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Original article

COMPARISON OF BLOOD SERUM BIOCHEMICAL PARAMETERS IN HOLSTEIN-FRIESIAN AND NORMANDE CALVES IN BULGARIA

I.-A. GAYDAROVA[1] & R. PETROV[1,2]

[1]Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria;
[2]Green Balkans Wildlife Rescue Centre, Stara Zagora, Bulgaria

Summary

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The study evaluated the differences in some biochemical blood serum parameters between Holstein-Friesian calves from two farms in Bulgaria (Korten, n=78; Bozveliysko, n=51) and Normande calves from a third farm (Slivnitsa, n=13) with emphasis on the effects of factors such as altitude, feeding schedule, and natural suckling conditions. A comparison was made between the Holstein-Friesian calves in the two farms with regard to farm altitude and diet. Blood glucose, urea, AST, ALT, cholesterol, calcium, and total protein levels showed significant variations between the groups. Total protein and liver enzyme levels were higher in suckling Normande calves, which may indicate that the type of milk has an impact of the biochemical parameters in the blood serum of the calves. Blood calcium concentrations were the greatest in Normande calves, followed by Holstein-Friesian calves from Bozveliysko Farm, whereas Holstein-Friesian calves from the Korten farm were the lowestcalves. These results provide important information about the metabolic status in the early development of the young animals, which can direct breed-specific feeding plans for the calves and better herd management. These patterns highlight the influence of farm-specific factors and breed on metabolic profiles in early calf development.

Key words: comparative blood biochemistry, Holstein-Friesian calves, Normande calves

INTRODUCTION

Calves, like all animals in their early stages of life adaptation, are going through rapid physiological changes, with the biggest development occurring in the digestive, immune, and metabolic systems. During these early life stages, blood biochemical parameters monitoring provides vital information about the nutritional and health status of young calves and reflects the influence of both environmental and genetic factors. Breed differences (genetic factors), farm altitude, and feeding practices as environmental factors – particularly the type of milk consumed – have a significant impact on the calves' development and shape their metabolic profiles (Dhabhar, 2018). Nutrition is a determining factor in animal development, and the supply of milk to calves is essential since it provides the necessary nutrients for the further development of tissues (Kertz *et al.*, 2017). Several metabolic parameters in Holstein calves – including blood glucose, cholesterol, and transaminases – have been shown to respond to both seasonal shifts and milk feeding practices, suggesting a high metabolic sensitivity in the breed (Hadžimusić, 2024). Known for their high milk production, Holstein-Friesian calves are frequently raised on standardised milk substitutes that are available on the market. Preweaning nutritional strategies in Holstein calves, particularly the level of milk replacer offered, have been shown to modulate markers of hepatic and protein metabolism (Leal *et al.*, 2021).

Normande calves, on the other hand, are renowned for their dual-purpose utility (milk and meat). They frequently nurse straight from their mothers, which can be beneficial in terms of obtaining richer or more breed-specific nutrients. Normande calves are known for their balanced metabolic profile and adaptability under diverse environmental and feeding conditions (Chester-Jones, 2006). Previous studies have shown that, on average, the daily gain of *ad libitum*-fed calves was greater compared to limited-access calves, and milk allowance can affect the behavior exhibited by calves as well (Arens, 2022). In addition, some studies have shown that *ad libitum* milk access improved daily gain and modified the behaviour and stress response in calves in contrast to restricted feeding systems (Hammon, 2002). These early-life differences can predetermine the calves' long-term physiological trajectory and productivity. Previous studies of ours (Petrov, 2025) have reported differences in blood biochemical indicators in Holstein-Friesian and Galloway breeds showing that metabolic profiles in young calves can serve as predictors for immune resilience and growth efficiency. Thus, understanding how breed and nutrition interact during this critical window can provide insights for optimising calf rearing practices.

This study aimed to compare the blood biochemical parameters of Normande calves raised in Slivnitsa under natural suckling conditions and Holstein-Friesian calves raised on two farms (Korten and Bozveliysko), which differ primarily in the altitude of the farms and the feeding practices and thus, to find how environmental conditions, diet, and breed factors affected blood biochemical parameters of calves in the early stages of life.

MATERIALS AND METHODS

For this study, 142 clinically healthy calves from three Bulgarian farms were sampled: 78 Holstein-Friesian calves from Farm 1 (Korten); 51 Holstein-Friesian calves from Farm 2 (Bozveliysko); and 13 Normande calves from Farm 3 (Slivnitsa). Similar stall-based housing arrangements were used for all calves in order to eliminate environmental variation brought on by management of the three farms. The animals were clinically healthy at the time of blood collection and were fed by experienced workers in the farm. The sampling events took place during the summer months June and July. The age of calves ranged from 2 to 7 months across the three farms. Located in the southern foothills of the Sredna Gora mountain range in the Upper Thracian Plain, Korten is roughly 193 meters above sea level. Approximately 111 meters above sea level, Bozveliysko is situated in the Provadia municipality of Varna district. At a height of roughly 525 meters above sea level, Slivnitsa is located in Sofia district in western Bulgaria. In Farm 1 (Korten), calves were kept in standard stall-housing conditions and fed a commercial milk replacer (CONFOR, 50% dried milk content) beginning in the first week of life. It consisted of 21.5% crude protein; 18% crude fat; 7.4% crude ash; 0.8% Ca; 0.5% sodium; 0.7% phosphorus; additives (vitamin A, D3, E, zinc, iodine, selenium). In addition to milk, calves received a pre-starter starting on the 14^{th} day of life until a week after weaning (Microstart CCC, Alltech) and a structured feeding programme including Royal Baby Musli PRO as solid feed until five months of age in the mornings and silage in the evening. Calves were provided *ad libitum* access to water. Weaning took place on the 8^{th} week. Similarly, calves at Bozveliysko's Farm 2 also received a milk replacer CONFOR, with 50% dried milk. From day 15, they were also provided MICROSTART CCC complementary feed which included oats, maize, sunflower meal, vitamins, and minerals to promote rumen development. After the milk phase, the feeding progressed to morning muesli and evening silage, followed by silage only after weaning (8^{th} week). At Farm 3 (Slivnitsa), Normande calves were allowed to suckle naturally and were exclusively fed maternal milk, without milk replacers or concentrated feed. They were managed under a cow-calf system and remained with their dams in a loose-housing system. During the suckling phase, calves had *ad libitum* access to silage forage and received no concentrate supplements. The calves suckling period in the farm lasted up to 6 months.

Blood samples were collected from each calf by jugular venipuncture, using sterile 4 mL tubes without anticoagulant. A volume of 2 mL of blood was drawn per animal. Samples were immediately stored at 4 °C in a refrigerated container during transportation to the laboratory. Biochemical analysis was performed on blood serum using the Mindray Vetube 30 Veterinary Automatic Biochemistry Analyzer with the Health-Check configuration on the following parameters: glucose (Glu) – mmol/L, total protein (TP) – mmol/L, albumin (Alb) – g/L, urea – mmol/L, creatinine – μmol/L, aspartate aminotransferase (AST) – U/L, alanine aminotransferase (ALT) – U/L, alkaline phosphatase (ALP) – U/L, gamma-glutamyl transferase (GGT) – U/L, creatine kinase (CK) – U/L, calcium (Ca) – mmol/L, chlorides (Cl) – mmol/L, phosphorus (P) – mmol/L, magnesium (Mg) – mmol/L, cholesterol (Chol) – mmol/L. The analyzer was calibrated following the standards of the manufacturer. The system provided printed results within 10 minutes of analysis. Data collection and results archiving were managed through the installed Mindray Data Management software. The biochemical results were grouped by farm and breed.

Statistical comparisons were performed using SPSS v25 with descriptive analysis (mean ± standard deviation), and ANOVA with *post hoc* Tukey test to compare the group means for multiple comparisons. The significance level was set at $P<0.05$. Normality of data distribution was verified using the Shapiro-Wilk test.

RESULTS

Although both Holstein groups were managed in similar living conditions (indoor stalls), multiple serum biochemical parameters showed statistically significant differences in the ($P<0.05$), highlighting the impact of the breed, as well as environmental factors such as altitude and feeding plans (Table 1). In terms of serum glucose the Korten group demonstrated higher levels (4.50±0.61 mmol/L) compared to Bozveliysko calves (4.10± 0.71 mmol/L), likely pointing to differences in energy density or carbohydrate composition of the used milk replacer. The mean levels of total protein, additionally proved to be higher in Bozveliysko calves (value =77.64 g/L in Bozveliysko and 74.70 g/L in Korten), The AST levels varied among the farms – 103.53 U/L in Korten and 126.11 U/L in Bozveliysko; and ALT values were elevated in the calves at the farm in Bozveliysko (31.86 U/L) relative to those in Korten (21.08 U/L). GGT values between the Holstein-Friesian calves from both groups were within the reference values with greater mean activity in Bozveliysko compared to Korten calves (24.20 U/L and 19.92 U/L respectively).

Electrolyte levels also varied significantly. Serum calcium concentrations were higher in the calves from Bozveliysko (3.44±0.61 mmol/L) compared to the the calves in Korten (2.84 ±0.12 mmol/L, P<0.05), similarly to the levels of phosphorus and magnesium, suggesting differences in mineral supplementation or absorption efficiency. Moreover, urea levels were shown to be higher in the samples from the Korten farm (4.62 ±0.72 mmol/L, p < 0.05 vs 4.25 ±5.58 mmol/L in Bozveliysko). In Korten calves, serum CK was also considerably lower (389.81 ±214.87 U/L) than in the samples from Bozveliysko (423.10±9.57 U/L). Furthermore, serum chlorides, a marker of acid-base balance and hydration, showed a statistically significant decrease in Bozveliysko (94.63 ±0.61 mmol/L) compared to Korten (101.55 ±4.22 mmol/L). Despite the similarity in the conditions (such as

Table 1. Comparison Between Holstein-Friesian Calves: Farm 1 (Korten) and Farm 2 (Bozveliysko)

Parameter (unit)	Farm 1: Holstein-Friesian calves	Farm 2: Holstein-Friesian calves	Reference values	P value
Glucose (mmol/L)	4.5±0.61	4.10 ±0.71	3.0–5.5	<0.05
Total protein (g/L)	74.70±4.20	77.64 ±15.61	51–78	0.200
Urea (mmol/L)	4.62±0.72	4.25 ±5.58	2.5–6.5	0.613
Creatinine (µmol/L)	84.10±11.40	107.10 ±40.10	88–180	<0.05
AST (U/L)	103.53±0.80	126.11 ±8.60	78–132	<0.05
ALT (U/L)	21.08±4.40	31.86±1.61	20–35	<0.05
ALP (U/L)	467.80±4.39	242.31±0.66	30–500	<0.05
GGT (U/L)	19.92±5.81	24.20±0.09	10–100	<0.05
CK (U/L)	389.81±214.87	423.10±9.57	100–600	0.176
Calcium (mmol/L)	2.84±0.12	3.44±0.61	2.3–3.0	<0.05
Chlorides (mmol/L)	101.55±4.22	94.60±0.61	90–105	<0.05
Phosphorus (mmol/L)	2.71±0.35	2.91±5.75	1.3–2.6	0.805
Magnesium (mmol/L)	0.92±0.05	1.32±3.43	0.74–1.23	0.409
Cholesterol (mmol/L)	2.00±0.51	1.40±6.73	1.6–5.2	0.528

Table 2. Comparison between Normande calves (Farm 3) and Holstein-Friesian calves (Farm 1). Data are presented as mean ±SD

Parameter (unit)	Farm 3: Normande calves	Farm 1: Holstein-Friesian calves	Reference values	P value
Glucose (mmol/L)	3.12±0.79	4.50±0.61	3.0–5.5	<0.05
Total protein (g/L)	80.15±7.08	74.70±4.20	51–78	0.01
Urea (mmol/L)	2.30±1.17	4.62±0.72	2.5–6.5	<0.05
Creatinine (μmol/L)	101.00±19.11	84.11±11.4	88–180	0.008
AST (U/L)	122.76±5.03	103.52±0.81	78–132	<0.05
ALT (U/L)	30.73±20.63	21.11±4.42	20–35	0.119
ALP (U/L)	234.46±3.64	467.81±4.39	30–500	<0.05
GGT (U/L)	18.08±3.75	19.91±5.81	10–100	0.153
CK (U/L)	417.00±7.29	389.82±214.87	100–600	0.269
Calcium (mmol/L)	5.00±0.77	2.83±0.12	2.3–3.0	<0.05
Chlorides (mmol/L)	118.30±17.69	101.53±4.22	90–105	0.331
Phosphorus (mmol/L)	1.66±8.80	2.71±0.35	1.3–2.6	0.678
Magnesium (mmol/L)	3.54±2.22	0.92±0.05	0.74–1.23	<0.05
Cholesterol (mmol/L)	1.41±2.61	2.00±0.51	1.6–5.2	0.432

stall housing), the established differences reinforce the sensitivity of calf biochemical profiles to farm-specific factors, and highlight the importance of biochemical testing of the serum in early-life management programmes.

The comparison of Holstein-Friesian (n=129) and Normande calves (n=13) revealed significant differences ($P<0.05$) for most of the analysed blood biochemical parameters (Table 2 and 3). Normande calves, nursed naturally by their mothers, had considerably higher concentrations of calcium, phosphorus, magnesium, and total protein compared to Holstein-Friesian calves from the other two farms. The differences suggest that natural maternal feeding may provide a more nutritionally complex or bioavailable source of nutrients to support hepatic function and enables higher rate of protein synthesis.

In contrast, the levels of urea (2.30 mmol/L), and glucose (3.12 mmol/L) were significantly lower in the Normande calves compared to the other two farms with Holstein-Friesian calves. Blood CK of the Normande calves (417.00 U/L), is in the middle of the reference range and was similar to both Holstein-Friesian groups. The activities of AST (122,76 U/L) and ALT (30,73 U/L) were within the norms, however were more elevated compared to Farm 1 (Korten), which can likely reflect increased metabolic activity in the liver or skeletal muscle, possibly due to richer milk composition or greater physical activity in pasture-based or less confined settings. Interestingly, while electrolyte profiles varied significantly between groups, no statistically significant differences were observed in chlorides, suggesting that acid-base balance remained within physiological norms. As a parameter of interest, serum calcium was higher in the samples from the Normande calves: 5.00 ±0.77. Serum total protein in

Table 3. Comparison between Normande calves (Farm 3) and Holstein-Friesian calves (Farm 2). Data are presented as mean ±SD

Parameter (unit)	Farm 3: Normande calves	Farm 2: Holstein-Friesian calves	Reference values	P value
Glucose (mmol/L)	3.12±0.79	4.10±0.71	3.0–5.5	<0.05
Total protein (g/L)	80.15±7.08	77.61±15.62	51–78	0.390
Urea (mmol/L)	2.30±1.17	4.21±5.58	2.5–6.5	<0.05
Creatinine (μmol/L)	101.00±19.11	107.10±40.11	88–180	0.434
AST (U/L)	122.76±5.03	126.12±8.63	78–132	0.079
ALT (U/L)	30.73±20.63	38.71±1.62	20–35	0.189
ALP (U/L)	234.46±3.64	242.31±0.66	30–500	<0.05
GGT (U/L)	18.08±3.75	24.21±0.09	10–100	<0.05
CK (U/L)	417.00±7.29	423.10±9.57	100–600	0.01
Calcium (mmol/L)	5.00±0.77	3.40±0.61	2.3–3.0	<0.05
Chlorides (mmol/L)	118.30±17.69	94.60±0.61	90–105	0.401
Phosphorus (mmol/L)	1.66±8.80	2.91±5.75	1.3–2.6	0.637
Magnesium (mmol/L)	3.54±2.22	1.30±3.43	0.74–1.23	0.007
Cholesterol (mmol/L)	1.41±2.61	1.40±6.73	1.6–5.2	0.993

Farm 3 (Normande calves) – 80.15 g/L had a higher mean value compared to the other two farms (Farm 1 74.70 g/L and Farm 2 77.64 g/L). Altogether, these findings emphasise how breed physiology, combined with feeding method and milk type, can significantly shape the early biochemical landscape of calves. They underscore the need to customise feeding strategies to both breed and developmental goals, especially in the first month of life when nutritional programming has lasting effects.

DISCUSSION

Despite being from the same breed and raised under similar housing conditions, the blood biochemical differences between Holstein-Friesian calves from Farm 1 (Korten) and Farm 2 (Bozveliysko) suggest that local environmental factors, particularly altitude, dietary practices, and milk replacer formulations, could play a critical role in shaping early metabolic development. The use of fortified milk replacers and starter feeds in Korten likely contributed to the elevated serum glucose, calcium, and total protein observed in this group. Milk replacers with high protein and fat content have been associated with increased glucose availability and improved energy balance (Michałek *et al.*, 2014). Quigley *et al.* (2006) demonstrated that increasing the volume of milk replacer significantly enhanced growth rates and elevated serum glucose and urea levels, aligning with Korten group's biochemical profile. Additionally, the early introduction of yeast-supplemented concentrates and prestarter diets in this farm may have speeded the development of the rumen, influencing creatinine turnover and urea recycling mechanisms. In contrast, the Bozveliysko calves, while genetically identical, showed lower glucose levels but elevated AST, ALT and electrolyte levels, which could potentially be linked to mild hepatic stress, variations in mineral intake, or differences in feed composition. These differences suggest that even within the same breed, micro-environmental and nutritional factors might be a reason for divergent physiological outcomes. In support of this, previous studies have shown that feeding system significantly influences glucose and protein profiles in calves, with milk replacer-fed Holstein crosses showing reduced values compared to naturally reared counterparts (Wielgosz-Groth, 2015) The blood biochemical values observed in Holstein-Friesian calves generally remained within the reference physiological ranges for healthy dairy calves, particularly in terms of urea and total protein (Klinkon & Ježek, 2012). This is consistent with the notion that the observed variations reflect adaptive metabolic adjustments rather than pathological states. Urea and CK blood values are especially sensitive to age, diet, and rumen development (Jainudeen & Hafez, 2000), and provide a valuable information on nitrogen metabolism. When comparing Holstein-Friesian and Normande calves (Slivnitsa), greater metabolic differences became evident. Normande calves, raised under natural suckling conditions, exhibited higher serum total protein, cholesterol, ALT, calcium, phosphorus, and magnesium, which may reflect the nutrient-dense composition of Normande dam's milk. It was found that calves fed whole milk gained significantly more weight than those fed milk replacer (Casagrande *et al.*, 2023). Similarly, calves on whole milk had elevated serum total protein and cholesterol levels, indicating enhanced lipid metabolism, immune development, and possibly stronger hepatic activity (Göncü *et al.*, 2023). The lower serum glucose and urea in Normande calves suggest a slower but more efficient metabolic rate, likely influenced by delayed rumen maturation, reduced dietary nitrogen load, or enhanced nutrient utilisation. This interpretation is supported by a report that Normande calves under organic/natural feeding showed greater glucose efficiency and superior weight gain compared to more intensively managed breeds (Kienitz, 2016). Normande calves also showed elevated ALT activity, which may reflect increased hepatic activity in response to higher milk fat and protein intake (Rahman *et al.*, 2023). The age range of the sampled calves which varies from 2 to 7 months could have an effect on some of the differences found between the groups.

Our findings on significant breed-based differences in AST, urea, and cholesterol further support historical studies, such as (McGillivray, 1981), who reported similar inter-breed metabolic profiles in European cattle. Urea level comparison between Korten and Bozveliysko samples can be an indicator for reduced nitrogen excretion. This can be potentially linked to altered protein digestion or microbial efficiency. Notably, the elevated levels of calcium, magnesium, and phosphorus in Normande calves are consistent with findings of Pollesel *et al.* (2020), who demonstrated that regional variation in forage mineral content, especially in higher altitude farms, can impact electrolyte balance in calves. The higher serum urea and creatinine levels in Normande calves may also suggest increased muscle development or turnover, characteristic of dual-purpose breeds. This is in line with the observations by Alugongo *et al.* (2022), who emphasised the importance of the feeding system design in optimising nitrogen metabolism and health. A previous study between the colour variations of Holstein-Friesian calves showed that the blood serum calcium, glucose, creatinine, chlorides, AST, ALT, ALP, GGT, CK, cholesterol, triglycerides, and potassium from the red and white calves had higher mean values . On the contrary, the serum of the black and white colour calves proved to be higher in phosphorus, magnesium, total protein, albumin, urea, total bilirubin, and sodium (Petrov, 2025). These findings collectively underscore the interconnected effects of breed, feeding system, and environment on the biochemical parameters in calves' serum, as well as their overall development. Monitoring blood biochemical markers provides a powerful tool for detecting early physiological shifts, evaluating nutritional balance, and could play a role in predicting long-term outcomes and possible changes. Further research is warranted to investigate the carry-over effects of these early differences into later growth phases, disease resistance, and productive lifespan.

CONCLUSIONS

The study revealed significant biochemical differences between Holstein-Friesian calves raised under different environmental and feeding conditions. When fed milk substitute and supplemental feed, the calves from Bozveliysko showed different metabolic profiles compared to those from Korten, suggesting that even subtle management variations can alter physiological outcomes. Additionally, the Slivnitsa-born Normande calves, who were nursed naturally by their mothers, displayed distinct biochemical values that were likely influenced by breed-specific milk composition and maternal care. The Normande group exhibited more balanced blood urea, creatinine, and cholesterol, which may reflect a slower, more regulated metabolic adaptation consistent with the breed's dual-purpose. Conversely, the elevated glucose and protein levels in Holstein calves, particularly those from Korten, indicate an accelerated metabolism likely driven by energy-dense milk replacers and early concentrate intake. Enzymatic blood markers such as AST and ALT also showed between-group differences potentially signalling differing hepatic loads or levels of growth-related stress. The results emphasise the crucial significance of feeding strategies, altitude, and breed selection in shaping the early metabolic health of calves. Understanding and adapting nutritional practices based on these factors could enhance calf welfare and improve long-term productivity. Future research should explore the longitudinal physiological effects of early-life biochemical patterns and investigate how early metabolic programming influences later performance, disease resistance, and reproductive health.

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Correspondence:

Dr. Ivana-Antoniya Gaydarova
Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria
e-mail: ivana.antoniya.gaydarova.19@trakia-uni.bg