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Anadolu Mandası (*Bubalus bubalis*) Kolostrumunda Fizikokimyasal Parametreler, Yağ Asitleri ve Minerallerin Değerlendirilmesi

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ÖZET

Bu çalışmanın amacı, Anadolu Mandası (*Bubalus bubalis*) kolostrumunun fizikokimyasal parametrelerini, yağ asidi kompozisyonunu ve mineral içeriklerini belirlemektir. Kolostrum, yalnızca yavrulara bağışıklık desteği sağlamakla kalmayıp aynı zamanda besin içeriği açısından zengin ilk besin olması nedeniyle de önemlidir. Bu yüzden Anadolu Mandalarında yapılan kapsamlı ilk öncü çalışma olması yanında, kolostrum besinsel içeriğinin bilinmesinde zootekni, hayvan beslenmesi, sağlığı ve gastronomik açıdan sonraki araştırmalar için bir temel bulgu oluşturması amaçlanmıştır. Çalışmada, Türkiye'de yetiştirilen *Bubalus bubalis* türüne ait kolostrum örnekleri kullanılmıştır. Bu türe ait tek yerli ırk Anadolu Mandasıdır. İkinci laktasyonuna başlayan Anadolu Mandası anaçlarının doğumu takip eden 12. saatlerinde kolostrum örnekleri alınmıştır. Fizikokimyasal bileşimleri, yağ asitleri ve mineral elementleri analizi sırasıyla FOSS MilkoScan™ FT-120, Gaz Kromatografisi (GC) ve İndüktif Eşleşmiş Plazma Kütle Spektrofotometresi (ICP-MS) cihazları ile gerçekleştirilmiştir. Toplam 27 yağ asidi tanımlanmıştır. Bunlar arasında başlıca yağ asitleri C16:0 (palmitik asit, %31,77), C18:1n9c (oleik asit, %26,60), C14:0 (miristik asit, %13,22) ve C18:0 (stearik asit, %8,76) olup, toplam yağ asitlerinin %80,35'ini oluşturmaktadır. Yağ asidi gruplarının dağılımı doymuş yağ asitleri (ΣSFA, %64,81), tekli doymamış yağ asitleri (ΣMUFA, %30,59) ve çoklu doymamış yağ asitleri (ΣPUFA, %4,61) şeklindedir. Ayrıca sekiz mineral belirlenmiştir: Mn (0,12 ppm), Zn (20,42 ppm), Fe (4,79 ppm), Mg (185,21 ppm), Na (761,59 ppm), K (1206,64 ppm), Ca (587,01 ppm) ve P (1733,05 ppm). Sonuçlar, Anadolu Mandası kolostrumunun yüksek oranda doymuş ve tekli doymamış yağ asitleri ile birlikte önemli düzeyde temel mineraller içerdiğini göstermektedir. Bu bulgular, Anadolu Mandası kolostrumunun yalnızca manda yavrularının büyüme ve sağlığında değil, aynı zamanda insan beslenmesinde de önemli bir potansiyele sahip olduğunu ortaya koymaktadır. Anadolu Mandası kolostrumunun besinsel profili, fonksiyonel bir gıda kaynağı olarak önemini vurgulamaktadır. Elde edilen veriler, manda kolostrumu ve süt bileşimi üzerine yapılacak gelecekteki çalışmalara temel teşkil edebilir. Özellikle biyoaktif bileşikler, antioksidan özellikler ve insan beslenmesindeki potansiyel uygulamalara yönelik araştırmaların yapılması önerilmektedir.

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Anahtar Kelimeler
Anadolu Mandası
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Yağ Asidi
Mineral Maddeler

Assessment of Physicochemical Parameters, Fatty Acids and Minerals in Anatolian Water Buffalo (*Bubalus bubalis*) Colostrum

ABSTRACT

This study aimed to determine the physicochemical parameters, fatty acid composition, and mineral contents of Anatolian water buffalo (*Bubalus bubalis*) colostrum. Colostrum is important not only as the first secretion that provides immune support for newborns but also as a nutrient-rich food source. Therefore, in addition to being the first pioneering study conducted on Anatolian Water Buffaloes, this research aims to provide fundamental data for future studies in animal science, animal health and nutrition, as well as gastronomic and human nutrition

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research through a comprehensive evaluation of the nutritional composition of colostrum. Colostrum samples were obtained from *Bubalus bubalis* bred in Türkiye. The Anatolian Water Buffalo is the only native breed of this species in Turkey. Colostrum samples were collected within 12 hours after parturition from Anatolian Water Buffalo cows at the beginning of their second lactation. Analyses of physicochemical composition, fatty acid profiles, and mineral elements were performed using FOSS MilkoScan™ FT-120, Gas Chromatography (GC), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), respectively. A total of 27 fatty acids were identified. Among these, the major fatty acids were C16:0 (palmitic acid, 31.77%), C18:1n9c (oleic acid, 26.60%), C14:0 (myristic acid, 13.22%), and C18:0 (stearic acid, 8.76%), accounting for 80.35% of the total fatty acids. The fatty acid groups were distributed as saturated fatty acids (ΣSFA, 64.81%), monounsaturated fatty acids (ΣMUFA, 30.59%), and polyunsaturated fatty acids (ΣPUFA, 4.61%). Eight minerals were determined, including Mn (0.12 ppm), Zn (20.42 ppm), Fe (4.79 ppm), Mg (185.21 ppm), Na (761.59 ppm), K (1206.64 ppm), Ca (587.01 ppm), and P (1733.05 ppm). Discussion: The results revealed that colostrum of the Anatolian Water Buffalo is characterized by a high proportion of saturated and monounsaturated fatty acids, together with significant levels of essential minerals. These findings suggest that buffalo colostrum has a unique nutritional profile, which may play a critical role not only in the health and growth of buffalo calves but also in supporting human nutrition. The nutritional profile of the Anatolian Water Buffalo colostrum highlights its importance as a functional food source. The obtained data can serve as a basis for future studies on buffalo colostrum and milk composition. Further research focusing on bioactive compounds, antioxidant properties, and potential applications in human nutrition is recommended.

Keywords
Anatolian Water Buffalo
Colostrum
Fatty Acids
Minerals

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INTRODUCTION

In Turkey, the only local buffalo breed is the Anatolian Water Buffalo (*Bubalus bubalis*, AWB), which is believed to have been introduced to Anatolia in the 7th century AD (Şahin, 2015; Nagarajan et al., 2015). The colostrum of Anatolia Water Buffalo (AWBC) exhibits morphological differences compared to water buffalo colostrum from Mediterranean countries, which also reflect in variations in productivity traits (Borghese & Moioli, 2021). Under the national breeding project (Project No. TAGEM/47MANDA2012-01), studies have been conducted for over ten years to enhance the developmental and productivity traits of animals owned by breeders in Turkey (Ağyar et al., 2022). Enhancements in nutritional and environmental conditions are complementing genetic selection-based improvements in productivity traits in AWB (Okuyucu et al., 2018; Yılmaz & Kara, 2019).

In addition, modern husbandry enterprises have proliferated across Türkiye in the last decade, accompanied by emerging approaches and greater awareness of animal care and nutrition. There has been growing attention to the importance of postpartum nutrition, especially in the development of buffalo calves (Cingöz et al., 2025). Given that only the abomasum is functional in the rumen of neonatal buffalo calves, colostrum serves as their first and fundamental nutritional source. Colostrum contains rich nutritional components essential for calf development (Elfstrand et al., 2002). While neonatal calf mortality is often attributed to difficult labor, unhygienic housing, and hypothermia, insufficient colostrum intake remains the most critical factor (Cuttance & Laven, 2019). Conversely, the administration of colostrum in the neonatal period significantly reduces mortality rates in buffalo calves by approximately 40% (Lotito et al., 2023). Calf growth performance and survival are influenced not only by the nutritional components of colostrum but also by its non-nutritional components and calf serum factors (Al-Najjar, 2022).

Colostrum composition and yield are affected by multiple factors. The quantity and quality of colostrum intake are vital for protecting calves from infectious agents as they transition from a sterile uterine environment to the

outside world. Key indicators of colostrum quality include its physicochemical properties—especially fatty acid and mineral profiles—and immunoglobulin content. Colostrum consists predominantly of nutrients, such as lipids, proteins, carbohydrates, vitamins, and minerals (Erdem et al., 2023).

Historically, colostrum has been valued in animal nutrition for its unique contribution to the development of buffalo calves (Abd El-Fattah et al., 2012). Consequently, despite forming only about 0.5% of lactation milk yield, its rich fatty acid and mineral content positions colostrum not only as essential for calf nutrition but also as a high-potential animal-based food source for human nutrition (Bondoc & Ramos, 2022). Modern consumption patterns increasingly include colostrum in daily diets due to its nutritional benefits. Although many dairy products have reached market saturation, buffalo dairy products have begun to gain prominence in both national and international markets. Under such conditions, colostrum could represent a novel and valuable addition to buffalo dairy offerings.

The most critical quality attributes of colostrum include its fatty acid and mineral contents (Kul et al., 2022), as well as the total concentrations of lactose, protein, and fat. Lipids enrich the sensory and nutritional quality of various food products (Sucak et al., 2020). Buffalo milk is characterized by higher total lipid content compared to other farm animals (Coroian et al., 2013; Özkaya et al., 2018), and this proportional difference holds true in fatty acid profiles as well. Fatty acids are crucial for cell membrane development, energy provision, and calf growth (De Carvalho & Caramujo, 2018). They also exert antibacterial, antiviral, and anticarcinogenic effects on calf metabolism (Cichosz & Czeczot, 2020; Startseva et al., 2023; Moon et al., 2024).

Building on our previous biochemical analyses of AWB milk components, the present study outlines a comprehensive investigation into the physicochemical and biochemical composition of AWB colostrum. Here, for the first time, AWBC—with its array of nutrients vital for calf growth and development—is subjected to detailed analysis.

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Animal Material

The animal material of this study consisted of Anatolian Water Buffaloes (AWB) raised under the National Anatolian Water Buffalo Development Program (TAGEM/49 MANDA 2012-01), coordinated by the Republic of Turkey, Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies, in enterprises located in the Muş province. Ethical approval for this study was obtained from the Adıyaman University Animal Experiments Department Local Ethics Committee (Protocol No: 2025/033). Animal selection for colostrum sampling was based on two criteria. First, animals had to be enrolled in the Breeding Improvement Project. Second, they had to be Anatolian Water Buffalo dams with at least one previous parturition. Accordingly, second-lactation breeding females that calved in June were selected, taking into account the available project animal stock. Generally, Anatolian Water Buffaloes calve seasonally. The Muş Plain, with its wide meadows and pastures, provides favorable conditions for the extensive breeding of AWB. The animals in the study group were fed exclusively on meadow and pasture forage, similar to other buffaloes in the breeding region, without any additional feed supplements. Muş is located in Eastern Anatolia, between 39°29′–38°29′ N latitudes and 41°06′–41°47′ E longitudes, with an altitude ranging between 1250 and 1400 m above sea level. The Murat and Karasu rivers, tributaries of the Euphrates, create a suitable environment for the reed beds and meadows used as pasture by AWB in the Muş Plain.

Colostrum Sampling

Colostrum samples were collected from AWB broodstock in their second lactation, 12 h after parturition. A total of 100 samples were collected and placed in 50 mL sterile glass bottles. Samples were transported under cold chain conditions to the laboratory, where they were stored at −20 °C until analysis. No preservatives or pre-treatment were applied.

Analytical Methods

The FOSS MilkoScan™ FT-120 (Foss Electric, Denmark), calibrated with appropriate buffalo standards, was used to determine solid-not-fat (SNF, %), protein (%), fat (%), and lactose (%). The FTIR analytical technology employed in the MilkoScan™ FT-120 complies with the International Dairy Federation (IDF) guidelines and Association of Official Analytical Chemists (AOAC) official procedures (Latimer Jr, 2023). All analyses were performed at the Central Research Laboratory of Adıyaman University (ADYU-MERLAB).

Mineral Analysis

Colostrum samples (2 mL) were transferred into DAP60-K PTFE containers of a microwave digestion system,

followed by the addition of 4 mL HNO_3 (65%, w/v) and 1 mL $HClO_4$ (60%, w/v). Digested samples were diluted to a final volume of 20 mL with 0.1 M HNO_3 in volumetric flasks. Blank samples were prepared and digested using the same procedure. The final solutions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Çiftçi et al., 2009).

Fatty Acid Analysis

Oil extraction and methyl esterification of fatty acids from AWB colostrum were performed according to established methods. Briefly, colostrum samples were homogenized in a hexane/isopropanol (3:2, v/v) mixture (Hara & Radin, 1978; Christie, 1989). Lipid extracts were centrifuged at 10,000 rpm for 5 min, and the solvents were evaporated at 40 °C. Oil extracts were stored at −25 °C until fatty acid analysis. For each sample, three replicate measurements were performed. Fatty acids in the lipid extract were methylated with 2% sulfuric acid in methanol and subsequently extracted with hexane. Fatty acid methyl esters were analyzed by gas chromatography (Shimadzu GC-2025, flame ionization detector) using a TR-CN 100 capillary column (60 m × 0.20 mm i.d., 25 µm film thickness; Teknokroma, Spain).

Statistical Analysis

Descriptive statistics were performed using SPSS software (version 25.0; IBM Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation± standard error.

RESULTS and DISCUSSION

The physicochemical analysis of Anatolian Water Buffalo colostrum (AWBC) revealed distinct compositional characteristics (Table 1). Mean concentrations were determined as follows: fat (5.50%), solids-not-fat (SNF) (9.89%), protein (4.11%), and lactose (5.89%). Especially, the high protein content (4.11%) underscores the immunological and nutritional importance of AWBC for neonatal water buffalo calves.

Çizelge 1. Anadolu mandası kolostrumu kimyasal kompozisyonu (n = 100)
Table 1. Chemical composition of Anatolian Water Buffalo colostrum (n = 100)

Item	Mean	Standard Deviation	Range	
			Minimum	Minimum
Fat (%)	5.50	1.24	4.06	9.69
Snf (%)	9.89	1.44	7.21	12.87
Protein (%)	4.11	0.76	3.00	6.22
Lactose (%)	5.89	1.29	4.00	9.77

Values refer to 12 h post-parturition milking for all individuals

Elemental Composition of AWBC

Comprehensive mineral profiling identified eight essential elements in AWBC (Table 2). Macrominerals showed the following concentrations: phosphorus (P) (1733.05 ppm), the most abundant mineral; potassium (K) (1206.64 ppm); sodium (Na) (761.59 ppm); calcium (Ca) (587.01 ppm); and magnesium (Mg) (185.21 ppm). Trace elements were detected at physiologically relevant levels: zinc (Zn) (20.42 ppm), iron (Fe) (4.79 ppm), and manganese (Mn) (0.12 ppm). High phosphorus and calcium concentrations are critical for the development of the skeletal system in newborn babies, while zinc and iron support postnatal hematopoiesis and immune functions. (Chang et al., 2020; Arslan et al., 2021; Okuyucu & Erdem, 2025).

Çizelge 2. Anadolu Mandası kolostrumu mineral konsantrasyonlarının tanımlayıcı istatistikleri (n:100)
Table 2. Descriptive statistics of mineral concentrations in Anatolian Water Buffalo colostrum (ppm) (n:100)

Item	Mean	Standard Deviation	Range	
			Minimum	Maximum
Mn	0.12	0.01	0.09	0.15
Zn	20.42	2.48	14.39	24.61
Fe	4.79	1.65	2.19	7.80
Mg	185.21	28.61	124	230
Na	761.59	144.26	517	1035
K	1206.64	186.22	898	1534
Ca	587.01	104.12	369	732
P	1733.05	272.22	1145	2242

Values refer to 12 h post-parturition milking for all individuals

Fatty Acid Content of AWBC

This study provides the first comprehensive fatty acid profile of AWBC (Table 3). The lipid fraction was characterized by a predominance of saturated fatty acids (SFA) comprising 64.81%, with palmitic acid (C16:0) as the major component at 31.77%.

Çizelge 3. Anadolu Mandası kolostrumdaki yağ asitleri kompozisyonu (%)
Table 3. Fatty acids composition of Anatolian Water Buffalo colostrum (%)

Fatty Acids	**% (Mean ± SD)**
C4:0 (Butyric acid)	1.65±0.46
C6:0 (Caproic acid)	1.37±0.34
C8:0 (Caprylic acid)	0.78±0.27
C10:0 (Capric acid)	1.29±0.33
C11:0 (Undecanoic acid)	0.12±0.06
C12:0 (Lauric acid)	2.94±0.63
C13:0 (Tridecanoic acid)	0.12±0.03
C14:0 (Myristic acid)	13.22±2.71
C15:0 (Pentadecanoic acid)	1.15±0.22
C16:0 (Palmitic acid)	31.77±1.94
C17:0 (Heptadecanoic acid)	1.10±0.22
C18:0 (Stearic acid)	8.76±1.89
C20:0 (Arachidic acid)	0.15±0.33
C22:0 (Behenic acid)	0.25±0.27
C23:0 (Tricosanoic acid)	0.14±0.07
Saturated Fatty Acids (ΣSFA)	**64.81±2.15**
C18:2n6c (Linoleic acid)	2.89±0.64
C18:3n3 (Gamma linolenic acid)	0.07±0.18
C18:3n6 (Alpha linolenic acid)	1.28±0.25
C20:3n3 (Cis-11.14.17 eicosatrienoic acid)	0.13±0.08
C20:4n6 (Arachidonic acid)	0.13±0.12
C22:6n3 (Cis-4.7.10.13.16.19-docosahexaenoic acid) DHA	0.11±0.09
(Polyunsaturated Fatty Acids (ΣPUFA)	**4.61±0.72**
C14:1 (Myristoleic acid)	0.78±0.30
C16:1 (Palmitoleic acid)	2.24±0.28
C18:1n9t (Elaidic acid)	0.63±0.21
C18:1n9c (Oleic acid)	26.60±4.55
C20:1n9 (Cis-11-eicosenoic acid)	0.15±0.05
C22:1n9 (Erucic acid)	0.19±0.18
Monounsaturated Fatty Acids (ΣMUFA)	**30.59±4.22**
Unsaturated Fatty Acids (ΣUSFA)	**35.20±4.48**

Values refer to 12 h post-parturition milking for all individuals

Unsaturated fatty acids comprised 35.20% of total fatty acids, including monounsaturated fatty acids (MUFA) at 30.59% - dominated by oleic acid (C18:1n9c) at 26.60% - and polyunsaturated fatty acids (PUFA) at 4.61%. Notably, essential fatty acids including linoleic acid (C18:2n6c, 2.89%) and alpha-linolenic acid (C18:3n6, 1.28%) were identified, along with trace amounts of long-chain omega-3 fatty acids such as DHA (C22:6n3, 0.11%) and arachidonic acid (C20:4n6, 0.13%).

In this study, 27 fatty acid types were identified. The major fatty acids were C16:0 (31.77%), C18:1n9c (26.60%), C14:0 (13.22%), and C18:0 (8.76%), representing 80.35% of the total fatty acids. The rates of ΣSFA, ΣUSFA, ΣMUFA, and ΣPUFA in AWBC were 64.81%, 35.20%, 30.59%, and 4.61%, respectively. The SFA composition included C4:0 (1.65%), C6:0 (1.37%), C8:0 (0.78%), C10:0 (1.29%), C11:0 (0.12%), C12:0 (2.94%), C13:0 (0.12%), C14:0 (13.22%), C15:0 (1.15%), C16:0 (31.77%), C17:0 (1.10%), C18:0 (8.76%), C20:0 (0.15%), C22:0 (0.25%), and C23:0 (0.14%). MUFAs were C14:1 (0.78%), C16:1 (2.24%), C18:1n9t (0.63%), C18:1n9c (26.60%), C20:1n9 (0.15%), and C22:1n9 (0.19%), while PUFAs were C18:2n6c (2.89%), C18:3n3 (0.07%), C18:3n6 (1.28%), C20:3n3 (0.13%), C20:4n6 (0.13%), and C22:6n3 (0.11%).

The biochemical composition of colostrum in the early postpartum period is crucial for the nutrition and

development of AWB calves. One of the key biochemical components is fat, which was determined as 5.50% in this study. Previous studies reported fat contents of 4.87% in Chinese water buffalo and 4.38% in Philippine water buffaloes (Bondoc & Ramos, 2022; An et al., 2023), which are lower than our findings. Conversely, higher fat contents were reported in Iranian (10.81%) and Indonesian (7.45%) buffaloes (Salar et al., 2021; Siregar et al., 2020). The SNF content of AWBC was 9.89%, while previous studies reported 16.15% and 23.63% (Bondoc et al., 2021; An et al., 2023). The protein content in our study was 4.11%, compared with 19.23% (An et al., 2023), 12.96% (Bondoc et al., 2021), 8.73% (Coroian et al., 2013), and 5.44% (Bernabucci et al., 2013) in previous studies. Lactose content in AWBC was 5.89%, whereas in other studies it ranged from 2.50% to 3.78% depending on the breed (Yonis et al., 2014; Coroian, 2013; Salar et al., 2020; Bernabucci et al., 2013).

Regarding minerals, previous studies reported K (102.20 ppm), Mg (29.20 ppm), P (53.00 ppm), Ca (261.80 ppm), Fe (0.76 ppm), and Zn (0.20 ppm) in Egyptian buffalo colostrum (Abd El-Fattah et al., 2012). In China, mineral contents were reported as K (12.45 g/100g), Na (10.30 g/100g), Mg (1.89 g/100g), Ca (6.02 g/100g), P (10.12 g/100g), and Fe (10.99 mg/100g) (An et al., 2023). In our study, AWBC mineral levels appear to be consistent with literature values. In previous research on AWB milk, K (1797.50 ppm), Na (675.66 ppm), Mg (243.91 ppm), Ca (3043.00 ppm), P (1831.33 ppm), Fe (1.59 ppm), Zn (9.30 ppm), and Mn (0.60 ppm) were detected, supporting the compatibility with AWBC mineral levels (Ağyar et al., 2022). Notably, trace elements Mn, Zn, and Fe were higher in AWBC than in AWB milk, potentially supporting faster metabolic activity in neonatal AWB calves (Silva et al., 2023; Yasmin et al., 2024).

Fatty acids are another critical biochemical component in AWBC. Most fatty acids contain an even number of carbons due to their composition of 2-carbon acetyl units. Saturated fatty acids directly contribute to energy metabolism, while polyunsaturated fatty acids (PUFAs) are essential for cell membrane structure, calf development, and neurological protection (Sucak et al., 2020; Batista da Silva Galdino et al., 2021). In this study, individual SFA levels were C12:0 (2.94%), C14:0 (13.22%), C15:0 (1.15%), C16:0 (31.77%), C17:0 (1.10%), C18:0 (8.76%), C20:0 (0.15%), and C22:0 (0.25%). These results are comparable to those reported for Murrah buffalo colostrum in the Philippines (Bondoc & Ramos, 2022), although minor differences may arise due to breed and environmental factors. Some fatty acids (C4:0, C6:0, C8:0, C10:0, C11:0, C13:0, and C23:0) detected in this study were not reported in Bondoc & Ramos (2022).

ΣSFA, ΣPUFA, and ΣMUFA rates were 64.81%, 4.61%, and 30.59%, respectively. PUFA composition included C18:2n6 (2.89%), C18:3n3 (0.07%), C20:4n6 (0.13%), and C22:6n3 (0.11%). These results are generally compatible with the literature, though minor differences in C18:3n6 and C20:3n3 were noted. MUFAs comprised C14:1 (0.78%), C16:1 (2.24%), C18:1n9 (26.60%), C20:1n9 (0.15%), and C22:1n9 (0.19%), consistent with previous findings (Bondoc & Ramos, 2022).

Based on the results of this study, further research should be conducted to establish quality criteria for colostrum storage conditions and calf feeding protocols. Studies can be planned to determine the time-dependent changes in physicochemical, mineral, and fatty acid composition of colostrum between 0 and 72 hours postpartum, as well as the optimal collection time interval for maximum immunoglobulin and nutrient density. Furthermore, systematic comparisons of colostrum from other ruminant breeds should be conducted to determine their nutritional advantages and the potential for cross-species colostrum supplementation strategies. Similarly, the potential for nutritional supplementation for human health and development is another area requiring further investigation.

CONCLUSION

In our research, we concluded that determining the physicochemical and biochemical components of AWBC using devices such as the FT-120 milk analyzer, GC, and ICP-MS was highly successful. This was confirmed by comparisons with our previous study on AWB milk and with relevant literature. Additionally, the nutritional content of AWBC demonstrates its importance in the feeding of buffalo calves. The results are expected to be valuable for future studies, as no previous research has been conducted specifically on AWBC.

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