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Original article

Comparison of IgG and lactoferrin levels in the serum and colostrum of holstein and buffalo cows and in their calves

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Abstract

This study compared immunoglobulin G (IgG) and lactoferrin concentrations in the serum and colostrum of cows and buffaloes, as well as in the serum of their calves. A total of 20 dams (10 Holstein cows and 10 buffaloes) and their 20 calves (10 Holstein calves and 10 buffalo calves) were included. Blood samples were collected from all calves on days 0 (before colostrum intake), 1, 2, 7, 14, and 30. In addition, blood and colostrum samples were obtained from the dams on days 0, 1, and 2. IgG and lactoferrin concentrations were measured using enzyme-linked immunosorbent assay (ELISA) with species-specific bovine kits.

Buffalo calves exhibited higher IgG and lactoferrin concentrations than Holstein calves, particularly during the first days after birth (IgG: Holstein calves: 29.10±7.20; Buffalo calves: 38.80±7.40) (Lactoferrin: Holstein calves: 122.00±31.00; Buffalo calves: 273.00±45.00). In both species, IgG and lactoferrin peaked on days 1-2 and then declined. Although IgG concentrations did not differ significantly between cows and buffaloes (p=0,100), buffaloes showed markedly higher lactoferrin concentrations (p=0.007). These findings suggest that buffalo colostrum may provide greater immunological support to calves in the early stages of life compared with Holstein colostrum.

Keywords: buffalo, calves, cow, IgG, lactoferrin

Introduction

The water buffalo (Bubalus bubalis) is an important livestock species in many parts of Asia, the Mediterranean, and South America, contributing significantly to rural livelihoods and food security (Campos de Souza et al. 2020). Similar to other ruminants, buffaloes give birth to agammaglobulinemic or hypogammaglobulinemic calves due to the syndesmochorial placenta, which prevents transplacental transfer of immunoglobulins (de Souza et al. 2019). Therefore, passive immunity must be acquired through the timely ingestion and intestinal absorption of colostrum (Wooding et al. 1997, Campanella et al. 2009). The ability of the neonatal intestine to absorb immunoglobulins decreases sharply within 12 h and ceases around 24 h after birth (Bush and Staley 1980).

Colostrum differs markedly from normal milk in composition, being richer in dry matter, protein, fat, and particularly immunoglobulins (Erdem and Atasever 2005, Yılmaz and Kaşıkçı 2013). The concentration of immunoglobulin G (IgG) is a key determinant of colostrum quality (Erdem and Atasever 2005). As the main immunoglobulin, IgG provides passive immunity by protecting mucosal surfaces against pathogens and mediating immune functions such as complement activation and opsonization (Gapper et al. 2007).

Lactoferrin, another major colostral glycoprotein, also contributes to neonatal defense. It exhibits antimicrobial, antiviral, antifungal, antioxidant, immunomodulatory, and anti-inflammatory activities, and regulates iron absorption (Yıldırım et al. 2011). Together, IgG and lactoferrin represent critical bioactive components that support calf health and development during the early postnatal period (Zarcula et al. 2010, Morrill et al. 2015). Although numerous studies have addressed colostrum composition in ruminants, few have directly compared IgG and lactoferrin concentrations in cows and buffaloes and their offspring. Most available research focuses on a single species, colostrum alone, or primarily on IgG. The aim of this study was to determine and compare IgG and lactoferrin levels in the colostrum and blood serum of Holstein cows and buffaloes during the first three days postpartum, as well as in the serum of their calves before colostrum intake and on days 2, 3, 14, and 30 after birth.

Materials and Methods

All experimental procedures and animal care protocols were carried out appropriately and approved by the Local Ethics Committee of Burdur Mehmet Akif Ersoy University (Protocol number: 2021/710).

In this study, a total of 20 dams (10 Holstein cows and 10 buffaloes) and a total of 20 calves (10 cow calves and 10 buffalo calves) were evaluated. Anatolian buffaloes and Holstein cows and their calves were selected from Burdur Mehmet Akif Ersoy University Research and Application Farm. Burdur has a continental Mediterranean climate with cold, snowy winters and hot, long and dry summers. The average temperature was between 3.9°C and 25°C, 51.2% relative humidity, with an annual precipitation average of 440 mm. Burdur is one of the provinces in Turkey with the highest cattle population.

All animals were in an semi open system with a concrete floor and housed in free stalls. The calves were housed in barns in groups of 5. All Holstein cows and buffaloes were in the first lactation and all of them were subjected to the same vaccination program before this study and they were non-vaccinated during the study and calving. All animals were fed with wheat straw, corn silage, and concentrate. Holstein and buffaloe calves received colostrum within half an hour after birth.

All Holstein and buffalo calves had venous blood samples taken on the day before colostrum (Day 0) and on days 1, 2, 7, 14, and 30. In addition, blood and colostrum samples were collected from Holstein cows and buffaloes immediately after calving (day 0) and on days 1., and 2.

Blood samples (10 mL) were collected by jugular venipuncture after local antisepsis. A vacuum collection system in siliconized tubes without anticoagulant (Vacutainer, Bencton Dickinson, Franklin Lakes, USA) was used. Blood samples were centrifuged at 2,000 g for 10 min, and 2.0 mL aliquots of blood serum were separated and stored in plastic tubes, labeled and maintained at -20°C until the time of the laboratory tests.

Collection of colostrum samples

Colostrum samples were collected from cows immediately after calving and before allowing the calf to nurse, within 2 hours postpartum. The quality of colostrum was assessed using a refractometer, and only samples with a dry matter content between 24-26% were included in the study. From each cow, a 50 ml aliquot of the first milking colostrum was obtained. To ensure representativeness, colostrum from all four quarters of the udder was pooled prior to analysis.

Following collection, samples were immediately frozen at the farm and subsequently transported to the laboratory, where they were stored at -30°C until analysis.

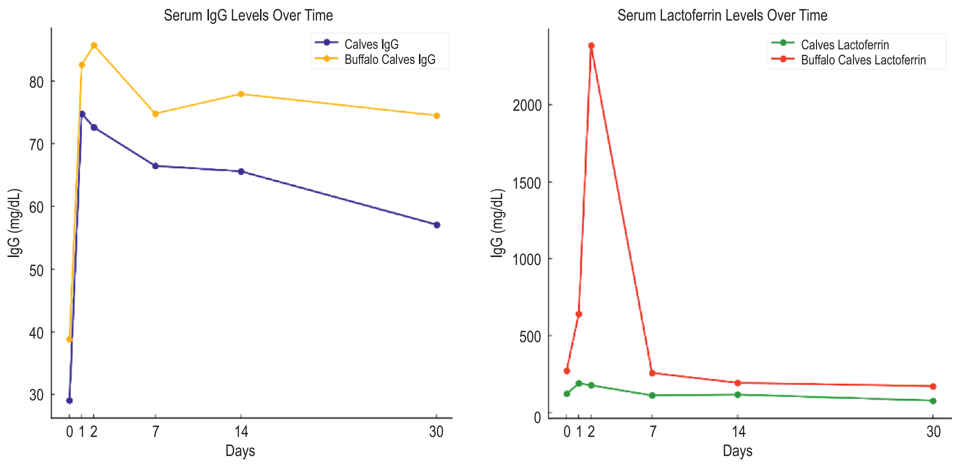

Fig. 1. The IgG and lactoferrin values in the serum of Holstein calves and buffalo calves were compared over time (Days 0, 1, 2, 7, 14, and 30). A. Serum IgG levels, B. Serum lactoferrin values.

Table1. IgG and lactoferrin values in the serum of Holstein calves and buffalo calves.

		Day 0	Day 1	Day 2	Day 7	Day 14	Day 30
IgG (mg/dL)	Holstein Calves	29.10±7.20	74.8±3.30	72.60±4.00	66.47±2.90	65.60±4.50	57.10±4.20
	Buffalo calves	38.80±7.40	82,63±2,20	85.70±0.72	74.80±8.10	77.93±2.50	74.50±4.30
	p value	p=0.357	p=0.071	p=0.011[a]	p=0.353	p=0.032[a]	p=0.010•
Lactoferrin (mg/L)	Holstein Calves	122.00±31.00	191.00±44.00	178.00±51.00	112.00±19.00	117.80±27.00	7.6±8.20
	Buffalo calves	273.00±45.00	642.00±186.00	2384.00±2000.00	257.80± 31.00	194.00±34.00	172.40±31.00
	p value	p=0.014[a]	p=0.040[a]	p=0.299	p=0.001[a]	p=0.093	p=0.016[a]

a < 0.05

Method of analysis

IgG and lactoferrin concentrations in blood serum and colostrum were measured using bovine-specific ELISA kits (BioX® Diagnostics S.A., 38 Rue de la Calestienne, 5580 Rochefort, Belgium) according to the manufacturer's instructions. All samples were first thawed at 4°C, then heated to 20-25°C, and prepared for analysis. Blood sera were diluted 1:10 for IgG and 1:5 for lactoferrin measurements, while colostrum samples were diluted 1:31.6 for IgG determination. Duplicate measurements were performed for all assays, and absorbance readings were recorded at 450 nm using a BioTek Epoch ELISA reader (USA).

Statistical analysis

All statistical analyses were performed using the Minitab statistical software package (Minitab® Release 16, Minitab Inc., USA). Prior to group comparisons, the Shapiro–Wilk test was applied to verify the assumption of normality. Differences between groups were evaluated using the two-sample t-test (independent samples t-test). Data are expressed as mean ± standard error (Mean ± SEM). A p-value < 0.05 was considered statistically significant.

Results

Buffalo calves consistently exhibited higher IgG levels than Holstein calves on Days 0, 1, 2, 7, 14, and 30, with both groups reaching a peak around Days 1-2, followed by a gradual decline over time. Similarly, lactoferrin concentrations were significantly higher in buffalo calves, particularly on Days 1 and 2. In buffalo calves, lactoferrin showed a marked increase on Day 2 before gradually decreasing, whereas Holstein calves maintained relatively stable levels with only a slight reduction by Day 30 (Table 1, Fig. 1).

In cows, IgG levels gradually decreased over time. In buffaloes, IgG levels remained relatively stable, showing a slight increase on Day 2. No statistically significant differences were observed in IgG values between cows and buffaloes on any day ($p>0.05$).

In cows, lactoferrin levels increased on Day 1 but dropped significantly on Day 2. In buffaloes, lactoferrin levels showed a more consistent trend, peaking on Day 0 and remaining higher than in cows on Day 2. Significant differences were observed on Day 0 ($p=0.043$) and Day 2 ($p= 0.007$) (Fig. 2, Table 2).

In cows, IgG levels showed a gradual decrease from Day 0 to Day 2. Similarly, in buffaloes, IgG levels decreased over time but were slightly higher on Day 0 compared to cows. The differences in IgG levels

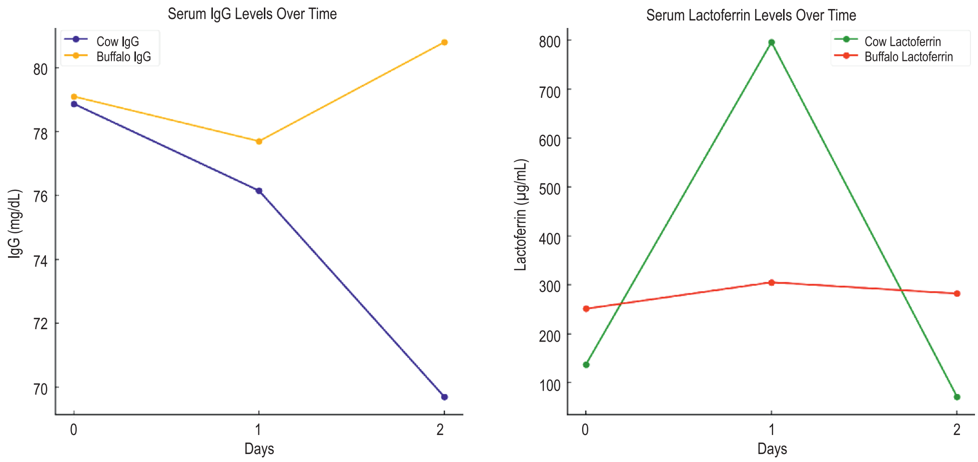

Fig. 2. The IgG and lactoferrin values in the serum of cows and buffaloes on Days 0, 1, and 2.
A. Serum IgG levels, B. Serum lactoferrin values

Table 2. IgG and lactoferrin values in the serum of cows and buffaloes.

		Day 0	Day 1	Day 2
IgG (mg/dL)	Cow	78.87± 1.60	76.15± 2.10	69.70± 4.80
	Buffalo	79.10±4.30	77.70±4.30	80.80±4.20
	p value	p= 0.961	p= 0.754	p= 0.100
Lactoferrin (µg/ml)	Cow	136.90±24.00	796.00±504.00	71.10±8.60
	Buffalo	251.00±44.00	305.00±70.00	282.00±59.00
	p value	p= 0.043[a]	p= 0.360	p= 0.007[a]

a < 0.05

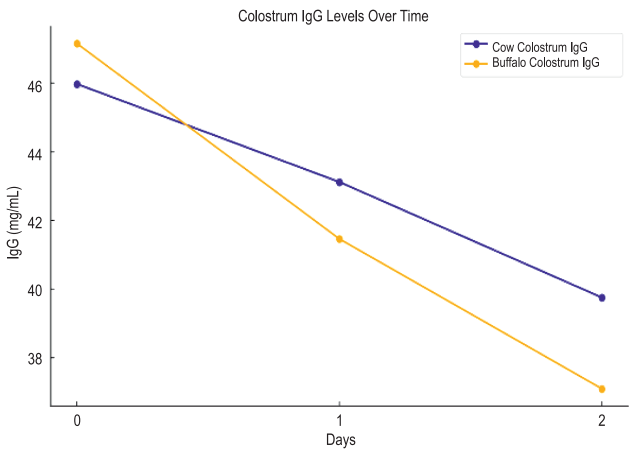

Fig. 3. The IgG levels in the colostrum of cows and buffaloes were compared over Days 0, 1, and 2.

Table 3. IgG levels in the colostrum of cows and buffaloes.

IgG (Colostrum) (mg/ml)	Day 0	Day 1	Day 2
Cow	45.98±1.50	43.12±1.60	39.75±1.50
Buffalo	47.16±1.50	41.46±1.90	37.09±1.90
p value	p=0.594	p=0.507	p=0.282

between cows and buffaloes were not statistically significant on any day (p>0.05) (Fig. 3, Table 3).

Discussion

In previous studies, the volume and timing of colostrum intake significantly influenced serum IgG concentrations in neonatal calves. For instance, Morin et al. (1997) reported that calves receiving 4 L of high-quality colostrum (60.1 mg/mL IgG) at birth and 2 L at 12 hours reached serum IgG concentrations of 10.3 mg/mL at 4 hours and an average of 31.1 mg/mL at 24 hours. Calves receiving only 2 L at birth and 2 L at 12 hours exhibited lower concentrations (7.4 mg/mL at 4 hours and 23.5 mg/mL at 24 hours). In our study, Holstein calves demonstrated similar dynamics, with average serum IgG levels of 29.1±7.2 mg/mL on day 0, peaking on day 1. This pattern aligns with previous reports showing a rapid postnatal increase in IgG levels (Holloway et al. 2002, Morrill et al. 2012, Nowak et al. 2012), although our values were comparatively higher.

Some studies reported peak IgG levels at later time points. While Pecka-Kiełb et al. (2025) reported a significant increase in serum IgG levels on the 3rd day of life in calves, our study demonstrated that serum IgG levels started to decline on the 3rd day of life in both Holstein and buffalo calves. Aydogdu (2014) observed that IgG concentrations in Holstein calves peaked on day 2 and gradually declined thereafter, whereas our study identified the highest IgG levels on day 1. Similarly, Osaka et al. (2014) found that calves receiving colostrum within 1 hour after birth reached the highest 24-hour IgG concentrations (26.8±6.0 mg/mL), consistent with the early peak observed in our study. Comparable serum IgG levels were reported in Charolais calves (30.57±24.68 mg/mL) at 24-48 hours (Martin et al. 2021). Overall, our findings support the established concept that serum IgG peaks within the first day post-colostrum intake, followed by a gradual decline (Wilm et al. 2018).

In buffalo calves, a similar early postnatal IgG peak was observed. Mastellone et al. (2011) reported 31.0±2.4 mg/mL at 24 h, while de Souza et al. (2019) found higher levels in calves born to multiparous dams, with concentrations gradually declining by day 30. Other studies also reported early increases in Murrah buffalo calves, although baseline pre-colostrum IgG values were lower than those observed in our study (Verma et al. 2018, Campos de Souza et al. 2020, Giammarco et al. 2021, Agrawal et al. 2022). Importantly, our measurements extended to day 30, offering a longitudinal perspective that has rarely been reported in previous studies. At all time points, buffalo calves showed higher serum IgG concentrations than Holsteins, with statistically significant differences on days 2, 14, and 30.

Serum lactoferrin patterns paralleled IgG dynamics, peaking on day 1 in both Holstein calves (Minea et al. 2020, Şensoy and Şahinduran 2022) and buffalo calves (Campos de Souza et al. 2020), followed by a gradual decline. Buffalo calves maintained consistently higher levels, with significant differences observed on days 0, 1, 7, and 30.

In cows, serum IgG and lactoferrin peaked around calving or on day 1 and declined thereafter. Buffaloes maintained higher levels than cows at all measured time points, with significant differences on days 0 and 2. Postpartum lactoferrin levels in both cows and buffaloes remain largely unreported, underscoring the novelty of our findings.

Colostral IgG concentrations varied widely among studies and species. In cows, first-milking IgG ranged from 34.9±12.2 mg/mL to 138.5±32.6 mg/mL (Quigley et al. 1995, Holloway et al. 2002, Kehoe et al. 2007, 2011, Gulliksen et al. 2008, Morrill and Tyler 2012, Quigley et al. 2013, Bartier et al. 2015, Cabral et al. 2016, Taniguchi et al. 2016, Dunn et al. 2017, Ceniti et al. 2019, Costa et al. 2021, Martin et al. 2021), while buffalo colostrum ranged from 35.25 to 71.4 mg/mL depending on breed and parity (Abd El-Hady et al. 2006, Dang et al. 2009, Chaudhary et al. 2016, Verma et al. 2018, Campos de Souza et al. 2020, Giammarco 2021, Agrawal et al. 2022, An et al. 2023). In most studies, buffalo colostrum contained higher IgG than cow colostrum, although differences decreased over time (Abd El-Fattah et al. 2012, Wang et al. 2019). In our study, first-milking colostral IgG was higher in buffaloes than cows, consistent with previous reports, and gradually declined during subsequent milkings.

Taken together, these results demonstrate species-specific differences in passive immunity dynamics. Both IgG and lactoferrin levels peak within the first 24 hours, with buffalo calves generally receiving higher serum concentrations than Holstein calves. Our longitudinal measurements up to day 30 highlight that while IgG and lactoferrin decrease over time, buffalo calves maintain relatively higher immune parameters than Holsteins and cows. These findings underscore the importance of early colostrum management and provide novel insights into postnatal immune development in calves and buffaloes.

Conclusion

This study demonstrates that, particularly in the early postnatal period, buffalo calves exhibited higher IgG and lactoferrin levels compared to Holstein calves at all time points, although the differences were not statistically significant. In both species, IgG and lactoferrin levels peaked on days 1-2 and subsequently declined. While colostral IgG levels did not differ significantly between cows and buffaloes, lactoferrin levels were found to be notably higher in buffaloes. These findings highlight the potential advantages of buffalo colostrum, suggesting that it may provide stronger immunological support to buffalo calves in the early stages of life compared to Holstein calves.

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