results

Serum creatinine concentration was significantly affected by season ($p < 0.001$), breed ($p = 0.044$), and days of observation ($p = 0.013$), with a significant breed $\times$ day interaction ($p < 0.001$). The highest creatinine concentration was observed in summer-born Vrindavani (SV) calves on day 14 (3.53 ± 0.74 mg/dl), whereas the lowest value was recorded in winter-born Vrindavani (WV) calves on day 42 (0.57 ± 0.16 mg/dl). Summer-born calves generally maintained higher creatinine concentrations throughout the study, whereas winter-born calves exhibited a decline during the early postnatal period, followed by variable changes

thereafter (Table 1). Serum urea and BUN concentration was significantly influenced by season ($p = 0.004$), breed ($p = 0.002$), and days of observation ($p = 0.023$), whereas all interaction effects were non-significant ($p > 0.05$). The urea concentrations ranged from 25.34 ± 2.40 to 38.30 ± 4.26 mg/dl and BUN values ranged from 11.84 ± 1.12 to 17.90 ± 1.99 mg/dl during the study period (Table 1). The highest value for urea and BUN was observed in summer-born Vrindavani (SV) calves on day 28, whereas the lowest value was observed in WT calves on day 14. Overall, the summer-born and Vrindavani calves exhibited higher urea and BUN concentrations than the winter-born and Tharparkar calves, and the urea levels tended to increase with age (Table 2 and 3).

Table 1: Season $\times$ Breed $\times$ Age effects on Nitrogen Metabolism and Renal Biomarkers of Calves (Mean $\pm$ SEM)

Creatinine (mg/dl)	0	14	28	42	56
ST	2.96 ± 0.67^{abc}	3.24 ± 0.42^{ab}	2.57 ± 0.29^{abcd}	3.14 ± 0.16^{ab}	3.05 ± 0.34^{ab}
SV	3.14 ± 0.28^{ab}	3.53 ± 0.74^a	2.1 ± 0.1^{abcde}	2.19 ± 0.25^{abcde}	2.1 ± 0.1^{abcde}
WT	1.05 ± 0.19^{cde}	1.43 ± 0.28^{bcde}	0.67 ± 0.19^{de}	2.95 ± 0.25^{abc}	2.48 ± 0.09^{abcde}
WV	3.05 ± 1.1^{ab}	1.52 ± 0.53^{bcde}	0.86 ± 0.16^{de}	0.57 ± 0.16^e	0.67 ± 0.1^{de}
Means with different superscripts in a row and column differ significantly ($0.05 \leq p < 0.1$)					
Urea (mg/dl)	0	14	28	42	56
ST	26.43 ± 0.78	31.96 ± 3.11	31.49 ± 1.8	31.68 ± 1.78	30.17 ± 2.38
SV	25.34 ± 2.4	37.59 ± 2.79	38.3 ± 4.26	31.49 ± 0.71	35.13 ± 1.17
WT	26.05 ± 1.16	25.39 ± 1.07	28.22 ± 2.31	26.86 ± 0.95	25.91 ± 1.12
WV	28.04 ± 1.78	29.27 ± 2.26	31.3 ± 0.29	31.3 ± 1.52	33.19 ± 7.21
BUN (mg/dl)	0	14	28	42	56
ST	12.35 ± 0.36	14.94 ± 1.45	14.72 ± 0.84	14.8 ± 0.83	14.1 ± 1.11
SV	11.84 ± 1.12	17.57 ± 1.3	17.9 ± 1.99	14.72 ± 0.33	16.42 ± 0.55
WT	12.17 ± 0.55	11.87 ± 0.5	13.19 ± 1.08	12.55 ± 0.45	12.11 ± 0.52
WV	13.1 ± 0.83	13.68 ± 1.06	14.63 ± 0.14	14.63 ± 0.71	15.51 ± 3.37

Table 2: Breed and Season effect on Nitrogen Metabolism and Renal Biomarkers of calves (Mean $\pm$ SEM)

Breed (Urea)		Breed (BUN)		Season (Urea)		Season (BUN)	
T	28.42 ± 0.66^b	T	13.28 ± 0.31^b	S	31.96 ± 0.97^a	S	14.93 ± 0.45^a
V	32.09 ± 1.09^a	V	15 ± 0.51^a	W	28.55 ± 0.85^b	W	13.34 ± 0.4^b
Means with different superscripts in a column differ significantly ($p < 0.05$)							

Table 3: Age effect on Nitrogen Metabolism and Renal Biomarkers of calves (Mean $\pm$ SEM)

Days	0	14	28	42	56
Urea	26.47 ± 0.76^b	31.05 ± 1.69^a	32.33 ± 1.57^a	30.33 ± 0.83^a	31.1 ± 1.96^a
BUN	12.37 ± 0.36^b	14.51 ± 0.79^a	15.11 ± 0.73^a	14.17 ± 0.39^a	14.53 ± 0.92^a
Means with different superscripts in a row differ significantly ($p < 0.05$)					

4. Discussion

Serum creatinine, an indicator of muscle metabolism and renal function, was significantly influenced by the season, breed, and age. Higher creatinine concentrations during early life likely reflect greater muscle catabolism and maternal influences, whereas subsequent declines indicate maturation of renal function and metabolic adaptation in neonatal calves (Knowles *et al.*, 2000; Mohri *et al.*, 2007) [2, 3]. The significant breed effect and breed $\times$ days interaction suggest genetic differences in muscle metabolism and postnatal adaptation patterns (Kalyani *et al.*, 2018) [1]. Higher creatinine concentrations in summer-born calves than in winter-born calves may be attributed to seasonal variations in metabolic activity, hydration status, and environmental stress, which are known to influence the biochemical parameters of blood in calves (Tejaswi *et al.*, 2022) [4]. Urea and Blood urea nitrogen (BUN) reflects protein metabolism, nitrogen utilization, and renal excretory

functions. The increase in urea and BUN concentration with advancing age may be attributed to enhanced dietary protein intake, increased ruminal and tissue protein metabolism, and maturation of hepatic urea synthesis in growing calves. Higher urea and BUN concentrations in summer-born calves suggest seasonal differences in nutrient utilization and metabolic activity (Tejaswi *et al.*, 2022) [4]. The significant breed effect indicates genetic variation in nitrogen metabolism and protein turnover in calves (Kalyani *et al.*, 2018) [1]. Similar age-related increases in urea and BUN concentrations have been reported in neonatal calves, which are associated with physiological maturation and changes in protein metabolism during early growth (Knowles *et al.*, 2000; Mohri *et al.*, 2007) [2, 3].

5. Conclusion

Serum creatinine, urea, and blood urea nitrogen undergo significant physiological changes during the first 56 days of

life and are influenced by birth season, breed, and postnatal age. Higher concentrations of creatinine, urea, and BUN observed in summer-born calves indicate that prenatal and early postnatal exposure to elevated environmental temperatures alters renal function and nitrogen metabolism. Vrindavani calves consistently exhibited higher renal biomarker concentrations than Tharparkar calves, reflecting greater metabolic sensitivity to heat stress, whereas the indigenous Tharparkar breed demonstrated superior physiological adaptation.

The progressive increase in urea and BUN with age corresponds to normal postnatal maturation, increased protein metabolism, and development of renal function. The significant breed $\times$ age interaction for creatinine further indicates genetic variation in renal and metabolic adaptation during neonatal development. The present findings establish reference information on early postnatal renal biomarkers in Tharparkar and Vrindavani calves and highlight the importance of considering breed, birth season, and age when interpreting biochemical indicators of renal function and nitrogen metabolism. These biomarkers may serve as practical tools for monitoring neonatal health, evaluating adaptation to prenatal thermal stress, and improving management strategies for dairy calves under changing climatic conditions.

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