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The Influence of Physiological and Lactational Factors and Their Association with Milk Yield, Color Parameters and Composition in Holstein Cows

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Abstract | This study aimed to evaluate the impact of both physiological and productive factors on milk color characteristics, milk yield, and its composition in Holstein cow milk under hot climatic conditions, using color analysis technology via a smartphone device. A total of 142 milk samples were collected from 40 cows and categorized according to lactation stage (early: <90 days, mid: 90–180 days, and late: ≥180 days), cow age (<48 months, 48–72 months, and ≥72 months), and parity (first parity, second to third parity, and fourth or higher). Daily milk yield was measured, and analyses of the chemical composition (fat, protein, lactose, and ash content) and physical properties (freezing point, density, and electrical conductivity) were conducted using standard methods. In addition, the "Color Grab App" was employed to convert the images of milk samples into CIELAB values (L*, a*, and b*). The results indicated that an increase in cow age, parity, and progression through the lactation stage is significantly associated with a reduction in daily milk yield, while the concentrations of milk solids and minerals (calcium and potassium) increased accordingly. Milk color parameters (L* = 81.96 ± 0.64; a* = -0.86 ± 0.17; b* = 12.54 ± 0.47) were found to be influenced by both productive and physiological factors; cows in their first parity, younger ages, and during the early lactation stage recorded the highest values of lightness (L*) and yellowness (b*). These changes suggest that an increase in the concentration of milk solids, resulting from a decrease in water content, leads to a reduction in L* values, which affects light absorption and reflection. Statistical analyses further revealed a positive correlation between L* values and daily milk yield, and a negative correlation with the concentrations of chemical and mineral components. The study confirms that milk color parameters can serve as a non-invasive indicator to assess the productive and health status of dairy cows, as well as to determine the quality of the produced milk. Furthermore, the results indicate that the physiological and productive changes affecting the chemical and physical composition of milk will inevitably be reflected in noticeable alterations in its color properties. Overall, these findings provide a robust scientific basis for the development of integrated management and nutritional strategies aimed at enhancing milk quality and achieving sustainable production in challenging climatic environments.

Keywords | Milk color, Milk yield, Lactation, Physiological, Holstein cows

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INTRODUCTION

Dairy cattle are the cornerstone of worldwide milk production, contributing approximately 80.98% of the global milk supply (FAO. FAOSTAT, 2025). Their substantial contribution is fundamental to ensuring food security and addressing the increasing consumption demand for dairy products. Bovine milk delivers a comprehensive spectrum of essential nutrients, offering high biological value proteins, along with fats and lactose as rich energy sources. Moreover, it contains a variety of vitamins critical for supporting neurological health and enhancing immune function (Linehan *et al*., 2024). The characteristic white appearance of bovine milk primarily arises from the dispersion of light within its colloidal structure, which consists of casein micelles (averaging 0.1–5 μm) and homogenized fat globules (Smoczyński *et al*., 2012). The yellowish undertones in milk are primarily varieties due to β-carotene levels that are affected by cattle breed and dietary carotenoid intake, while riboflavin (vitamin B_2) in whey imparts subtle greenish hues, particularly evident in skim milk (Dhar *et al*., 2024). Milk's natural whiteness is primarily enhanced by essential minerals like calcium and phosphorus, while its high-water content (~85%) further influences optical properties, with increased water content contributing to greater lightness. Abnormal color shifts, such as reddish or bluish tints, often indicate microbial contamination or metabolic changes (Diaz-Olivares *et al*., 2024). Monitoring these color characteristics is crucial for assessing the health and productivity of dairy cattle, as it provides valuable insights into nutritional status, disease susceptibility, and overall, well-being, thereby facilitating targeted interventions to optimize herd management and milk quality (D'Alessandro *et al*., 2011).

Milk production and composition are influenced by a complex interplay of genetic, productive, and physiological factors, each contributing to variations in its chemical and physical properties. Among these, physiological responses to environmental challenges, particularly heat stress, have been widely recognized as key determinants of milk quality. Studies indicate that such stressors disrupt metabolic processes, altering nutrient partitioning, enzymatic activity, and hormonal balance, which collectively influence milk composition, particularly fat and protein levels (Al-Kanaan *et al*., 2015; Kipp *et al*., 2021; Sangor *et al*., 2024).

Milk composition, including its fat, protein, and lactose content, is modulated by both genetic and non-genetic factors such as breed, age, and lactation stage (Tirfie, 2023). Cow age, parity, and stage of lactation represent principal physiological and production-related factors that significantly influence the chemical composition of milk. During early lactation, milk yield is typically lower, and total solids reduced due to hormonal fluctuations (Abdulsada *et al*., 2024) and significant structural changes in the mammary gland (Perumal *et al*., 2023). Since milk color results from the combination of its chemical and physical properties, any alteration in milk composition inevitably modifies its color parameters (Al-Hilphy *et al*., 2021, 2022), making milk color a reliable indicator of overall herd health, nutritional adequacy, and animal stress exposure. Younger cows tend to produce milk with a whitish or pale-yellow hue attributable to lower fat content, whereas increased milk yellowness in older cows reflects elevated fat and carotenoid concentrations (Méndez-Cid *et al*., 2017; Alajwadi and Owaid, 2024). Similarly, first-parity cows generally secrete lighter-colored milk, while repeated calvings contribute to a progressive increase in β-carotene, further deepening the milk's yellowness. Recent genetic studies have further demonstrated that polymorphisms in genes such as the growth hormone gene significantly affect milk composition which, in turn, influence its physical characteristics, including color (Yousief *et al*., 2023).

The physical characteristics of milk, including the freezing point, electrical conductivity, and density, act as important markers that reveal the physiological and productive levels of dairy cows as well as the quality of the milk produced. The freezing point is used precisely to assess changes in the concentration of total solids in milk, which may decline due to physiological stress or compositional modifications associated with advancing cow age and increased parity (Chetri *et al*., 2024). Meanwhile, conductivity indicates the ionic balance of minerals such as sodium and chloride, varying according to milk yield and udder health (Yesil and Goncu, 2024). Furthermore, milk density reflects the content of milk solids and is affected by a range of factors, including the production stage and physiological state (Velayudhan *et al*., 2023). In addition, mineral elements such as calcium and potassium are of special significance for milk quality, with calcium playing an essential role in protein assembly and milk stability, and potassium thereby modifying the ionic equilibrium and osmotic pressure within the udder (Voronina *et al*., 2022).

Color perception is initiated by the interaction of light -whether emitted directly, reflected, or transmitted -interacts with surfaces, triggering visual response that depends on its wavelength. Given the subjective variability in human interpretation of color, the development of objective measurement frameworks has become essential (Milovanovic *et al*., 2020, 2021a, b). To this end, the International Commission on Illumination (CIE) established a standardized colorimetry system, that translates optical properties into quantitative values. A fundamental model within this, the CIELAB system (ISO/CIE, 2019) which characterizes color through three primary parameters: lightness (L*; 0–100 scale), red-green axis (a*), and blue-yellow axis (b*) (Chudy *et al*., 2020).

In dairy research, spectrophotometric techniques based on the CIELAB system enable precise evaluation of milk color by correlating subtle chromatic variations with the product's chemical and physical properties (Figueroa *et al.*, 2020). Ultimately, this standardized approach enhances the reproducibility of color assessments and supports quality control, offering valuable insights for innovation in livestock product industries (Green, 2023).

The assessment of milk colorimetry employs both conventional and advanced analytical approaches. While colorimeters quantify chromatic attributes (L*, a* and b*) through reflected light analysis (de Souza Gomes *et al.*, 2024), spectrophotometry further evaluates light absorption/reflectance spectra to correlate color with compositional properties (Bielaszka *et al.*, 2024). Additionally, image-based Computer Vision Systems and Mid-Infrared Spectroscopy enable high-throughput analysis (Franzoi *et al.*, 2023), whereas complementary methods like turbidity, refractometry and traditional colorimetric tests provide rapid, cost-effective quality indicators (Smirnova *et al.*, 2020). However, the high cost and the operational complexity of conventional colorimeters necessitate the adoption of affordable, user-friendly alternatives, such as smartphone-based solutions (Rayhan *et al.*, 2025). In this regard, the Color Grab App represents a significant advancement in color analysis, merging laboratory-grade precision with user-friendly accessibility. Thus, it has proven effective in spectral color analysis for assessing milk quality and quantifying calcium in food and environmental samples as a cost-efficient alternative to traditional spectrophotometry (Peng *et al.*, 2019). Also, its reliability in dairy analysis was validated, showing a deviation of less than <2% compared to reference spectrophotometers and a 95% accuracy achieving ±0.5 CIELAB precision in correlating milk color with lipid-protein profiles (Silva and Rocha, 2020), and hydrogen peroxide as a milk adulterant (Lima *et al.*, 2020). This approach enhances real-time color analysis and facilitates cloud-based data sharing, making it a valuable, accessible tool for food quality assessment and resource-limited research.

This study aims to evaluate the color parameters of Holstein cow milk in Iraq utilizing a cost-effective smartphone-based device. It examines how key productive and physiological factors (such as cow age, parity, and lactation stage) influence colorimetry of milk and its correlation with milk yield, and chemical and physical composition. These interactions are crucial in hot climates to cope with ongoing climatic changes, impacting milk quality and dairy productivity, and informing targeted herd management strategies to improve sustainability in challenging conditions.

MATERIALS AND METHODS

The study was conducted at Al-Khalis Grand Cattle Station in Diyala Governorate, Iraq, from November 11, 2023, to January 11, 2024, using 142 milk samples collected from 40 Holstein dairy cows with ranging in ages, parities, and lactation stages.

Study design

Cows were categorized into three groups based on their lactation stage: Early lactation (<90 days), mid-lactation (90–180 days), and late lactation (≥180 days). They were also classified by age into three groups: <48 months, 48–72 months, and ≥72 months, and by parity into three groups: 1st parity, 2nd to 4th parity, and ≥ 4th parity.

Sample collection and analysis

Milk samples were collected according to a predetermined schedule during the morning milking. Daily milk yield for each cow was measured every two weeks using two 60-mL containers. The first container was sent directly to the station's dairy laboratory for chemical and physical analysis of milk, while the second container was transported in a refrigerated box for further color and mineral analyses.

Chemical and physical analysis

Milk composition was analyzed using a Milkotester (Master PRO-P1, Bulgaria) that measured fat%, protein%, lactose% and ash%, as well as physical parameters such as freezing point (°C), density (lactometer reading) and electrical conductivity (mS/cm).

Color analysis

Milk color parameters were assessed using a smartphone-based colorimetric method under controlled and standardized conditions. Measurements were performed within a custom-designed, light-shielded apparatus to minimize ambient light interference and ensure consistent sample illumination and presentation geometry. A Samsung Galaxy A12, equipped with a 48-megapixel rear camera (aperture f/2.0) and a 6.5-inch display (720×1560 pixels), operating on Android 13 with a MediaTek Helio P35 processor and a 5000 mAh battery, was employed. Furthermore, the Color Grab App (Loomatix, Version 3.9.2, 2023) was used to convert images into RGB values and analyze the fundamental color parameters (L*, a*, b*) within a 32×32-pixel region centered on the test tube.

Mineral analysis

Minerals were analyzed using a specialized assay kit from Bio Research for Medical Diagnostics (Jordan) to measure calcium and potassium through spectrophotometric analysis.

Statistical analysis

Data were analyzed using a mixed model approach in IBM SPSS Statistics for Windows 11, Version 29.0 (IBM Corp., Armonk, NY, USA) to evaluate the effects of non-genetic factors on milk traits. Cow ID was included as a random effect to account for individual variability, while fixed effects included cow age, parity, and lactation stage. Multiple comparisons among means were performed using the adjusted LSD test. Results are presented as mean ± standard error, with differences considered statistically significant at P < 0.05.

RESULTS AND DISCUSSION

Milk production

The average daily milk yield for Holstein cows in this study was 12.07 ± 0.40 kg. This mean production level is consistent with findings from other studies in Iraqi Holstein cows, which report daily milk yields ranging from 8.74 kg (Al-Taiy, 2025) to 23.15 kg (Dragh *et al.*, 2023). However, it is notably lower than yields reported in intensive dairy systems in regions with similar agricultural and climatic conditions, such as 30.34 kg in Egypt (Faid-Allah *et al.*, 2025) and 41.24 to 46.87 kg in Saudi Arabia (Mohammed *et al.*, 2024). The considerable variability in milk production observed in our study (range 5.25-25.75 kg) may reflect individual animal factors alongside nutritional and environmental influences, particularly the harsh climatic conditions prevalent in Iraq.

Figure 1 elucidates the influence of lactation stage, cow age, and parity on average daily milk yield in the Holstein cows under this investigation. The results clearly indicate that lactation stage is a major factor in milk production. As depicted in Figure 1A, cows in early lactation (< 90 days) exhibited the highest average daily milk yield (19.09 kg), followed by a significant decline (P < 0.05) in mid-lactation (90-180 days) to 14.40 kg, reaching the lowest level in late lactation (≥ 180 days) at 9.91 kg. This production pattern aligns with the typical lactation curve observed in dairy cows, characterized by peak yield in early stages and a gradual decline thereafter (Akers, 2016). The gradual decline in milk yield throughout lactation is a consequence of natural physiological mechanisms in dairy cows. These processes involve a shift in energy and nutrient allocation away from milk production and towards other physiological priorities (Connolly *et al.*, 2023). Notably, the reduced milk output during the late lactation phase plays a vital biological role, allowing the cow to prioritize nutrient allocation for fetal growth during late pregnancy and facilitate mammary gland involution and body tissue replenishment, particularly during the dry period, in preparation for the subsequent lactation cycle (Kotsampasi *et al.*, 2024).

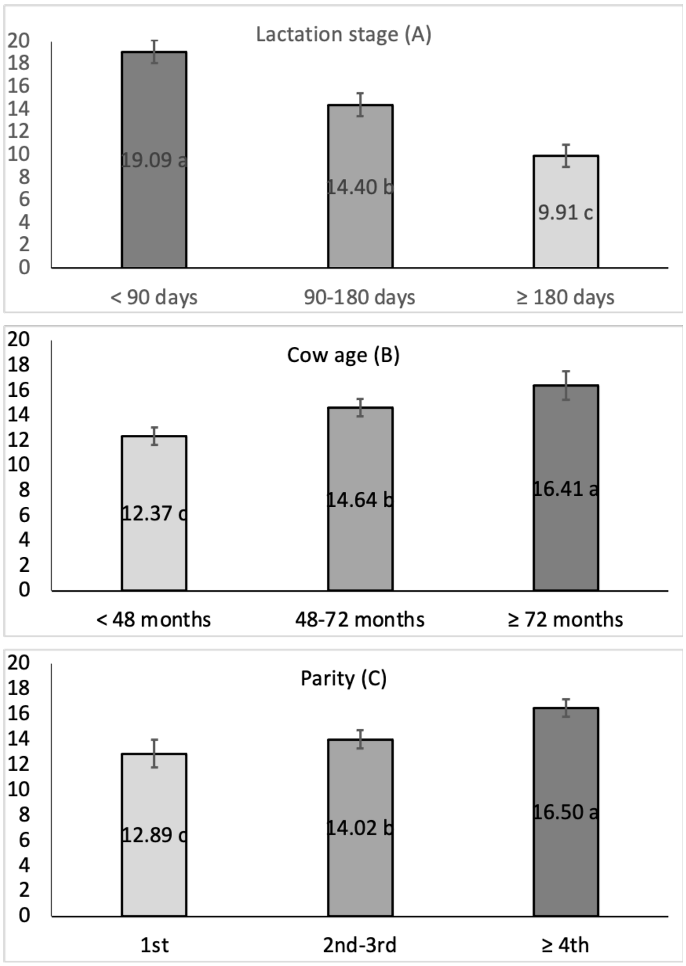

Figure 1: Effect of lactation stage (A), age (B) and parity of cow on daily milk production (Kg/day/cow) in Holstein cattle.

Cow age also significantly affected daily milk yield (Figure 1B). In contrast to typical expectations of declining productivity with age, older cows (≥ 72 months) exhibited the highest average daily milk yield (16.41 kg), followed by mid-aged (14.64 kg) and younger cows (12.37 kg) (P < 0.05). This finding can be explained by the specific herd dynamics under study. The herd composition with a lower proportion of aged or clinically compromised cows, combined with effective management practices, likely contributed to the sustained high productivity observed in older animals (Dallago *et al.*, 2021).

Parity also significantly influenced daily milk yield (Figure 1C). Cows in parity ≥ 4th parity had the highest average daily milk yield (16.50 kg), significantly exceeding cows in parities two and three (14.02 kg), and primiparous cows (12.89 kg). This trend aligns with established knowledge that milk production typically increases with parity, reaching a peak at the third or fourth lactation before a gradual decline (Mayasari *et al.*, 2024). The parity-associated increase in milk yield can be attributed to the progressive maturation of the mammary gland and its enhanced milk synthesis capacity across lactations, alongside the cow's increasing metabolic efficiency and physiological adaptation to lactation demands (Quaresma and Payan-Carreira, 2021).

Chemical composition of milk

Table 1 displays the effects of lactation stage, cow age, and parity on the chemical composition of milk. As anticipated, lactation stage significantly affected all chemical components examined (P< 0.05). As anticipated, lactation stage significantly affected all chemical components examined (P < 0.05), with a general trend of gradual increase in fat (4.62%), protein (3.41%), lactose (5.23%), and ash (0.79%) percentages were observed with advancing lactation stage, reaching peak concentrations in late lactation. Conversely, early lactation milk exhibited the lowest percentages of these components (2.53% for fat, 2.60% for protein, 4.46% for lactose, and 0.60% for ash). This trend aligns with the gradual increase in total milk solids and the concomitant decrease in daily milk yield observed in Figure 1A, reflecting a concentration effect of milk solids at the expense of water content as lactation progresses. This variation in milk components reflects the natural physiological and hormonal shifts inherent to different lactation stages, ultimately serving to meet the evolving demands of milk production while maintaining milk quality and nutritional balance (Connolly *et al*., 2023). Furthermore, advances in ultrafiltration have improved milk composition by increasing protein concentration while reducing moisture, lactose, and salt (Al-Hatim *et al*., 2020).

Also, a comparable trend of increasing fat, lactose, and ash percentages with advancing age was evident, with older cows (≥ 72 months) exhibiting the highest mean values (3.80%, 5.26%, and 0.77%, respectively) and younger cows (< 48 months) displayed the lowest (3.15%, 4.65%, and 0.64%) levels. This age-related progressive increase in milk components may be attributed to the limited capacity of younger cows to synthesize milk constituents, particularly fat, due to their incomplete body development combined with a smaller digestive system (Johansson *et al*., 2024). In contrast, milk protein remained constant with advancing cow age, potentially suggesting a distinct regulatory mechanism for milk protein synthesis compared to the other components under study (Voronina *et al*., 2022).

Regarding parity, a similar trend to lactation stage and age emerged, with multiparous cows (≥ 4th parity) recording the highest percentages of fat (3.77%), lactose (5.12%), and ash (0.75%), while primiparous cows recorded the lowest values (2.96%, 4.76%, 0.66%, respectively). This upward trend in milk solids concentration with parity can be attributed to enhanced metabolic efficiency and progressive mammary gland development across lactations (Van Eetvelde *et al*., 2020). Multiparous cows are postulated to acquire an improved capacity for accumulating and synthesizing key milk components due to accumulated physiological expertise and metabolic adaptations (Quaresma and Payan-Carreira, 2021). Furthermore, greater udder capacity and more developed glandular tissue in multiparous cows may contribute to higher solids concentration per milk volume (Jaswal *et al*., 2022). The similar effect of cow age and parity on milk chemistry suggests shared underlying physiological mechanisms related to mammary development and enhanced milk solids production with advancing age and parity (Van Eetvelde *et al*., 2020). Consistent with prior research on Iraqi indigenous breeds, Faraj *et al*. (2023) demonstrated that parity, age, and lactation stage significantly affect milk composition, particularly percentages of fat, protein, lactose, and total solids. These compositional variations, as elucidated by their genetic research, underlie the physiological mechanisms driving mammary gland development and efficient milk solids synthesis during lactation.

Table 1: Effects of lactational and physiological factors on chemical composition (Fat%, Protein%, Lactose% and Ash%) of Holstein milk.

		Fat%	Protein%	Lactose%	Ash%
Lactation	< 90 days	2.53±0.10 [a]	2.60±0.09 [a]	4.46±0.06 [a]	0.60±0.01 [a]
	90-180 days	3.15±0.07 [b]	3.25±0.06 [b]	5.14±0.04 [b]	0.73±0.01 [b]
	≥ 180 days	4.62±0.08 [c]	3.41±0.07 [c]	5.23±0.05 [b]	0.79±0.01 [c]
Age	< 48 months	3.15±0.07 [a]	2.92±0.06 [a]	4.65±0.04 [a]	0.64±0.01 [a]
	48-72 months	3.35±0.07 [b]	3.16±0.06 [b]	4.91±0.04 [b]	0.70±0.01 [b]
	≥ 72 months	3.80±0.12 [c]	3.18±0.09 [b]	5.26±0.07 [c]	0.77±0.01 [c]
Parity	1	2.96±0.10 [a]	3.00±0.12 [a]	4.76±0.06 [a]	0.66±0.01 [a]
	2-3	3.57±0.08 [b]	3.09±0.06 [a]	4.95±0.04 [b]	0.70±0.01 [b]
	≥ 4	3.77±0.08 [c]	3.17±0.05 [a]	5.12±0.05 [c]	0.75±0.01 [c]

Table 2: Effects of lactational and physiological factors on physical properties (freezing point, conductivity and density) of Holstein milk.

		Freezing point (°C)	Conductivity (mS/cm)	Density (lactometer reading)
Lactation	< 90 days	-0.58±0.01 [a]	5.01±0.06 [a]	32.74±0.45 [b]
	90-180 days	-0.60±0.01 [b]	4.99±0.04 [a]	32.77±0.32 [b]
	≥ 180 days	-0.61±0.01 [b]	5.10±0.04 [a]	31.27±0.37 [a]
Age	< 48 months	-0.58±0.01 [a]	5.00±0.04 [a]	31.57±0.33 [a]
	48-72 months	-0.60±0.01 [a]	5.06±0.04 [a]	32.54±0.32 [b]
	≥ 72 months	-0.60±0.01 [a]	5.07±0.06 [a]	32.67±0.54 [b]
Parity	1st	-0.59±0.01 [a]	5.07±0.06 [a]	32.71±0.48 [a]
	2nd-3rd	-0.59±0.01 [a]	4.98±0.04 [a]	31.94±0.35 [a]
	≥ 4th	-0.59±0.01 [a]	5.07±0.04 [a]	32.13±0.36 [a]

Physical properties of milk

Table 2 illustrates the influence of lactation stage, cow age, and parity on the physical properties of Holstein milk, notably freezing point, electrical conductivity, and density. Freezing point significantly decreased with advancing lactation, reaching its lowest in late lactation (-0.61 ± 0.01 °C) compared to early lactation (-0.58 ± 0.01 °C). This decline may reflect osmotic pressure and dissolved solids concentration shifts as lactation progresses, potentially due to altered total solids content (Voronina *et al*., 2022) and physiological stress in late lactation (Chetri *et al*., 2024). Furthermore, this trend may be explained by an increase in daily milk yield during late stage of lactation (Figure 1A) and a corresponding rise in water percentage accompanied by a decrease in total solids content (Supplementary Table 1) as lactation across its end and toward cows dried off. Density reflected this trend, decreasing in late lactation (31.27 ± 0.37 lactometer reading) relative to early lactation (32.74 ± 0.45 lactometer reading), consistent with the impact of milk solids concentration, where higher solids mean greater density (Velayudhan *et al*., 2023), and early lactation milk's lower water content contributes to higher density. Older cows (≥ 72 months) produced denser milk (32.67 ± 0.54) than younger cows (< 48 months) (31.57 ± 0.33), aligning with increased fat, protein, lactose, ash, and total solids with age (Supplementary Table 1), indicating more concentrated milk in older cows lactation (Chetri *et al*., 2024). Conversely, electrical conductivity showed no significant variation across factors (4.98-5.10 mS/cm), suggesting stable ionic composition likely due to good udder health. While conductivity remained stable in this study, it's noteworthy that milk conductivity is recognized as a valuable indicator for mastitis detection, as highlighted by machine learning-based prediction models for clinical mastitis (Tian *et al*., 2024), implying that consistent conductivity here may reflect effective herd management and overall udder health (Dallago *et al*., 2021).

Table 3: Effects of lactational and physiological factors on minerals (calcium and potassium) of Holstein milk.

		Calcium	Potassium
Lactation	< 90 days	2.44±0.04 [a]	1.08±0.02 [a]
	90-180 days	2.97±0.03 [b]	1.31±0.01 [b]
	≥ 180 days	3.21±0.03 [c]	1.42±0.02 [c]
Age	< 48 months	2.63±0.03 [a]	1.16±0.01 [a]
	48-72 months	2.84±0.03 [b]	1.25±0.01 [b]
	≥ 72 months	3.15±0.05 [c]	1.39±0.02 [c]
Parity	1st	2.70±0.05 [a]	1.19±0.02 [a]
	2nd-3rd	2.87±0.03 [b]	1.27±0.01 [b]
	≥ 4th	3.06±0.03 [c]	1.35±0.01 [c]

Calcium and potassium in milk

Table 3 demonstrates a significant influence of lactation stage, cow age, and parity on calcium and potassium concentrations in Holstein milk, with a notable significant increase in the concentration of both minerals as influenced by the studied factors. Milk calcium and potassium content significantly increased with advancing lactation stage. Cows in late lactation (≥ 180 days) exhibited the highest averages for both minerals (Calcium: 3.21±0.03, Potassium: 1.42±0.02), significantly surpassing milk from mid-lactation (90-180 days; Calcium: 2.97±0.03, Potassium: 1.31±0.01) and early lactation (< 90 days; Calcium: 2.44±0.04, Potassium: 1.08±0.02). This progressive increase can be partially attributed to the decrease in milk yield associated with advancing lactation stage (Figure 1), potentially leading to a relative concentration effect of milk solids (Table 1 and Supplementary Table 1). Similarly, older cows (≥ 72 months) produced milk with a higher concentration of calcium (3.15±0.05) and potassium (1.39±0.02) compared to younger cows (< 48 months; Calcium: 2.63±0.03, Potassium: 1.16±0.01). This trend may reflect the cumulative effect of age on the development of body condition and digestive functions in cows, potentially enhancing the efficiency of feed digestion, mineral absorption, and their transfer to milk. This aligns with findings by Dallago *et al.* (2021), indicating that older cows may possess higher efficiency in nutrient digestion and absorption. Furthermore, Voronina *et al.* (2022) highlighted the physiological adaptations occurring within the mammary gland during different stages of milk production. Parity also showed a similar pattern, where milk from cows in their ≥ 4th parity recorded the highest levels of calcium (3.06±0.03) and potassium (1.35±0.01), exceeding milk from first-parity cows (Calcium: 2.70±0.05, Potassium: 1.19±0.02), which may suggest improved mammary gland efficiency in multiparous cows (Jaswal *et al.*, 2022).

Given the crucial role of calcium in milk protein stability and potassium in maintaining ionic balance (Voronina *et al.*, 2022), these concentration increases may contribute to preserving milk quality and its processing characteristics. Other studies have corroborated the significance of these productive and physiological factors in milk composition. El-Qaliouby *et al.* (2024) indicated a similar effect of cow age and parity on milk solid and mineral components, while Walter *et al.* (2022) confirmed the influence of lactation stage on metabolic markers, and Zyl *et al.* (2024) demonstrated the sensitivity of milk properties to managerial and environmental factors interacting with the physiological stage. These results are consistent with the analysis by Mayasari *et al.* (2024) regarding the relationship between parity, lactation period, and milk production. Collectively, these findings underscore the complex physiological regulation of mineral secretion into milk and its modulation by key productive and physiological factors, warranting further research to elucidate the precise mechanisms and their implications for milk quality (Toscano *et al.*, 2023). Understanding these effects is crucial for optimizing dairy cow feeding and management to achieve superior milk mineral quality across various physiological and lactational stages. This underscores the importance of alternative nutritional strategies that enhance productivity, economic efficiency, and sustainability in animal production (Al-Kanaan, 2022).

Milk color parameters

In the current study, the overall mean values for CIELAB milk color parameters were L*= 81.96 ± 0.64 (68.09 - 95.38), a*= -0.86 ± 0.17 (-4.80 - 1.60), and b*= 12.54 ± 0.47 (4.17 - 22.00) (Table 4). As the first investigation into the colorimetric properties of Holstein cow's milk in Iraq, this study addresses a gap in regional dairy research. This research elucidates how physiological and lactational factors influence milk color as an indicator of quality and herd health under Iraq's unique environmental and managerial conditions. Amidst challenges facing the Iraqi dairy sector, this study's significance lies in providing valuable insights for decision-makers and dairy farmers, thereby offering a scientifically grounded foundation for optimizing herd management to enhance milk quality and economic value. Furthermore, the adoption of the smartphone Color Grab App provides a methodologically innovative, accessible, and cost-effective method for milk color assessment, with broad applications for dairy quality control and research (Peng *et al.*, 2019; Lima *et al.*, 2020; de Souza Gomes *et al.*, 2024; Rayhan *et al.*, 2025). These values fall within the ranges previously reported by Al-Hilphy *et al.* (2022) for local Iraqi cattle (L*: 69.22 - 75.3, a*: -0.71 - 4.71, b*: 7.06 - 11.53), though some variations were noted. Differences between the two studies may be attributed to breed-related factors, as local Iraqi cattle typically produce milk with higher fat content and lower yield than Holstein cows (Yousief *et al.*, 2024), which could influence colorimetric properties. Additionally, variations in environmental conditions, feeding regimes, and management practices between the two studies may have contributed to the observed variations.

Table 4: Effects of lactational and physiological factors on CIELAB color characteristics (L*, a*, and b*) in Holstein milk.

Main Factors	Categories	L* (Mean ± SE)	a* (Mean ± SE)	b* (Mean ± SE)
Lactation	< 90 d	87.12±0.82 [a]	-1.26±0.22 [a]	16.71±0.80 [a]
	90-180 d	81.72±0.60 [b]	-0.63±0.14 [b]	11.32±0.52 [b]
	≥ 180 d	76.57±0.71 [c]	-0.72±0.16 [b]	8.71±0.61 [c]
age	< 48 mo	83.98±0.63 [a]	-0.93±0.15 [a]	12.68±0.55 [a]
	48-72mo	81.45±0.59 [b]	-0.79±0.14 [a]	13.01±0.53 [a]
	≥ 72 mo	79.55±1.02 [b]	-0.88±0.24 [a]	11.06±0.90 [a]
Parity	1st	84.18±0.80 [a]	-0.96±0.27 [a]	13.71±0.93 [a]
	2nd-3rd	82.07±0.60 [b]	-0.74±0.15 [a]	12.12±0.56 [ab]
	≥ 4th	79.16±0.66 [c]	-0.90±0.13 [a]	10.91±0.5[1] b

Color coordinates were measured using the CIELAB system: L* (lightness), a* (red-green axis), b* (blue-yellow axis). Superscripts ([a], [b], [c]) denote significant differences (P < 0.05, Bonferroni-adjusted LSD) of column means in mixed model with cow as random effect, and age, parity, and lactation stage as fixed effects.

Lactation stage significantly affected milk color, particularly L* and b* values. Cows in early lactation exhibited the highest L* values (87.12 ± 0.82), which declined progressively to 76.57 ± 0.71 in milk of late lactation. Similarly, b* values declined from 16.71 ± 0.80 in early lactation to 8.71 ± 0.61 in late lactation, suggesting a reduction in β-carotene levels as lactation progresses. The a* values were considerably more negative in early lactation (-1.26 ± 0.22), indicating greener hue, but stabilized during mid (-0.63 ± 0.14) and late lactation (-0.72 ± 0.16), possibly due to fluctuations in riboflavin content. According to Al-Muhja *et al.* (2023), the third lactation stage in Iraqi Holstein cows is characterized by increased protein, lactose, and total solids levels compared to earlier stages. These changes in milk composition could partially explain variations in its colorimetric properties, emphasizing the relevance of lactation stage when assessing milk quality via color measurements

Age also exerted a notable influence on milk lightness and yellowness. Milk from younger cows (< 48 months) exhibited significantly higher L* values (83.98 ± 0.63) compared to older cows (≥ 72 months: 79.55 ± 1.02, $P < 0.05$), potentially reflecting lower intramammary fat content in younger animals. This age-related difference in lightness aligns with Scarso *et al.* (2017) who suggested that milk color is influenced by both genetic and non-genetic factors, including fat content. Conversely, b* values peaked in middle-aged cows (48–72 months: 13.01 ± 0.53), suggesting increased milk yellowness during these years, which could be indicative of more efficient carotenoid absorption, particularly relevant considering the dietary influences on milk color emphasized by Kilcawley *et al.* (2018). Notably, a* values remained statistically consistent across age groups, indicating a less pronounced age-related impact on red green chroma.

Parity significantly determined L* and b* color parameters: primiparous cows (first parity) exhibited the highest L* values (84.18 ± 0.80) and b* values (13.71 ± 0.93), while multiparous cows (≥ 4th parity) showed significantly lower L* values (79.16 ± 0.66) and declining b* values (10.91±0.51). This aligns with established findings associating higher fat and total solids in multiparous milk with concentrated fat-soluble pigments, thus reducing lightness and yellowness. Consistent with this, Milovanovic *et al.* (2020) suggest that primiparous milk's typically lower fat and solid content may, in turn, be reflected in its lighter and more yellow appearance. Conversely, multiparous milk's higher concentration of these components results in darker, less pronounced yellow hues, despite potentially containing more fat-associated pigments.

These findings underscore the influence of lactational and physiological factors on milk color attributes, which could have implications for milk quality assessment and processing characteristics in Holstein cows. Collectively, these results indicate that both lactational and physiological factors play a critical role in determining the colorimetric properties of Holstein milk. The progressive decline in L* and b* values with advancing lactation stage, increasing cow age, and higher parity suggests that milk becomes darker and less yellow as the cow's physiological status changes over time. This could be associated with increased concentrations of milk solids, such as fat and proteins, and alterations in mineral and pigment composition. Such compositional changes likely reflect underlying metabolic and hormonal adaptations, which are known to impact milk quality and overall dairy productivity.

The significant variations observed in the CIELAB parameters underscore the potential utility of milk color as a non-invasive, rapid indicator of milk quality and cow health. These findings not only corroborate previous literature (Al-Hilphy *et al.*, 2021, 2022) but also highlight new perspectives for dairy management. Future research should focus on elucidating the biochemical pathways that drive these color changes and explore the practical applications of colorimetry in monitoring dairy herd performance under diverse environmental conditions.

Correlation analysis of the studied traits

Table 5 presents the correlation matrix showing the relationships between milk color parameters, milk yield, physicochemical composition, and mineral content, reflecting the interaction of these variables within the study. The current study focuses on the CIELAB color properties of milk (L*, a*, b*) and attempts to understand how these characteristics correlate with other milk components. In this context, the L* value (lightness) emerges as a fundamental color attribute of particular importance. The findings reveal a significant positive correlation between L* and milk yield ($r = 0.79$, $p<0.01$), indicating that cows with high milk yield produce milk with greater lightness than cows with low milk yield. This can be attributed to the dilution effect, whereby increased volume of milk (Figure 1) distributes the solid content over a larger amount of water, leading to lower concentrations of total solids (Table 1) and minerals (Table 3) compared to milk from low-yielding cows (Voronina *et al.*, 2022; Chetri *et al.*, 2024). Conversely, L* shows significant negative correlations ($p<0.01$) with the chemical components of milk-fat ($r = -0.73$), protein ($r = -0.60$), lactose ($r = -0.66$), ash ($r = -0.68$), calcium ($r = -0.86$), and potassium ($r = -0.68$). This indicates that milk with higher L* value has lower levels of these components, which may be attributed to a dilution effect resulting from increased water content (Supplementary Table 1). Physically, milk with lower density, reflecting a lower concentration of solids, tends to reflect more light and therefore appears lighter, resulting in higher L* values. In contrast, milk with a higher solid content exhibits reduced light reflection and appears darker, consistent with the density measurements shown in Table 3 (Figueroa *et al.*, 2020; Velayudhan *et al.*, 2023).

Regarding the b* value (yellowness), a positive correlation with milk yield ($r = 0.70$, $p<0.01$) and negative correlations with chemical components were observed. This may be due to the optical dilution effect, where increased milk production results in a broader distribution of solid

Table 5: Pearson correlation matrix for Holstein milk: Color parameters, milk yield, milk composition, physical properties, and mineral content.

	L*	a*	b*	Milk prod	Fat	Protein	Lactose	Ash	Freezing	Conduct	Density	Calcium
L*												
a*	-0.15											
b*	0.53**	-0.27**										
Milk prod	0.79**	-0.13	0.70**									
Fat	-0.73**	0.08	-0.59**	-0.90**								
Protein	-0.60**	0.11	-0.62**	-0.78**	0.56**							
Lactose	-0.66**	0.14	-0.58**	-0.78**	0.51**	0.70**						
Ash	-0.68**	0.17*	-0.60**	-0.78**	0.60**	0.72**	0.80**					
Freezing	0.26**	0.05	0.22**	0.39**	-0.19*	-0.52**	-0.50**	-0.34**				
Conduct	-0.02	0.06	-0.01	-0.06	0.05	0.03	0.08	-0.01	-0.23**			
Density	0.04	-0.11	0.03	0.02	-0.30**	0.29**	0.32**	0.09	-0.82**	0.21*		
Calcium	-0.68**	0.17*	-0.60**	-0.78**	0.60**	0.72**	0.80**	0.99**	-0.34**	-0.01	0.01	
Potassium	-0.68**	0.17*	-0.60**	-0.78**	0.60**	0.72**	0.80**	0.99**	-0.34**	-0.01	0.01	0.99**

compounds, which in turn affects light absorption and reflection, altering the perceived yellowness. Additionally, the complex interaction among fat, protein, and water might modify light refraction within the milk, influencing the yellow color perception. Whereas, for a* value color parameters, shows only weak correlations with other traits, suggesting that variations along the red-green axis are less influential overall milk composition.

The correlations between physical and mineral traits further highlight the strong association between density and solids. A significant positive correlation between fat and density suggests that higher solids contribute to greater milk density, as reflected in Table 2. Moreover, the negative correlations of calcium and potassium with milk yield and their positive associations with all major milk components underscore their importance in maintaining the chemical and physical balance of milk. These minerals play a crucial role in stabilizing proteins and fats, enhancing milk productivity and nutritional quality, and serving as indicators of mineral absorption efficiency and optimal physiological status in dairy cows (Voronina *et al*., 2022). Finally, the stability of electrical conductivity values further reflects robust udder health and animal well-being, underscoring the integral role of animal welfare in maintaining milk quality and overall productivity.

Overall, these findings highlight that milk color is not merely a visual attribute influencing consumer acceptance but is also closely linked to the concentrations of chemical and physical components. This suggests that any change in milk color inevitably reflects alterations in its nutrient profile. Thus, milk color can serve as a reliable indicator of milk composition and quality, as well as an effective tool for assessing the production, physiological, and health status of dairy cows. These findings underscore the importance of using milk color assessment as a non-invasive, practical tool for monitoring milk quality and herd health, providing a scientific basis for improving management strategies in livestock production systems (Figueroa *et al*., 2020).

CONCLUSIONS AND RECOMMENDATIONS

The results of the study indicate that both productive and physiological factors play a pivotal role in influencing daily milk yield, its chemical and physical composition, and the concentration of minerals. Our findings reveal that as cows age, progress in parity, and advance through the lactation phase, there is a significant decline in daily milk yield, while concurrently, the concentrations of milk solids (fat, protein, lactose, and ash) and minerals (calcium and potassium) exhibit a notable increase. This alteration is reflected in the milk's color characteristics, as evidenced by decreases in lightness (L*) and yellowness (b*) values, corresponding proportionally to the increased concentration of solids and their consequent effects on light absorption and reflection. As the first study of its kind to investigate the colorimetric properties of Holstein cow milk reared in Iraq, these findings underscore that the observed color attributes are not merely superficial visual traits but rather serve as vital indicators that accurately reflect the underlying productive and physiological changes occurring throughout the management period under local environmental conditions. The results further demonstrate that an elevated L* value correlates with increased daily milk yield accompanied by lower concentrations of milk constituents, whereas a reduced L* value indicates a higher concentration of milk solids. Moreover, the effective utilization of the Color Grab App validates the reliability of smartphone applications as precise and cost-effective tools for color analysis, thereby offering a practical means for monitoring of milk quality. These findings provide a robust scientific foundation for the development of innovative herd management strategies that emphasize environmental sustainability, particularly in challenging hot climates such as those found in Iraq, in order to enhance both milk quality and sustainable productive performance.

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NOVELTY STATEMENT

This study establishes that milk color parameters, quantified via a cost-effective smartphone application, function as powerful non-invasive indicators for key productive and physiological traits in Holstein cows. The research demonstrates that variations in milk lightness (L*) and yellowness (b*) are not merely superficial qualities but are strongly correlated with daily milk yield, concentrations of milk solids (fat, protein), and mineral content (calcium, potassium). These findings present a novel and practical approach for real-time milk quality assessment, offering a valuable tool for optimizing herd management and enhancing productivity, particularly in challenging climatic environments.

AUTHOR'S CONTRIBUTION

AJJ Al-Kanaan: Conceptualization, methodology, supervision, validation, writing review and editing.
AJA Al-Rabiey: Investigation, data curation, formal analysis, software, writing original draft.
All authors have read and approved the final version of the manuscript for publication.

Generative AI and AI-assisted technology statement

This manuscript was prepared with the assistance of generative AI technologies and AI-assisted tools to improve language clarity and facilitate the editing process. The authors confirm that all scientific content, data interpretation, and conclusions are the original work of the authors. The use of AI tools was limited to language refinement only and did not influence the scientific integrity, analysis, or results of this study. The authors remain fully responsible for the accuracy and integrity of the research reported

Conflict of interest

The authors have declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Abdulsada HM, Kassim WY, Hassan KA (2024). Effect of birth season and lactation stages on the level of some hormones and milk yield of Holstein x Janoubi cows. IOP Conf. Ser. Earth Environ. Sci., 1371: 072030. https://doi.org/10.1088/1755-1315/1371/7/072030

Akers RM (2016). Lactation and the mammary gland. John Wiley and Sons. https://doi.org/10.1002/9781119264880

ALajwadi IMN, Owaid JM (2024). Polymorphism of the DGAT1 gene and its relationship to milk yield and some chemical properties of milk during the first stage of production in local cows and Holstein cows; a comparative study. J. Glob. Innov. Agric. Sci., 12: 101–104. https://doi.org/10.22194/JGIAS/12.1150

Al-Hatim RR, Al-Rikabi AK, Ghadban AK (2020). The physico-chemical properties of bovine and buffalo whey proteins milk by using ultrafiltration membrane technology. Basrah J. Agric. Sci., 33: 122–134. https://doi.org/10.37077/25200860.2020.33.1.10

Al-Hilphy AR, Ali HI, Al-IEssa SA, Gavahian M, Mousavi-Khaneghah A (2022). Assessing compositional and quality parameters of unconcentrated and refractive window concentrated milk based on color components. Dairy, 3: 400–412. https://doi.org/10.3390/dairy3020030

Al-Hilphy AR, Ali HI, Al-IEssa SA, Lorenzo JM, Barba FJ, Gavahian M (2021). Refractance window (RW) concentration of milk-Part II: Computer vision approach for optimizing microbial and sensory qualities. J. Food Process. Preserv., 45: e15702. https://doi.org/10.1111/jfpp.15702

Al-Kanaan A, König S, Brügemann K (2015). Effects of heat stress on semen characteristics of Holstein bulls estimated on a continuous phenotypic and genetic scale. Livest. Sci., 177: 15–24. https://doi.org/10.1016/j.livsci.2015.04.003

Al-Kanaan, G. A., Al-Bader, S. H., Al-Kanaan, A. J., & Al-Aqaby, H. S. (2021). Effects of different levels of vitamin C intake on some productive traits and blood parameters in rabbits. In AIP Conference Proceedings (Vol. 2372, No. 1, p. 030013). AIP Publishing LLC. https://doi.org/10.1063/5.0065393

Al-Kanaan AJJ (2022). Effects of adding different levels of hydroponic barley fodder on the productive performance and economic value of broiler chickens. Arch. Razi Inst., 77: 1853–1864.

Al-Muhja RK, Yousief MY, Al-Khauzai ALD (2023). A study of the relationship of polymorphisms of the PPARGC1A gene with milk production and its components, in Holstein Friesian cows. IOP Conf. Ser. Earth Environ. Sci., 1262: 072069. https://doi.org/10.1088/1755-1315/1262/7/072069

Al-Taiy MK (2025). Relationship of luteinizing hormone concentration with reproductive and productive performance in Holstein cows bred In Iraq. Al-Furat J. Innov. Agric. Sci., pp. 72–80. https://fjias.atu.edu.iq/index.php/dfg/article/

view/21
Bielaszka A, Staśkiewicz-Bartecka W, Kiciak A, Wieczorek M, Kardas M (2024). Color and its effect on dietitians' food choices: Insights from tomato juice evaluation. Beverages, 10: 70. https://doi.org/10.3390/beverages10030070
Chetri DK, Devkota NR, Basnet HB, Karki DB, Chitwan N (2024). Effects of feeding time, breed, parity, stage of lactation and milking methods on somatic cell count and milk constituents in dairy cows. Ethiop. Vet. J., 1: 101–112. https://www.gandakiuniversity.edu.np/wp-content/uploads/2024/09/CH11.pdf
Chudy S, Bilska A, Kowalski R, Teichert J (2020). Colour of milk and milk products in CIE L* a* b* space. Med. Weter., 76: 77–81. https://doi.org/10.21521/mw.6327
Connolly C, Yin X, Brennan L (2023). Impact of lactation stage on the metabolite composition of bovine milk. Molecules, 28: 1–13. https://doi.org/10.3390/molecules28186608
D'Alessandro A, Zolla L, Scaloni A (2011). The bovine milk proteome: cherishing, nourishing and fostering molecular complexity. An interactomics and functional overview. Mol. BioSyst., 7: 579–597. https://doi.org/10.1039/C0MB00027B
Dallago GM, Wade KM, Cue RI, McClure JT, Lacroix R, Pellerin D, Vasseur E (2021). Keeping dairy cows for longer: A critical literature review on dairy cow longevity in high milk-producing countries. Animals, 11: 1–26. https://doi.org/10.3390/ani11030808
de Souza Gomes BR, Coutinho MEB, Santos JM (2024). Development of rapid, simple and low-cost methods for quantification of reducing sugars in cow milk and derivatives using smartphone digital images. Food Anal. Methods, 17: 1161–1170. https://doi.org/10.1007/s12161-024-02637-2
Dhar H, Verma S, Dogra S, Katoch S, Vij R, Singh G, Sharma M (2024). Functional attributes of bioactive peptides of bovine milk origin and application of in silico approaches for peptide prediction and functional annotations. Crit. Rev. Food Sci. Nutr., 64: 9432–9454. https://doi.org/10.1080/10408398.2023.2212803
Diaz-Olivares JA, Gote MJ, Saeys W, Adriaens I, Aernouts B (2024). Near-infrared spatially-resolved spectroscopy for milk quality analysis. Comput. Electron. Agric., 219: 108783. https://doi.org/10.1016/j.compag.2024.108783
Dragh KT, Mohsin RH, Yousief MY (2023). Relationship of genotypes of csn3 gene in milk production and proportions of its components in Holstein Friesian cows bred in Iraq. J. Surv. Fish. Sci., 10: 3740–3745. https://zienjournals.com/index.php/tjabs/article/view/3533
El-Qaliouby HS, El-Kasrawy NI, Abouelghait HA, Hegazy MM, Abd El-Aziz AH (2024). Influence of calving age, parity, and calf gender on lactation metrics, reproductive and economic efficiency parameters in Egyptian buffaloes. J. Adv. Vet. Res., 14: 445–449. https://www.advetresearch.com/index.php/AVR/article/view/1694#google_vignette
Faid-Allah E, Mourad RS, Saddick EI, Eldahshan E (2025). Managerial factors affecting milking-abilities of Holstein cattle under intensive production system in Egypt. Trop. Anim. Health Prod., 57: 23. https://doi.org/10.1007/s11250-024-04271-w
FAO. FAOSTAT (2025). Statistical Database. (Livestock Manure). Extracted from: https://www.fao.org/faostat/en/#data/EMN
Faraj SH, Putra WPB, Tyasi TL (2023). Detection of SNPs in FABP4 gene and its relationship with milk quality traits in Iraqi Jenoubi cows. Basrah J. Agric. Sci., 36: 131–139. https://doi.org/10.37077/25200860.2023.36.1.11
Figueroa A, Caballero-Villalobos J, Angón E, Arias R, Garzón A, Perea JM (2020). Using multivariate analysis to explore the relationships between color, composition, hygienic quality, and coagulation of milk from Manchega sheep. J. Dairy Sci., 103: 4951–4957. https://doi.org/10.3168/jds.2019-17201
Franzoi M, Niero G, Meoni G, Tenori L, Luchinat C, Penasa M, De Marchi M (2023). Effectiveness of mid-infrared spectroscopy for the prediction of cow milk metabolites. J. Dairy Sci., 106: 5288–5297. https://doi.org/10.3168/jds.2023-23226
Green P (2023). Colorimetry and colour difference. In Fundamentals And Applications Of Colour Engineering (pp. 27–52). Fundamentals And Applications Of Colour Engineering. Wiley Online Library. Pages: 27-52. https://doi.org/10.1002/9781119827214.ch2
ISO/CIE (2019). Colorimetry Part 4: CIE 1976 L*a*b* colour space. International Organization for Standardization, Geneva, Switzerland; Commission internationale de l'éclairage, Vienna, Austria. https://www.iso.org/standard/74166.html
Jaswal S, Jena MK, Anand V, Jaswal A, Kancharla S, Kolli P, Mohanty AK (2022). Critical review on physiological and molecular features during bovine mammary gland development: Recent advances. Cells, 11: 3325. https://doi.org/10.3390/cells11203325
Johansson M, Lindberg M, Lundh Å (2024). Does keeping cows for more lactations affect the composition and technological properties of the milk? Animals, 14: 157. https://doi.org/10.3390/ani14010157
Kilcawley KN, Faulkner H, Clarke HJ, O'Sullivan MG, Kerry JP (2018). Factors influencing the flavour of bovine milk and cheese from grass based versus non-grass based milk production systems. Foods, 7: 37. https://doi.org/10.3390/foods7030037
Kipp C, Al-Kanaan A, Brügemann K, König S (2021). Genetic analyzes of physiological traits in relation to climatic stresses in German Black Pied cattle (Genetische Analysen von physiologischen Merkmalen in Bezug zu klimatischen Belastungen beim Deutschen Schwarzbunten Niederungsrind). Züchtungskunde, 93: 81–98. https://www.zuechtungskunde.de/archiv/genetische-analysen-von
Kotsampasi B, Karatzia MA, Tsiokos D, Chadio S (2024). Nutritional strategies to alleviate stress and improve welfare in dairy ruminants. Animals, 14: 2573. https://doi.org/10.3390/ani14172573
Lima MJA, Sasaki MK, Marinho OR, Freitas TA, Faria RC, Reis BF, Rocha FRP (2020). Spot test for fast determination of hydrogen peroxide as a milk adulterant by smartphone-based digital image colorimetry. Microchem. J., 157: 105042 https://doi.org/10.1016/j.microc.2020.105042.
Linehan K, Patangia DV, Ross RP, Stanton C (2024). Production, composition and nutritional properties of organic milk: A critical review. Foods, 13: 1–23. https://doi.org/10.3390/foods13040550
Mayasari N, Giffari HS, Ismiraj MR (2024). Exploratory analysis in the relationship of parity and lactation period on milk production. Iasi Univ. Life Sci., 1: 100–106. https://www.uaiasi.ro/firaa/Pdf/Pdf_Vol_81/N_Mayasari1.pdf
Méndez-Cid FJ, Lorenzo JM, Martínez S, Carballo J (2017). Oxidation of edible animal fats. Comparison of the performance of different quantification methods and of a proposed new semi-objective colour scale-based method.

Food Chem., 217: 743–749. https://doi.org/10.1016/j.foodchem.2016.09.009

Milovanovic B, Djekic I, Miocinovic J, Djordjevic V, Lorenzo JM, Barba FJ, Tomasevic I (2020). What is the color of milk and dairy products and how is it measured? Foods, 9: 1629. https://doi.org/10.3390/foods9111629

Milovanovic B, Tomovic V, Djekic I, Miocinovic J, Solowiej BG, Lorenzo JM, Tomasevic I (2021). Colour assessment of milk and milk products using computer vision system and colorimeter. Int. Dairy J., 120: 105084. https://doi.org/10.1016/j.idairyj.2021.105084

Milovanovic BR, Djekic IV, Tomovic VM, Vujadinovic D, Tomasevic IB (2021). Color measurement of animal source foods. Theory Pract. Meat Proc., 6: 311–319. https://doi.org/10.21323/2414-438X-2021-6-4-311-319

Mohammed AE-NA, Al-Shaheen T, Al-Saiady M, El-Waziry A (2024). Effect of dietary source of omega-3 fatty acids on milk production, fatty acid profiles and IGF-1 of lactating dairy cows in arid subtropics. Pak. J. Zool., 56: 2319. https://doi.org/10.17582/journal.pjz/20231210172024

Peng B, Zhou J, Xu J, Fan M, Ma Y, Zhou M, Zhao S (2019). A smartphone-based colorimetry after dispersive liquid–liquid microextraction for rapid quantification of calcium in water and food samples. Microchem. J., 149: 104072. https://doi.org/10.1016/j.microc.2019.104072

Perumal P, De AK, Bhattacharya D, Chakurkar EB (2023). Lactation stages modulate the hematological, serum biochemical, and endocrinological profiles and oxidative stress markers in crossbred cows under tropical humid island ecosystem of Andaman and Nicobar Islands. Trop. Anim. Health Prod., 55: 544. https://doi.org/10.1007/s11250-023-03544-0

Quaresma M, Payan-Carreira R (2021). Calf-sex influence in bovine milk production. Anim. Reprod. Vet. Med., https://doi.org/10.5772/intechopen.93966

Rayhan MS Al, Talukder A, Rani S, Easin K Bin, Hossain MA, Biswas PC (2025). Visible diffuse reflectance smartphone spectrometer with high spectral accuracy. Spectrochim. Acta A Mol. Biomol. Spectrosc., 326: 125240. https://doi.org/10.1016/j.saa.2024.125240

Sangor WKS, Al-Kanaan AJJ, Khudhair NA (2024). Effects of acetaminophen and vitamin C combination on clinical vital signs and behavioral traits of heat-stressed dairy buffalo. Adv. Life Sci., 11: 406–413. https://doi.org/10.62940/als.v11i2.2462

Scarso S, McParland S, Visentin G, Berry DP, McDermott A, De Marchi M (2017). Genetic and nongenetic factors associated with milk color in dairy cows. J. Dairy Sci., 100: 7345–7361. https://doi.org/10.3168/jds.2016-11683

Silva AFS, Rocha FRP (2020). A novel approach to detect milk adulteration based on the determination of protein content by smartphone-based digital image colorimetry. Food Contr., 115: 107299. https://doi.org/10.1016/j.foodcont.2020.107299

Smirnova A, Konoplev G, Mukhin N (2020). Milk as a complex multiphase polydisperse system: approaches for the quantitative and qualitative analysis. J. Compos. Sci., 4: 151. https://doi.org/10.3390/jcs4040151

Smoczyński M, Staniewski B, Kiełczewska K (2012). Composition and structure of the bovine milk fat globule membrane some nutritional and technological implications. Food Rev. Int., 28: 188–202. https://doi.org/10.1080/87559129.2011.595024

Tian H, Zhou X, Wang H, Xu C, Zhao Z, Xu W, Deng Z (2024). The prediction of clinical mastitis in dairy cows based on milk yield, rumination time, and milk electrical conductivity using machine learning algorithms. Animals, 14: 427. https://doi.org/10.3390/ani14030427

Tirfie FW (2023). A review of genetic and non-genetic parameter estimates for milk composition of cattle. Anim. Vet. Sci., 11: 64–70. https://pdfs.semanticscholar.org/0def/b60e94f4fbe9bd8198b4103936de54d14f01.pdf

Toscano A, Giannuzzi D, Pegolo S, Vanzin A, Bisutti V, Gallo L, Schiavon S (2023). Associations between the detailed milk mineral profile, milk composition, and metabolic status in Holstein cows. J. Dairy Sci., 106: 6577–6591. https://doi.org/10.3168/jds.2022-23161

Van Eetvelde M, de Jong G, Verdru K, van Pelt ML, Meesters M, Opsomer G (2020). A large-scale study on the effect of age at first calving, dam parity, and birth and calving month on first-lactation milk yield in Holstein Friesian dairy cattle. J. Dairy Sci., 103: 11515–11523. https://doi.org/10.3168/jds.2020-18431

Velayudhan SM, Brügemann K, Alam S, Yin T, Devaraj C, Sejian V, König S (2023). Molecular, physiological and hematological responses of crossbred dairy cattle in a tropical savanna climate. Biology, 12: 26. https://doi.org/10.3390/biology12010026

Voronina OA, Bogolyubova NV, Zaitsev SYU (2022). Mineral composition of cow milk. A mini review. Agric. Biol., 57: 681–693. https://doi.org/10.15389/agrobiology.2022.4.681eng

Walter LL, Gärtner T, Gernand E, Wehrend A, Donat K (2022). Effects of parity and stage of lactation on trend and variability of metabolic markers in dairy cows. Animals, 12: 1008. https://doi.org/10.3390/ani12081008

Yesil MI, Goncu S (2024). The determination of mastitis severity at 4-level using Milk physical properties: A deep learning approach via MLP and evaluation at different SCC thresholds. Res. Vet. Sci., 174: 105310. https://doi.org/10.1016/j.rvsc.2024.105310

Yousief MY, Al-Galbi HA, Al-Bataat HA (2024). The relationship of DGAT1 polymorphisms and milk fatty acids production of cows bred in Iraq (Local, cross and Holstein-Friesen). Iraqi J. Vet. Sci., 38: 7–14. https://doi.org/10.33899/ijvs.2023.139337.2929

Yousief MY, Owaid JM, Jaafer AA (2023). Relationship between polymorphisms of growth hormone gene with milk yield and its components in Iraqi cattle bred of Janoubi, Cross and Holstein. Iran. J. Ichthyol., 10: 144–149. https://www.ijichthyol.org/index.php/iji/article/view/931

Zyl CL Van, Eriksson HK, Knegsel ATM Van (2024). Consequences of weaning and separation for feed intake and milking characteristics of dairy cows in a cow-calf contact system. J. Dairy Sci.,

Supplementary Table 1: Effects of lactational and physiological Factors on Solids-Not-Fat (SNF), Total Solids (TS) and water of Holstein milk.

Main factors	Categories	NSF	TS	Water
Lactation	< 90 d	7.66±0.13 a	10.13±0.20 [a]	89.87±0.20 [a]
	90-180 d	9.08±0.09 b	12.21±0.14 [b]	87.79±0.14 [b]
	≥ 180 d	9.40±0.10 c	14.06±0.16 [c]	85.94±0.16 [c]
age	< 48 mo	8.19±0.10 [a]	11.34±0.14 [a]	88.66±0.14 [a]
	48-72 mo	8.74±0.09 [b]	12.08±0.14 [b]	87.92±0.14 [b]
	≥ 72 mo	9.22±0.15 [c]	12.98±0.23 [c]	87.02±0.23 [c]
Parity	1st	8.43±0.16 [a]	11.37±0.22 [a]	88.63±0.22 [a]
	2nd-3rd	8.73±0.09 [a]	12.33±0.14 [a]	87.67±0.14 [a]
	≥ 4th	8.98±0.09 [b]	12.69±0.14 [b]	87.31±0.14 [b]

Links Researchers