



J. Dairy Sci. TBC

<https://doi.org/10.3168/jds.2025-27467>

© TBC, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>).

The cost of being early or late: Biological and economic outcomes of age at first calving in dairy buffaloes

M. Santinello,¹ , A. Calanni Macchio,¹ , A. Lombardi,¹ , R. Matera,¹ , A. Paparella,²
S. Biffani,^{3*} , M. Gómez-Carpio,⁴ , G. Neglia,¹ and G. Campanile¹

¹Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy

²Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy

³Consiglio Nazionale delle Ricerche (CNR), Istituto di Biologia e Biotecnologia Agraria (IBBA), 20133 Milan, Italy

⁴Italian National Association of Buffalo Breeders (ANASB), 81100 Caserta, Italy

ABSTRACT

Age at first calving (AFC) is a key determinant of life-time productivity and profitability in dairy herds, yet its long-term effects in dairy buffaloes remain poorly documented. This study evaluated the influence of AFC on milk yield and composition, reproductive performance, and economic outcomes in Italian Mediterranean buffaloes reared under commercial conditions. Data included 576,028 test-day records from 27,744 buffaloes across 110 herds, collected over a 10-year period (2013–2023). Animals were grouped into 8 AFC classes and stratified by parity order: primiparous, mid-parity (second–third parities), and greater parity (≥ 4). Productive traits comprised daily milk yield, fat and protein content, SCS, and cumulative yields of milk, fat, and protein per lactation, and reproductive traits included calving interval (CIN) and days open (DO). Mixed-model analysis indicated that mid- and greater-parity buffaloes calving between 35 and 38 mo achieved higher daily milk yield, whereas in primiparous buffaloes, daily milk yield increased progressively with AFC. Milk fat content was unaffected by AFC, whereas the highest protein percentages were observed in mid-parity buffaloes calving at AFC between 32 and 34 mo. Somatic cell score significantly decreased as AFC increased across all parity groups, except in primiparous buffaloes, where an increase in SCS was observed in buffaloes with AFC between 37 and 38 mo. Moreover, CIN and DO were shortest in buffaloes calving the first time between 32 and 37 mo. The economic analysis integrated model-based estimates of milk yield with farm-level cost data from the Farm Accountancy Data Network covering the same 10-year period. Produc-

tion costs were organized into 3 categories: direct costs, overheads, and imputed costs. Based on this structure, the average production cost was estimated at €1.38/L of milk. Total production costs per lactation were calculated as the unit cost multiplied by the estimated cumulative milk yield for each AFC class and parity group. Revenues were obtained in the same way, by applying the actual farm-gate milk price (€1.50/L) to the estimated cumulative milk yields, providing a standardized framework for comparison across AFC classes. Net profit was calculated as the difference between total revenues and production costs, yielding an overall average of €314 per lactation across all AFC and parity groups. The highest net profit was recorded in mid-parity buffaloes whose first calving occurred between 37 and 38 mo of age, with an average net return of €339 per lactation. Overall, buffaloes calving the first time between 34 and 42 mo achieved significantly higher net profit compared with other AFC classes. Conversely, both early (< 30 mo) and late (> 42 mo) AFC values were associated with reduced net profits, driven by lower milk yields. Overall, these results indicate that targeting an AFC of 35 to 38 mo provides the most favorable balance between biological performance, udder health, reproductive efficiency, and farm profitability.

Key words: calving interval, culling risk, economic return, fertility

INTRODUCTION

Over the last 2 decades, global buffalo milk production has more than doubled, from 67 to 144 million tons, reinforcing its significance as a dynamic and expanding segment of the dairy industry (FAOSTAT, 2024). Renowned for its nutritional value and suitability for high-quality dairy products (Basilicata et al., 2018), buffalo milk has gained increasing consumer appeal, stimulated rural economies, and promoted the adoption of more sus-

Received August 21, 2025.

Accepted November 4, 2025.

*Corresponding author: stefano.biffani@cnr.it

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

tainable livestock practices (Kumar et al., 2020; Matera et al., 2025). These developments have coincided with growing attention to advances in husbandry, particularly in feeding strategies, reproductive efficiency, and animal welfare (Javed et al., 2022; Serrapica et al., 2022).

Italy is the leading European producer of buffalo milk, accounting for ~87% of the entire European buffalo population (FAOSTAT, 2024). The Italian buffalo population exceeds 400,000 animals (BDN, 2025), including ~121,000 lactating females annually monitored by the Italian Buffalo Breeders Association (ANASB, 2025). This sector consists of roughly 2,400 farms, each managing on average 170 to 180 head (BDN, 2025). Buffaloes are well adapted to Mediterranean environments, and are valued for their longevity, with productive lifespans that can reach up to 10 lactations. About 70% of the herd is in southern Italy, where milk is primarily processed into Mozzarella di Bufala Campana Protected Designation of Origin cheese (PDO), a product that earned PDO status in 1996. Over the last decade, production of this iconic cheese has increased by ~40%, reaching 55.7 million kg in 2024 (Napolitano et al., 2021; Consorzio di Tutela Mozzarella di Bufala Campana DOP, 2024).

Despite this steady growth and the increasing economic importance of the sector, reproductive efficiency continues to represent a critical challenge for Italian Mediterranean buffaloes. Indeed, seasonal fertility remains a major constraint in buffalo dairy systems, even with recent advancements in reproductive management (Neglia et al. 2020). As short-day breeders, buffaloes reach peak fertility in autumn and winter (Gasparrini, 2018; D'Occhio et al., 2020), whereas consumer demand for Mozzarella typically peaks in spring and summer (Otava et al., 2021). To address this mismatch and shift calving toward periods of higher milk demand, out-of-breeding-season mating techniques have been commonly applied in Italian Mediterranean buffaloes (Zicarelli, 1997). Within this framework, optimizing age at first calving (AFC) is crucial to maximize lifetime productivity and economic returns. Nutritional management of prepubertal BW plays a pivotal role in determining the timing of reproductive onset (Krpáľková et al., 2014a,b). Early-life nutritional strategies critically influence growth rates, metabolic development, and pubertal onset in both cattle (Bruinje et al., 2021) and buffaloes (Terzano et al., 2007a,b). In Italian Mediterranean buffaloes, the average AFC is ~35 to 36 mo (Gómez-Carpio et al., 2025). However, this value may vary across buffalo populations. For example, in Murrah buffaloes, it can range from 37 to 54 mo (Rautela et al., 2024). Although reducing AFC is often pursued to lower rearing costs (Campanile et al., 2006), early calving without adequate BW and physiological maturity can compromise body condition at parturition, impair repro-

ductive performance, and reduce milk yields in subsequent lactations. Recent findings from Calanni Macchio et al. (2025) showed that buffaloes calving the first time before 30 mo had longer calving intervals (CIN) and days open (DO), as well as lower milk yield during first lactation. Comparable trends have been observed in dairy cattle, where insufficient BW at calving is associated with suboptimal lactation and reproductive outcomes (Eastham et al., 2018; Kusaka et al., 2023). Conversely, higher AFC may enhance physiological development and improve first-lactation performance, but it may prolong the nonproductive period, delay the onset of milk production, and increase the risk of involuntary culling, postponing economic returns. Although the short-term effects of AFC on lactation performance and fertility are relatively well established in buffaloes, its long-term impact on lifetime productivity remains underexplored. In this context, Aspilueta-Borquis et al. (2022) provided valuable insights, reporting favorable genetic correlations between AFC and functional longevity in a population of 3,431 buffaloes across 6 herds. Furthermore, the authors highlighted the economic relevance of this trait, estimating potential gains of +\$102 to \$147 per animal when AFC is used as a selection criterion, thus supporting its integration into breeding programs aimed at enhancing productive lifespan and economic sustainability.

Therefore, this study investigates the influence of AFC on the lifetime performance of Italian Mediterranean buffaloes, with a focus on milk yield and composition, reproductive efficiency, and overall economic returns. By analyzing data from a large population over 10 years of observation, the study offers field-based evidence to inform management strategies and enhance the sustainability of buffalo dairy systems.

MATERIALS AND METHODS

Ethical approval from an animal welfare and use committee was not required for this study, as the data were obtained from routine animal recording practices conducted by the Italian National Association of Buffalo Breeders (ANASB).

Data and Editing

Data used in this study consisted of routine test-day records collected by ANASB between January 2013 and December 2023, using data from farms located in the 4 main provinces of the Mozzarella di Bufala Campana PDO-producing area (Caserta, Salerno, Latina, and Foggia), which together account for 90% of total PDO-certified output. Each test-day record included information

on fat and protein content (%), SCC, daily milk yield (kg), and animal characteristics such as parity order and DIM. To determine milk composition, milk samples were analyzed using mid-infrared spectroscopy (MilkoScan FT6000, Foss Electric A/S, Hillerød, Denmark) at the official AIA-certified laboratory in Benevento, Italy. Additionally, cumulative yields (kg) for milk, fat, and protein were available for each individual lactation. Reproductive data included complete birth and calving dates for each animal.

To ensure data reliability, a series of data quality control procedures were applied. Lactation data were retained only for buffaloes reared in herds with continuous and complete data reporting throughout the study period, restricted to farms with at least 40 lactating buffaloes. Records were further limited to buffaloes between the first and twelfth parity. Only lactations with a minimum of 3 test-day records between 5 and 305 DIM were included. To reduce variability due to small group sizes, herd-test-day (HTD) contemporary groups were considered only when comprising at least 5 lactating animals. Outlier values for milk yield, fat, and protein content, and cumulative lactation yields exceeding ± 3 SD from the mean were set to missing, following the method described by Calanni Macchio et al. (2025) and Costa et al. (2020). Somatic cell count was log-transformed into SCS using the standard formula provided by Ali and Shook (1980). The AFC was calculated as the number of days between birth and first calving. Implausible AFC values (< 23 or > 47 mo) were excluded, following Gómez-Carpio et al. (2023), to reduce potential bias related to voluntary delays in first insemination often adopted by farmers applying out-of-breeding-season mating techniques. Because AFC was normally distributed (Supplemental Figure S1, see Notes), it was categorized into 8 biologically and balanced classes, each comprising $\sim 12\%$ of the records: ≤ 30 , > 30 to ≤ 32 , > 32 to ≤ 34 , > 34 to ≤ 35 , > 35 to ≤ 37 , > 37 to ≤ 38 , > 38 to ≤ 42 , and > 42 mo. Calving interval was defined as the number of days between 2 consecutive calvings, and the date of conception was estimated by subtracting a fixed gestation length of 310 d from the date of the subsequent calving. Days open were then calculated as the interval between calving and conception dates. To further minimize the influence of extreme reproductive records and ensure consistency with the assumed gestation duration, DO values falling outside the mean ± 0.50 SD were excluded. This approach aligns with standard outlier-handling practices in reproductive studies on Mediterranean buffaloes (Calanni Macchio et al., 2025; Gómez-Carpio et al., 2025) and was designed to control for the effect of management decisions, such as seasonal breeding or voluntary waiting periods, that can influence reproductive intervals independently of the animal's physiological performance. After the editing

procedure, the final data set included 576,028 test-day records corresponding to 88,620 lactations from 27,744 buffaloes raised across 110 herds. Each buffalo contributed, on average, to 3 lactations, with ~ 7 test-day records per lactation (~ 6.50).

Economic Calculations: Cost Analysis

The cost analysis was conducted using microeconomic data collected from 2013 and 2023 through the Farm Accountancy Data Network (FADN), which annually compiles harmonized economic information from over 80,000 farms across Europe (FADN, 2010; Ciaian et al., 2013). The analysis focused on the production cost per liter of buffalo milk (€/L). A total of 517 farm-year observations were included and primarily engaged in buffalo milk production. Production costs were classified into 3 hierarchical levels to reflect their nature and allocation in buffalo milk production. First-level costs comprised variable expenses directly associated with production activities, including feed, veterinary services, reproduction management, energy, and other consumables (Supplemental Table S1, see Notes). Second-level costs represented general overheads that, though essential to farm operations, could be directly attributed to a specific production activity. These included administration, maintenance, and insurance (Supplemental Table S2, see Notes). Third-level costs referred to imputed values for internal production factors, specifically unpaid family labor and the notional rent of owned land. Labor costs were estimated using net hourly wage rates from comparable dairy farms, whereas land rent was derived from average regional values per hectare of utilized agricultural area reported in FADN. In accordance with standard managerial accounting practices, opportunity costs for non-land capital were excluded, given their marginal influence under prevailing economic conditions. Depreciation was calculated using the historical cost method to ensure comparability among farms with different investment timelines. For both second- and third-level costs, an allocation index was applied to proportionally assign expenses to buffalo milk production. This index was calculated as the ratio between the gross output from buffalo farming and the total gross output of the farm, thereby ensuring a consistent and economically sound cost allocation framework:

$$\text{Allocation index (I)} = \frac{\text{Total gross output, buffalo}}{\text{Total farm revenue}}.$$

Statistical Analyses

Descriptive statistics stratified by parity groups were computed for milk composition traits derived from test-day records, cumulative milk yields (Table 1), and reproductive traits (Table 2), with the aim of identifying and highlighting potential differences in both productive and reproductive performance. Animals were grouped into 3 parity classes: primiparous (parity 1), mid-parity (parities 2–3), and greater parity (parity ≥ 4), reflecting the distinct patterns observed in early lactations and the stabilization of performance from the fourth parity onward. For analytical purposes, DIM was grouped into 10 categories of 30 d each, with the first class covering 5 to 30 DIM and the last including records beyond 271 DIM. To assess the effects of AFC on test-day traits, separate linear mixed models with repeated measures were implemented for each parity group using Proc GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC):

$$y_{ijklmn} = \mu + AFC_i + DIM_j + Month_k + Year_l \\ + (AFC \times DIM)_{ij} + (AFC \times Month)_{ik} \\ + (DIM \times Month)_{jk} + Animal_ID_m + HTD_n + e_{ijklmn},$$

where μ is the population mean for daily milk yield, fat and protein content, and SCS; AFC_i , DIM_j , $Month_k$, and $Year_l$ represent the fixed effects of AFC (8 classes), DIM (10 classes), calving month, and calving year (2013–2023), respectively; $Animal_ID_m$ and HTD_n (herd-test-day) were included as independent random effects, assuming normal distribution with mean zero and variance σ_0^2 . Only the significant 2-way interactions were retained in the final model: $AFC \times DIM$, $AFC \times Month$, and $DIM \times Month$. Specifically, for each analyzed trait, separate models were applied to primiparous, mid-parity, and greater-parity buffaloes. This stratified modeling approach enabled a more precise and biologically relevant

assessment of AFC effects within each parity group, while avoiding overly complex interaction terms and improving the overall interpretability of the results. Normality and homoscedasticity of residuals were assessed using visual diagnostics and the Shapiro-Wilk test. An autoregressive covariance structure was specified in the repeated statement using the TYPE = AR(1) option of SAS. This structure was selected because it yielded the lowest Akaike's information criterion and Bayesian information criterion values among the tested models. Moreover, proc GLMSELECT in SAS was used to check for the absence of collinearity among fixed effects.

A similar approach was applied to cumulative yields for milk, fat, and protein, and reproductive traits evaluated separately within the same parity groups. For these traits, the model excluded DIM and related interactions:

$$y_{ijkl} = \mu + AFC_i + Month_j + Year_k \\ + (AFC \times Month)_{ij} + Herd_l + e_{ijkl},$$

where μ represent the population mean for cumulative yields for milk, fat and protein; CIN_1 , CIN_{2-3} , $CIN_{\geq 4}$, DO_1 , DO_{2-3} , $DO_{\geq 4}$: CIN_1 and DO_1 refer to reproductive intervals between the first and second calvings, CIN_{2-3} and DO_{2-3} correspond to the average between the second to third and third to fourth reproductive intervals, and $CIN_{\geq 4}$ and $DO_{\geq 4}$ indicate the average reproductive intervals from the fourth to fifth calvings and beyond; AFC_i , $Month_j$, and $Year_k$ represent the fixed effects of AFC (8 classes), calving month, and calving year (from 2013 to 2023); $Herd_l$ was modeled as a random effect only for reproductive and cumulative yields of first parity order model. In contrast, random effect of animal nested within herd was included to account for repeated measures of animals within the same herd in mid- and greater-parity groups. Due to their right-skewed distribution, generalized linear mixed models with gamma distribution and log-link function were used for all reproductive intervals

Table 1. Descriptive statistics of milk yield and composition according to parity groups¹

Parity ¹	No. animals	Trait	Mean	STD	Minimum	Maximum
1	9,599	Total milk yield (kg)	2,496	764	229	4,933
		Fat (%)	7.98	0.92	3.94	11.8
		Protein (%)	4.69	0.24	3.37	5.88
		SCS (unit)	2.96	1.68	−2.06	10.7
		Total milk yield (kg)	2,735	823	189	5,336
2–3	11,181	Fat (%)	7.92	0.96	4.05	11.6
		Protein (%)	4.66	0.25	3.46	5.82
		SCS (unit)	3.37	1.70	−2.06	10.7
		Total milk yield (kg)	2,563	826	40.0	5,188
		Fat (%)	7.72	1.00	4.06	11.3
≥ 4	6,964	Protein (%)	4.62	0.26	3.65	5.61
		SCS (unit)	3.63	1.74	−2.06	10.7

¹Parity group 1 included 62,394 test-day records from 9,599 lactations; parity group 2–3 included 145,353 test-day records from 22,362 lactations; parity ≥ 4 included 368,281 test-day records from 56,659 lactations.

Table 2. Descriptive statistics of reproductive intervals¹ (days) according to parity groups

Variable	No. animals	Mean	SD	Median	Quartile 1	Quartile 3
DO ₁	9,599	142	74.1	126	78.0	197
DO ₂₋₃	11,181	114	62.0	94.1	63.2	155
DO _{≥4}	6,964	105	57.3	88.0	60.1	142
CIN ₁	9,599	452	74.1	436	388	507
CIN ₂₋₃	11,181	424	62.0	404	373	464
CIN _{≥4}	6,964	415	57.1	398	371	453

¹DO₁ = average days open between the second and the first calvings; DO₂₋₃ = average days open between the second and third, and between the third and fourth calvings; DO_{≥4} = average days open calculated across all calvings from the fourth onward; CIN₁ = average interval between the second and the first calvings; CIN₂₋₃ = average interval between the second and third, and between the third and fourth calvings; CIN_{≥4} = average interval calculated across all calvings from the fourth onward.

(Diana et al., 2021). Results are reported as least squares means \pm standard error, and multiple comparisons were adjusted using the Bonferroni post-hoc correction ($P < 0.05$).

To evaluate the economic impact of AFC on farm profitability, the cost structure described previously was integrated with model-based estimates of milk yield per lactation. The analysis was stratified by AFC classes and parity groups to capture differences in productive and economic performance. Revenues were calculated by multiplying the predicted lactation total milk yield by the average market price of buffalo milk. Because historical farm-gate prices for the full 10-year period were not available, the current average price (€1.50/L; CLAL, 2025) was applied uniformly across all scenarios. This approach allowed us to simulate the economic returns of the observed production patterns under present market conditions, providing results that are both directly interpretable and relevant to contemporary decision-making. Although this method does not account for historical price fluctuations, it offers a standardized framework for comparing AFC classes under the same economic assumptions, thereby isolating the effect of AFC on profitability from confounding variations in milk prices over time. Net profit for each AFC class was obtained by subtracting the estimated production costs from the calculated revenues, enabling an AFC-specific assessment of economic return for the sampled farms. A comparative analysis among AFC classes was then performed to evaluate significant differences in profitability.

RESULTS

Descriptive Statistics

Descriptive statistics for milk yield and composition traits, stratified by parity groups, are summarized in Table 1. Mid-parity buffaloes produced the highest cumulative milk yield, averaging 2,735 kg, outperforming all other

parity groups. In contrast, primiparous buffaloes had the highest milk quality, reaching fat and protein contents of 7.98% and 4.69%, respectively. Somatic cell score increased progressively with parity, rising from 2.96 in primiparous to 3.63 in greater-parity buffaloes (parity ≥ 4). Table 2 presents the descriptive statistics of reproductive traits across parity groups. The greatest CIN and DO were recorded in primiparous buffaloes, averaging 452 and 142 d, respectively. These values decreased progressively as parity order increased. Mid-parity buffaloes exhibited moderate reproductive intervals (CIN₂₋₃: 424; DO₂₋₃: 114 d), which decreased in animals in their fourth parity or greater (CIN_{≥4}: 415; DO_{≥4}: 105 d).

Effect of AFC on Milk Yield and Composition Traits

Supplemental Table S3 (see Notes) showed the significance values for productive traits. Age at first calving significantly affected all the analyzed traits ($P < 0.05$), except protein content in primiparous buffaloes ($P = 0.550$) and fat content for all parity groups ($P > 0.05$). The association between AFC groups and productive traits is illustrated in Supplemental Figures S2 to S5 (see Notes). Multiparous buffaloes (mid- and greater parity) with AFC between 35 and 38 mo had the highest daily milk yield (Supplemental Figure S2; $P < 0.05$), whereas daily milk yield increased progressively with AFC in primiparous buffaloes ($P < 0.05$). Conversely, multiparous buffaloes (parity orders 2–3 and ≥ 4) that calved either too early (< 30 mo) or too late (> 38 – 42 mo) showed a significant reduction in daily milk yield within each parity group ($P < 0.05$). Although fat content was not significantly affected by AFC in all parity groups ($P > 0.05$; Supplemental Figure S3), it tended to increase in primiparous animals ($P = 0.05$). Protein content showed a clear dependence on both parity group and AFC ($P < 0.05$; Supplemental Figure S4). The highest protein content was observed in primiparous and mid-parity buffaloes with AFC between 32 and 34 mo, even though primiparous buffaloes maintained stable protein content

across AFC classes ($P > 0.05$). Moreover, multiparous buffaloes (parity groups 2–3 and ≥ 4) with AFC > 37 mo had significantly lower protein content compared with those calving earlier (< 37 mo). The effect of AFC on SCS is shown in Supplemental Figure S5. Regardless of AFC, SCS increased consistently as parity order increased ($P < 0.05$), and significantly decreased as AFC increased across all parity groups ($P < 0.05$), except in primiparous buffaloes, where a temporary increase in SCS was observed for buffaloes with AFC between 37 and 38 mo ($P < 0.05$).

Effect of AFC on Reproductive Traits

Supplemental Table S4 (see Notes) showed the significance of fixed effect of minimum selected models for reproductive traits. AFC significantly explains the variability of all the analyzed traits ($P < 0.05$). Figures 1 and 2 illustrate the relationship between AFC and reproductive intervals. The most favorable reproductive intervals (shorter CIN and DO) were observed in buffaloes with AFC between 32 and 37 mo ($P < 0.05$). Within this range, primiparous buffaloes recorded CIN_1 of 447 d and DO_1 of 133 d. Mid-parity animals had CIN_{2-3} of 424 d and DO_{2-3} of 113 d, and those in the greater-parity group (parity ≥ 4) showed the shortest reproductive intervals ($P < 0.05$; $CIN_{\geq 4}$: 399 d; $DO_{\geq 4}$: 87 d). In contrast, buffaloes calving at either younger (< 32 mo) or older AFC (> 37 mo) exhibited significantly extended reproductive intervals in all parity groups ($P < 0.05$).

Cumulative yields of milk, fat, and protein according to AFC classes and parity groups are presented in Figure 3. Buffaloes with AFC between 34 and 37 mo consistently showed the highest cumulative production across all traits ($P < 0.05$). This effect was particularly evident in mid-parity buffaloes, which achieved cumulative yields of 2,850 kg of milk, 225 kg of fat, and 130 kg of protein within this AFC range ($P < 0.05$). In contrast, both primiparous and multiparous buffaloes outside this range exhibited lower cumulative yields ($P < 0.05$). Overall, the lowest production was observed in animals with an AFC below 30 or above 38 mo.

Effect of AFC on Economic Return

The analysis showed that the average first-level cost amounted to €0.72/L of milk, with a median of €0.73/L (Supplemental Table S1). The allocation index, used to apportion second- and third-level costs to buffalo milk, averaged 0.95 ± 0.09 , highlighting the predominant contribution of buffalo milk to overall farm revenues. Based on this allocation, second-level costs averaged €0.51/L (median of €0.49/L), and third-level costs averaged €0.15/L (median: €0.13/L). The cost structure underlying

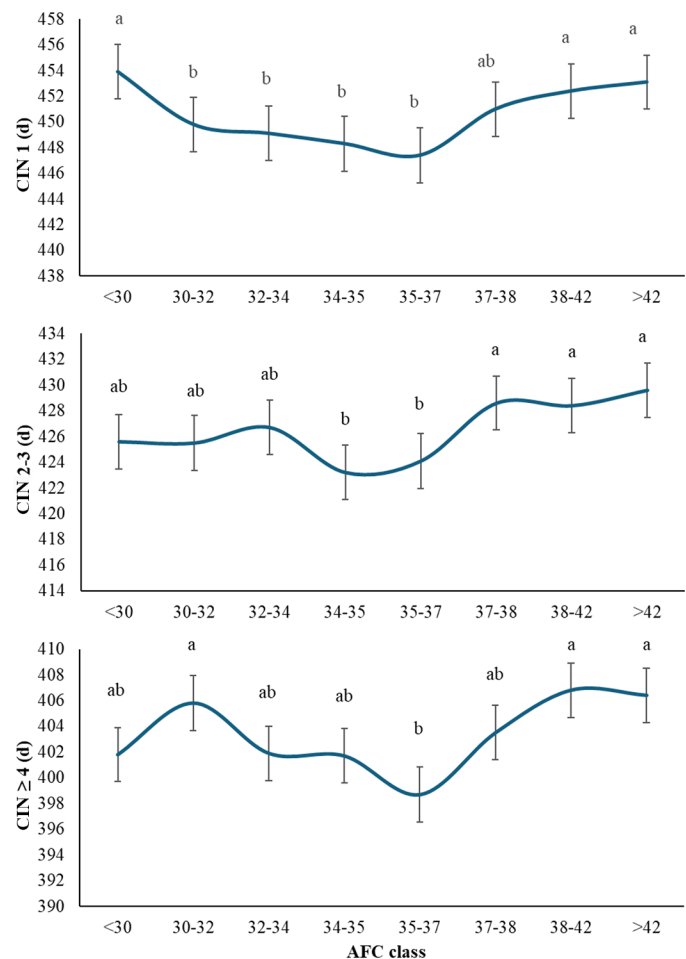


Figure 1. Least squares means of calving intervals (CIN; days) in relation to age at first calving (AFC) classes. CIN_1 represents the calving interval between the first and second calvings; CIN_{2-3} represents the calving intervals between the second to third and third to fourth calvings; $CIN_{\geq 4}$ represents the calving intervals for all calvings beyond the fourth. CIN_1 estimates were based 9,599 buffaloes, each contributing 1 calving; CIN_{2-3} estimates were based on 11,181 buffaloes contributing a total of 22,362 calvings; $CIN_{\geq 4}$ estimates were based on 6,964 buffaloes contributing 56,659 calvings. Age at first calving classes were categorized as follows: 1: ≤ 30 mo; 2: 30–32 mo; 3: 32–34 mo; 4: 34–35 mo; 5: 35–37 mo; 6: 37–38 mo; 7: 38–42 mo; and 8: > 42 mo. Error bars represent SE.

these results was characterized by first-level direct costs representing 52.2% of the total milk production cost, second-level general overheads accounting for 37.0%, and third-level imputed costs contributing the remaining 10.9%. Summing the average values from all 3 cost levels resulted in an estimated total production cost of €1.38/L of buffalo milk. Across the entire data set, the mean net return was €314 per lactation (Table 3). The highest net profit was recorded in mid-parity buffaloes, whose first calving occurred between 37 and 38 mo of age, with an average net return of €339 per lactation. Overall, buffaloes with AFC between 34 and 42 mo had the highest average net returns ($P < 0.05$). Within this optimal AFC

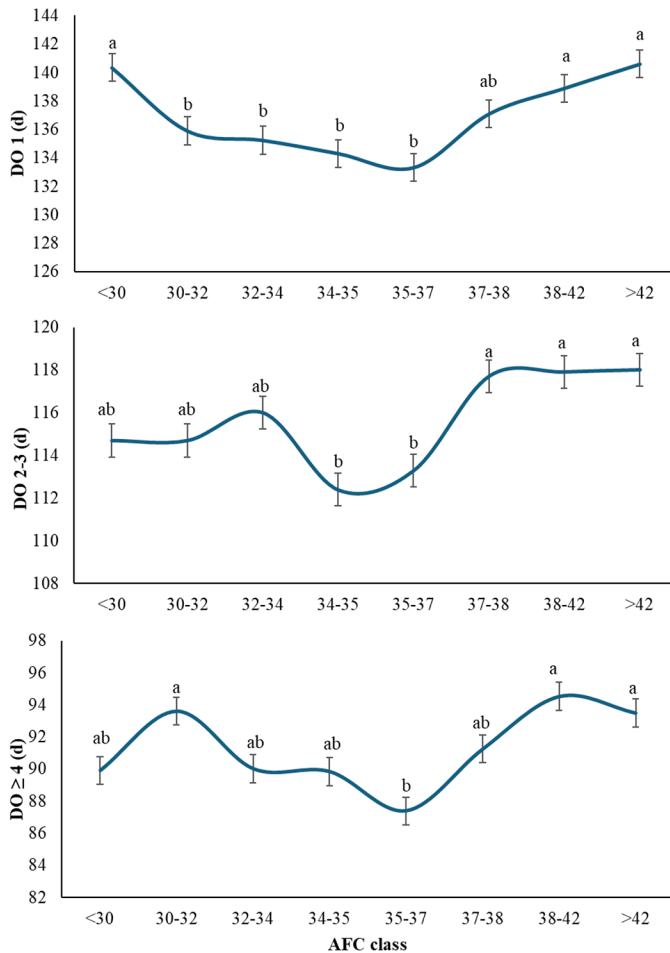


Figure 2. Least squares means of days open (DO; days) in relation to age at first calving (AFC) classes. DO₁ represents to the days open between the first and second calvings; DO₂₋₃ represents the days open between the second to third and third to fourth calvings; DO_{≥4} represents the days open for all calvings beyond the fourth. DO₁ estimates were based on 9,599 buffaloes, each contributing 1 calving; DO₂₋₃ estimates were based on 11,181 buffaloes contributing a total of 22,362 calvings; DO_{≥4} estimates were based on 6,964 buffaloes contributing 56,659 calvings. Age at first calving classes were categorized as follows. 1: <30 mo; 2: 30–32 mo; 3: 32–34 mo; 4: 34–35 mo; 5: 35–37 mo; 6: 37–38 mo; 7: 38–42 mo; and 8: >42 mo. Error bars represent SE.

range, primiparous buffaloes achieved an average net return of €305 per lactation, mid-parity animals reached €335, and multiparous buffaloes (parity ≥4) recorded €315 per lactation. Conversely, both early (<30 mo) and late (>42 mo) calvings were associated with significantly reduced net profits.

DISCUSSION

Milk yield peaked in mid-parity animals (2,735 kg), confirming this physiological stage as the apex of production performance in buffaloes (Costa et al., 2020). In contrast, the highest fat (7.98%) and protein (4.69%)

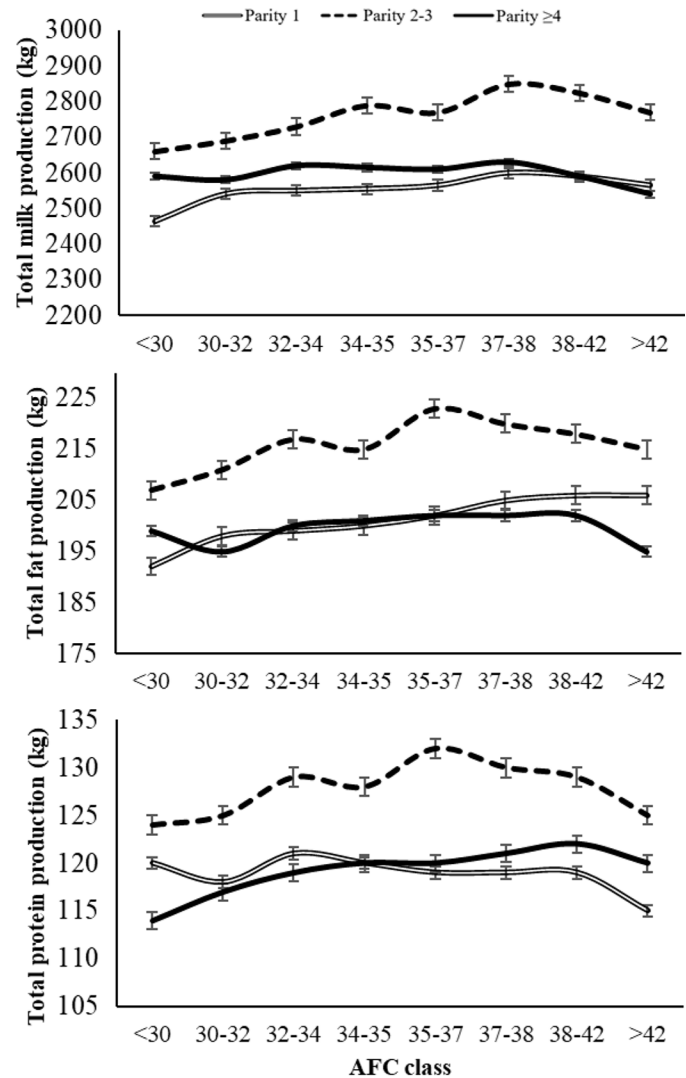


Figure 3. Least squares means (±SE) of cumulative milk, protein, and fat yields (kg), according to age at first calving (AFC) classes and parity groups. Age at first calving classes were categorized as follows. 1: <30 mo; 2: 30–32 mo; 3: 32–34 mo; 4: 34–35 mo; 5: 35–37 mo; 6: 37–38 mo; 7: 38–42 mo; and 8: >42 mo. Parity group 1 included 9,599 buffaloes, each contributing 1 lactation; parity group 2–3 included 11,181 buffaloes contributing a total of 22,362 lactations; and parity group ≥4 included 6,964 buffaloes contributing 56,659 lactations.

contents were recorded in primiparous animals, with values progressively declining in subsequent parity groups, likely reflecting the increasing metabolic demands associated with higher milk output. Concurrently, SCS rose from 2.96 in primiparous to 3.63 in greater-parity buffaloes, indicating a gradual deterioration of udder health and cumulative stress on mammary tissue with advancing parity (Moroni et al., 2006; Matera et al., 2025), a pattern also reported in dairy cattle (Dürr et al., 2008; Fonseca et al., 2025). These results support the choice to divide animals into 3 biologically coherent parity groups.

Table 3. Total milk yield and economic traits according to age at first calving (AFC) classes and parity groups; values calculated using a fixed milk price of €1.50/L and an average production cost of €1.38/L

Parity ¹	No. animals	AFC class ²	Total milk yield ³	Average total cost ⁴	Average total revenue ⁵	Net profit ⁶	95% CI ⁷
1	9,599	<30	2,364	3,262	3,546	284 ^b	[279–289]
		30–32	2,480	3,422	3,720	298 ^b	[291–303]
		32–34	2,475	3,416	3,713	297 ^b	[292–302]
		34–35	2,508	3,461	3,762	301 ^a	[296–306]
		35–37	2,502	3,453	3,753	300 ^a	[295–305]
		37–38	2,546	3,513	3,819	306 ^a	[300–311]
		38–42	2,589	3,573	3,884	311 ^a	[306–316]
		>42	2,478	3,420	3,717	297 ^b	[292–303]
2–3	11,181	<30	2,741	3,783	4,112	329 ^b	[324–334]
		30–32	2,742	3,784	4,113	329 ^b	[324–334]
		32–34	2,760	3,809	4,140	331 ^{ab}	[326–336]
		34–35	2,780	3,836	4,170	334 ^a	[328–339]
		35–37	2,779	3,835	4,169	333 ^a	[328–339]
		37–38	2,822	3,894	4,233	339 ^a	[333–344]
		38–42	2,793	3,854	4,190	335 ^a	[330–340]
		>42	2,682	3,701	4,023	322 ^b	[317–327]
≥4	6,964	<30	2,616	3,610	3,924	314 ^a	[307–321]
		30–32	2,615	3,609	3,923	314 ^a	[307–320]
		32–34	2,602	3,591	3,903	312 ^{ab}	[306–319]
		34–35	2,640	3,643	3,960	317 ^a	[310–323]
		35–37	2,630	3,629	3,945	316 ^a	[307–319]
		37–38	2,614	3,607	3,921	314 ^a	[307–320]
		38–42	2,626	3,624	3,939	315 ^a	[309–322]
		>42	2,467	3,404	3,701	296 ^b	[289–303]

^{a,b}Net profit values with different superscript letters within parity group differ significantly according to Bonferroni post-hoc multiple comparison adjustment ($P < 0.05$).

¹Parity group 1 included 9,599 buffaloes, each contributing 1 lactation; parity group 2–3 included 11,181 buffaloes contributing a total of 22,362 lactations; and parity group ≥4 included 6,964 buffaloes contributing 56,659 lactations.

²Age (mo) at first calving classes were categorized as follows. 1: ≤30 mo; 2: 30–32 mo; 3: 32–34 mo; 4: 34–35 mo; 5: 35–37 mo; 6: 37–38 mo; 7: 38–42 mo; and 8: >42 mo.

³Average total milk yield per lactation (kg): cumulative individual milk yield.

⁴Average total production costs (€): calculated by multiplying predicted total milk yields by the production cost of €1.38/L, including variable, overhead, and imputed costs, allocated using FADN data.

⁵Average total revenue (€): revenue per entire lactation, calculated by multiplying predicted total milk yields by the average milk market price (€1.50/L).

⁶Net profit (€): difference between average total revenue and average total costs.

⁷95% CI: 95% confidence intervals of net profit.

Indeed, primiparous buffaloes, still completing somatic growth and experiencing their first lactation, face unique metabolic challenges, including heightened risk of negative energy balance (Campanile et al., 2010). Mid-parity buffaloes generally achieve the most favorable balance between productivity and physiological stability, whereas buffaloes beyond the third parity show clear signs of functional decline, characterized by reduced milk quality and diminished udder resilience.

Effect of AFC on Milk Yield and Composition Traits

In the present study, daily milk yield increased with advancing AFC, corroborating previous findings in primiparous buffaloes (Rautela et al., 2024; Calanni Macchio et al., 2025). This trend likely reflects the greater physiological maturity and more advanced mammary

gland development achieved at calving by animals with greater AFC (Macias and Hinck, 2012; Challana et al., 2014). However, the influence of AFC on milk yield diminished in greater parities, as also reported by Zicarelli et al. (2007) in a cohort of 953 Italian Mediterranean buffaloes. This attenuation may be explained by the predominant effect of AFC during first lactation, when insufficient BW or suboptimal body conditions at calving can exacerbate negative energy balance and reduce milk yield potential. In agreement with previous studies in both buffaloes and dairy cows (Catillo et al., 2002; Bilal et al., 2014), fat content was largely unaffected by AFC in all parity groups. This stability is consistent with the tight physiological regulation of milk fat synthesis, which may depend more on energy balance than on developmental stage or parity order (Bauman and Griinari, 2003; Jenkins et al., 2008). Nonetheless, in primiparous

buffaloes, a slight numerical increase in fat content was associated with greater AFC, as also observed by Calanni Macchio et al. (2025). This effect may be linked to improved body reserves and greater metabolic readiness at the onset of lactation in animals calving with greater AFC. In contrast, protein content showed a slight but consistent decline in buffaloes with AFC above 37 mo ($\sim 0.02\%$ – 0.03%) for all parity groups except for primiparous. Although seemingly modest, such reduction can negatively affect cheese yield by impairing caseification efficiency (Fasale et al., 2017; Sales et al., 2021). This is particularly relevant in the Italian context, where buffalo milk is primarily destined for Mozzarella production (Catillo et al., 2002; Rosati and Van Vleck, 2002). Furthermore, SCS significantly decreased as AFC increased, except in primiparous buffaloes, which had higher SCS when calving between 37 and 38 mo. Previous studies have reported that greater AFC may be associated with increased SCS and greater mastitis susceptibility in greater parities, potentially compromising lifetime udder function and productivity (Costa et al., 2019). Our findings indicate also that anticipating AFC may have long-term negative effects on udder health in subsequent lactations, suggesting that excessively early calving could predispose buffaloes to higher SCS and mastitis risk later in life. Conversely, when AFC is excessively delayed, primiparous animals may experience suboptimal udder health and increase susceptibility to disorders. Overall, these results highlight the need to identify an optimal AFC range that balances early-lactation performance with long-term udder health and herd productivity.

Effect of AFC on Reproductive Traits, Cumulative Milk Yields

Although a lower AFC is often targeted to reduce rearing costs, and delayed calving may result from poor management or biological constraints, both extremes are associated with reduced longevity and an increased risk of premature culling, largely due to suboptimal milk yield and impaired reproductive performance (Galeazzi et al., 2010; Fodor et al., 2020). In dairy cattle, where physiological maturity is reached earlier than in buffaloes (~ 24 – 25 mo), excessively early first calving has been associated with higher incidence of fertility disorders and involuntary culling (Wathes et al., 2008). Conversely, achieving an optimal AFC can improve lifetime productivity and lower replacement rates, both of which are essential for sustainable buffalo farming (Aspilcueta-Borquis et al., 2022; Gómez-Carpio et al., 2023, 2025). In this study, buffaloes with AFC between 32 and 37 mo exhibited the most favorable reproductive performance, with significantly shorter CIN and DO, corroborating previous findings in primiparous animals (Calanni Mac-

chio et al., 2025). The greater total yields of milk, fat, and protein recorded in this AFC class may be partly attributable to the shorter DO, as a reduced interval between calving and conception increases the proportion of time spent in lactation within a given time frame. Thus, although greater AFC within this optimal range appears to support both improved reproductive efficiency and greater lactation yields, part of this production advantage may be mediated through its effect on DO, rather than being solely an independent outcome of AFC. This highlights the complex interdependence between reproductive management and productive performance.

Effect of AFC on Economic Return

Economic analysis showed that all AFC classes generated positive net profits, confirming the overall economic efficiency of buffalo farming under current production and market conditions. However, AFC between 34 and 42 mo were consistently associated with the highest net returns, especially in mid-parity buffaloes. These findings support the view that a moderate delay in first calving enhances physiological maturity and productive performance, thereby improving the cost-effectiveness of rearing investments. Recent large-scale evidence on more than 50,000 Italian Mediterranean buffaloes further confirmed that calving at 34 to 37 mo provides the most advantageous combination of shorter CIN, lower SCS, and sustained daily milk yields, ultimately boosting farm profitability (Calanni Macchio et al., 2025). By contrast, both early (<30 mo) and late (>38 – 42 mo) AFC can be associated with reduced productivity, prolonged CIN, and higher culling risk, which undermine long-term economic sustainability (Rautela et al., 2024). Although an earlier AFC may appear economically favorable by reducing rearing time and heifer management costs, these savings are often outweighed by lower milk yield, impaired reproductive efficiency, and greater metabolic stress in the first lactation when calving occurs before full physiological maturity (Sung et al., 2016).

From a cost-structure perspective, the total production cost of buffalo milk averaged €1.38/L, including direct, indirect, overhead, and imputed components, in line with incremental costing methodologies adopted by the Council for Agricultural Research and Economics (CREA) and supported in dairy economics literature (Ghelfi, 2000). Direct costs represented the largest share (52.2%), followed by overheads (37.0%) and imputed costs for family labor and land use (10.9%), highlighting the central role of operational expenditures in determining profitability. This distribution emphasizes that net returns depend not only on biological performance (milk yield, udder health, reproductive efficiency) but also on managerial choices related to feeding strategies, reproduction, and

resource allocation. In agreement, previous studies have shown that optimizing both animal performance and feed resource management can substantially increase profitability in buffalo systems (Ozturk et al., 2022). Overall, the evidence indicates that aligning AFC with the physiological development of animals, optimally between 35 and 38 mo, represents a key strategy to enhance both farm profitability and long-term economic resilience in buffalo dairy systems.

However, some limitations should be acknowledged when interpreting these findings. Although the models accounted for major fixed effects and herd variability, residual confounding by management strategies, nutritional plans, and body condition at calving cannot be fully excluded. Differences in farm management intensity, housing systems, and labor organization may also have influenced both biological performance and cost structure. The influence of herd-specific environmental and management factors on performance has long been recognized in animal breeding research, highlighting the challenge of disentangling genetic and environmental effects in field data (Weigel et al., 2017). Although the editing procedure helped to control potential confounding factors, selection bias might exist, as heifers reaching first calving at different ages could have followed distinct growth trajectories or been subject to varying culling pressures, factors not entirely captured by the available data. In this context, the results of the present study, supported by a large data set encompassing thousands of animals and lactations, provide a robust benchmark for defining AFC targets that optimize both biological efficiency and economic return, while underscoring the need for experimental validation across different production systems.

CONCLUSIONS

Age at first calving emerges as a strategic variable in the management of buffalo dairy systems, with long-term implications not only for individual animal performance but also for the economic sustainability of the entire herd. The present findings, based on observational evidence, indicate that the objective should not be to minimize AFC at all costs, but rather to identify an optimal window that maximizes both biological and economic efficiency. Given the observational nature of the data set, these results should be interpreted with caution and validated under farm-specific conditions. In practical terms, herd managers are encouraged to pursue the best possible balance between production and AFC. This includes careful monitoring of growth and body condition, nutritional plans that promote balanced development without compromising mammary gland integrity, and reproductive management based on readiness rather than chronologi-

cal age alone. Aligning AFC decisions with the animal's actual preparedness and the farm's production goals can help reduce early-life reproductive failures, improve lifetime milk yields, and reduce involuntary culling rates. Furthermore, incorporating AFC as a routine decision-making parameter in buffalo herd management and genetic selection programs may significantly enhance farm resilience and profitability. In this context, the results of this study provide a valuable framework for defining AFC targets that balance biological performance with economic sustainability, while underscoring the need for further experimental validation across different production environments.

NOTES

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-Generation EU (Piano Nazionale di Ripresa e Resilienza [PNRR]—Missione 4 Componente 2, Investimento 1.4—D.D. 1032 17/06/2022, CN00000022; CUP: E63C22000920005). This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them. Supplemental material for this article is available at [XXX]. Ethical approval from an animal welfare and use committee was not required for this study, as data were obtained from routine animal recording practices conducted by the Italian National Association of Buffalo Breeders (ANASB). The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: AFC = age at first calving; ANASB = Italian National Association of Buffalo Breeders; CIN = calving interval; DO = days open; FADN = Farm Accountancy Data Network; HTD = herd-test-day; PDO = Protected Designation of Origin cheese.

REFERENCES

- Ali, A. K. A., and G. E. Shook. 1980. An optimum transformation for somatic cell concentration in milk. *J. Dairy Sci.* 63:487–490. [https://doi.org/10.3168/jds.S0022-0302\(80\)82959-6](https://doi.org/10.3168/jds.S0022-0302(80)82959-6).
- ANASB (Associazione Nazionale Allevatori Specie Bufalina). 2025. ANASB Data. Accessed Feb. 7, 2025. <https://www.anasb.it/statistiche/>.
- Aspilcueta-Borquis, R., L. de Oliveira Seno, F. R. de Araujo Neto, D. J. de Abreu Santos, N. A. Hurtado-Lugo, and H. Tonhati. 2022. Lifetime productivity: Genetic study of longevity and its associations with economically important traits in dairy buffaloes. *Livest. Sci.* 259:104900. <https://doi.org/10.1016/j.livsci.2022.104900>.
- BDN (Banca dati nazionale dell'Anagrafe Zootecnica). 2025. Veterinary Information System—Statistics. Accessed Mar. 10, 2025. https://www.vetinfo.it/j6_statistiche/#/report-pbi/11.
- Basilicata, M. G., G. Pepe, E. Sommella, C. Ostacolo, M. Manfra, G. Sosto, G. Pagano, E. Novellino, and P. Campiglia. 2018. Peptidome profiles and bioactivity elucidation of buffalo-milk dairy products

- after gastrointestinal digestion. *Food Res. Int.* 105:1003–1010. <https://doi.org/10.1016/j.foodres.2017.12.038>.
- Bauman, D. E., and J. M. Grinari. 2003. Nutritional regulation of milk fat synthesis. *Annu. Rev. Nutr.* 23:203–227. <https://doi.org/10.1146/annurev.nutr.23.011702.073408>.
- Bilal, G., R. I. Cue, A. F. Mustafa, and J. F. Hayes. 2014. Effects of parity, age at calving, and stage of lactation on fatty acid composition of milk in Canadian Holsteins. *Can. J. Anim. Sci.* 94:401–410. <https://doi.org/10.4141/cjas2013-172>.
- Bruinje, T. C., J. P. Rosadiuk, F. Moslemipur, H. Sauerwein, M. A. Steele, and D. J. Ambrose. 2021. Differing planes of pre- and post-weaning phase nutrition in Holstein heifers: II. Effects on circulating leptin, luteinizing hormone, and age at puberty. *J. Dairy Sci.* 104:1153–1163. <https://doi.org/10.3168/jds.2020-18810>.
- Calanni Macchio, A., M. Santinello, G. Bifulco, R. Matera, S. Biffani, M. Gómez-Carpio, G. Campanile, and G. Neglia. 2025. The role of age at first calving in shaping production and reproductive outcomes in Italian buffaloes. *J. Dairy Sci.* 108:7235–7247. <https://doi.org/10.3168/jds.2025-26369>.
- Campanile, G., P. S. Baruselli, D. Vecchio, A. Prandi, G. Neglia, N. A. T. Carvalho, J. N. S. Sales, B. Gasparrini, and M. J. D'Occhio. 2010. Growth, metabolic status and ovarian function in buffalo (*Bubalus bubalis*) heifers fed a low energy or high energy diet. *Anim. Reprod. Sci.* 122:74–81. <https://doi.org/10.1016/j.anireprosci.2010.07.005>.
- Campanile, G., G. Neglia, R. Di Palo, B. Gasparrini, C. Pacelli, M. J. D'Occhio, and L. Zicarelli. 2006. Relationship of body condition score and blood urea and ammonia to pregnancy in Italian Mediterranean buffaloes. *Reprod. Nutr. Dev.* 46:57–62. <https://doi.org/10.1051/rnd:2005066>.
- Catillo, G., N. P. P. Macciotta, A. Carretta, and A. Cappio-Borlino. 2002. Effects of age and calving season on lactation curves of milk production traits in Italian water buffaloes. *J. Dairy Sci.* 85:1298–1306. [https://doi.org/10.3168/jds.S0022-0302\(02\)74194-5](https://doi.org/10.3168/jds.S0022-0302(02)74194-5).
- Cerón-Muñoz, M., H. Tonhati, J. Duarte, J. Oliveira, M. Muñoz-Berocal, and H. Jurado-Gómez. 2002. Factors affecting somatic cell counts and their relations with milk and milk constituent yield in buffaloes. *J. Dairy Sci.* 85:2885–2889. [https://doi.org/10.3168/jds.S0022-0302\(02\)74376-2](https://doi.org/10.3168/jds.S0022-0302(02)74376-2).
- Challana, A., A. Gupta, N. Bansal, and V. Uppal. 2014. Morphogenesis of mammary glands in buffalo (*Bubalus bubalis*). *Anat. Res. Int.* 2014:687936. <https://doi.org/10.1155/2014/687936>.
- Ciaian, P., S. Paloma, and J. Delincé. 2013. Literature review on cost of production methodologies. In First Scientific Advisory Committee Meeting. Accessed Dec. 7, 2024. https://www.fao.org/fileadmin/templates/ess/documents/meetings_and_workshops/GS_SAC_2013/Improving_methods_for_estimating_CoP/Improving_methods_for_estimating_cost_of_production_in_developing_countries_Report_JRC_Lit_Review.pdf.
- CLAL. 2025. Italy: Milk Prices. Accessed Dec. 7, 2024. https://www.clal.it/?section=latte_italia.
- Consorzio di Tutela Mozzarella di Bufala Campana DOP. 2024. Bufala campana, in Italia aumentano i consumi. Accessed Dec. 7, 2024. <https://www.mozzarelladop.it/consorzio/numeri-della-dop>.
- Costa, A., M. De Marchi, G. Campanile, R. Negrini, and G. Neglia. 2019. Effect of milk somatic cell level on lifetime milk related performances in Italian water buffaloes. *Ital. J. Anim. Sci.* 18:103 <https://hdl.handle.net/11577/3332319>.
- Costa, A., R. Negrini, M. De Marchi, G. Campanile, and G. Neglia. 2020. Phenotypic characterization of milk yield and quality traits in a large population of water buffaloes. *Animals (Basel)* 10:327. <https://doi.org/10.3390/ani10020327>.
- D'Occhio, M. J., S. S. Ghuman, G. Neglia, G. Della Valle, P. S. Baruselli, L. Zicarelli, J. A. Visintin, M. Sarkar, and G. Campanile. 2020. Exogenous and endogenous factors in seasonality of reproduction in buffalo: A review. *Theriogenology* 150:186–192. <https://doi.org/10.1016/j.theriogenology.2020.01.044>.
- Diana, A., M. Penasa, M. Santinello, F. Scali, E. Magni, G. L. Alborali, L. Bertocchi, and M. De Marchi. 2021. Exploring potential risk factors of antimicrobial use in beef cattle. *Animal* 15:100091. <https://doi.org/10.1016/j.animal.2020.100091>.
- Dürr, J. W., R. I. Cue, H. G. Monardes, J. Moro-Méndez, and K. M. Wade. 2008. Milk losses associated with somatic cell counts per breed, parity and stage of lactation in Canadian dairy cattle. *Livest. Sci.* 117:225–232. <https://doi.org/10.1016/j.livsci.2007.12.004>.
- Eastham, N. T., A. Coates, P. Cripps, H. Richardson, R. Smith, and G. Oikonomou. 2018. Associations between age at first calving and subsequent lactation performance in UK Holstein and Holstein-Friesian dairy cows. *PLoS One* 13:e0197764. <https://doi.org/10.1371/journal.pone.0197764>.
- FADN (Farm Accounting Data Network). 2010. Farm Accounting Data Network: An A to Z of Methodology. Version 4/11/2010. European Commission, Brussels, Belgium. Accessed Dec. 7, 2024. http://ec.europa.eu/agriculture/rica/pdf/site_en.pdf.
- FAOSTAT (Food and Agriculture Organization). 2024. Global buffalo milk production data. Accessed Dec. 7, 2024. <https://www.fao.org/faostat>.
- Fasale, A. B., V. S. Patil, and D. T. Bornare. 2017. Process optimization for mozzarella cheese from cow and buffalo milk. *Int. J. Food Ferment. Technol.* 7:165–173. <https://doi.org/10.5958/2277-9396.2017.00018.6>.
- Fodor, I., Z. Lang, and L. Ózsvári. 2020. Relationship of dairy heifer reproduction with survival to first calving, milk yield, and culling risk in the first lactation. *Asian-Australas. J. Anim. Sci.* 33:1360–1368. <https://doi.org/10.5713/ajas.19.0474>.
- Fonseca, M., D. Kurban, J. P. Roy, D. E. Santschi, E. Molgat, and S. Dufour. 2025. Usefulness of differential somatic cell count for udder health monitoring: Effect of intramammary infections, days in milk, quarter location, and parity on quarter-level differential somatic cell count and somatic cell score in apparently healthy dairy cows. *J. Dairy Sci.* 108:3878–3899. <https://doi.org/10.3168/jds.2024-25401>.
- Galeazzi, P. M., M. E. Z. Mercadante, J. I. V. Silva, L. G. de Albuquerque, G. M. F. de Camargo, and H. Tonhati. 2010. Analysis of culling probability in dairy buffalo using survival models. *Animal* 4:1325–1329. <https://doi.org/10.1017/S1751731110000406>.
- Gasparrini, B. 2018. Effects of reproductive season on embryo development in the buffalo. *Reprod. Fertil. Dev.* 31:68–81. <https://doi.org/10.1071/RD18315>.
- Ghelfi, R. 2000. Evoluzione delle metodologie di analisi dei costi aziendali in relazione alle innovazioni tecniche ed organizzative. Conf. XXXVII SIDEA, Bologna, Italy.
- Gómez-Carpio, M., A. Cesarani, G. Zullo, R. Cimmino, G. Neglia, G. Campanile, and S. Biffani. 2023. Genetic parameters for reproductive traits in the Italian Mediterranean buffalo using milk yield as a correlated trait. *J. Dairy Sci.* 106:9016–9025. <https://doi.org/10.3168/jds.2023-23257>.
- Gómez-Carpio, M., D. Rossi, R. Cimmino, Y. Gombia, D. Altieri, R. Di Palo, G. Campanile, S. Biffani, and G. Neglia. 2025. On the relationship among linear type traits and functional longevity in Italian Mediterranean buffalo. *J. Dairy Sci.* 108:1730–1746. <https://doi.org/10.3168/jds.2024-25232>.
- Javed, K., M. Salman, M. Sharif, H. Muneer, U. Muzammal, T. Najam, and U. Iqbal. 2022. Nutritional requirements of dairy buffalo. *Braz. J. Sci.* 1:1–8. <https://doi.org/10.14295/bjs.v1i9.86>.
- Jenkins, T. C., R. J. Wallace, P. J. Moate, and E. E. Mosley. 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *J. Anim. Sci.* 86:397–412. <https://doi.org/10.2527/jas.2007-0588>.
- Krpálková, L., V. E. Cabrera, J. Kvapilík, J. Burdych, and P. Crump. 2014a. Associations between age at first calving, rearing average daily weight gain, herd milk yield, and dairy herd production, reproduction, and profitability. *J. Dairy Sci.* 97:6573–6582. <https://doi.org/10.3168/jds.2013-7497>.
- Krpálková, L., V. E. Cabrera, M. Vacek, M. Štípková, L. Stádník, and P. Crump. 2014b. Effect of prepubertal and postpubertal growth and age at first calving on production and reproduction traits during the first 3 lactations in Holstein dairy cattle. *J. Dairy Sci.* 97:3017–3027. <https://doi.org/10.3168/jds.2013-7419>.
- Kumar, A., S. Mehrotra, G. Singh, V. P. Maurya, K. Narayanan, A. S. Mahla, R. K. Chaudhari, M. Singh, Y. K. Soni, B. L. Kumawat, S. K. Dabas, and N. Srivastava. 2016. Supplementation of slow-release melatonin improves recovery of ovarian cyclicity and conception in

- summer anoestrous buffaloes (*Bubalus bubalis*). *Reprod. Domest. Anim.* 51:10–17. <https://doi.org/10.1111/rda.12639>.
- Kusaka, H., T. Yamazaki, and M. Sakaguchi. 2023. Association of age at first calving with longevity, milk yield, and fertility up to the third lactation in a herd of Holstein dairy cows in Japan. *J. Reprod. Dev.* 69:291–297. <https://doi.org/10.1262/jrd.2023-012>.
- Macias, H., and L. Hinck. 2012. Mammary gland development. *Wiley Interdiscip. Rev. Dev. Biol.* 1:533–557. <https://doi.org/10.1002/wdev.35>.
- Matera, R., F. Pierro, M. Santinello, A. Iraci Fuintino, G. Pacelli, T. Norton, and G. Neglia. 2025. Precision livestock farming in buffalo species: A sustainable approach for the future. *Smart Agric. Technol.* 11:101060. <https://doi.org/10.1016/j.atech.2025.101060>.
- Moroni, P., C. Sgoifo Rossi, G. Pisoni, V. Bronzo, B. Castiglioni, and P. J. Boettcher. 2006. Relationships between somatic cell count and intramammary infection in buffaloes. *J. Dairy Sci.* 89:998–1003. [https://doi.org/10.3168/jds.S0022-0302\(06\)72165-8](https://doi.org/10.3168/jds.S0022-0302(06)72165-8).
- Napolitano, F., C. Pacelli, F. Grasso, A. Braghieri, and G. De Rosa. 2013. The behaviour and welfare of buffaloes (*Bubalus bubalis*) in modern dairy enterprises. *Animal* 7:1704–1713. <https://doi.org/10.1017/S1751731113001109>.
- Neglia, G., D. de Nicola, L. Esposito, A. Salzano, M. J. D’Occhio, and G. Fatone. 2020. Reproductive management in buffalo by artificial insemination. *Theriogenology* 150:166–172. <https://doi.org/10.1016/j.theriogenology.2020.01.016>.
- Otava, G., S. Squicciarini, S. Marc, T. Suici, G. William Onan, I. Hutu, I. Torda, and C. Mircu. 2021. Effects of age and season on conception rate of Mediterranean Italian Dairy Buffalo (*Bubalus bubalis*) following oestrus synchronization and fixed-time artificial insemination. *Reprod. Domest. Anim.* 56:1511–1518. <https://doi.org/10.1111/rda.14013>.
- Ozturk, N., O. Kocak, A. Peker, L. Serva, F. Kaygisiz, P. D. Kecici, H. Yalcintan, H. I. Kilic, and L. Magrin. 2022. Characteristics of buffalo farming systems in Turkey based on a multivariate aggregation of indicators: A survey study. *Animals (Basel)* 12:3056. <https://doi.org/10.3390/ani12213056>.
- Rautela, R., S. Kumar, R. K. Sharma, S. K. Phulia, R. Kumar, M. Singh, R. Katiyar, A. Bharadwaj, and T. K. Datta. 2024. Impact of age at first calving on fertility and production performance in Murrah buffalo. *Reprod. Domest. Anim.* 59:e14691. <https://doi.org/10.1111/rda.14691>.
- Rosati, A., and L. D. Van Vleck. 2002. Estimation of genetic parameters for milk, fat, protein and mozzarella cheese production for the Italian river buffalo (*Bubalus bubalis*) population. *Livest. Prod. Sci.* 74:185–190. [https://doi.org/10.1016/S0301-6226\(01\)00293-7](https://doi.org/10.1016/S0301-6226(01)00293-7).
- Sales, D., S. Urbano, Do. de Lima Júnior, J. Galvão Júnior, A. Brito, C. Cipolat-Gotet, L. Borba, and A. Rangel. 2021. Factors affecting buffalo Mozzarella cheese yield: A study using regression analysis. *Food Sci. Technol. (Campinas)* 41:852–855. <https://doi.org/10.1590/fst.25620>.
- Serrapica, F., F. Masucci, G. De Rosa, A. Braghieri, F. Sarubbi, F. Garofalo, F. Grasso, and A. Di Francia. 2022. Moving buffalo farming beyond traditional areas: Performances of animals, and quality of mozzarella and forages. *Agriculture* 12:1219. <https://doi.org/10.3390/agriculture12081219>.
- Sung, M.-K., S.-C. Lee, J.-K. Jeong, I.-S. Choi, S.-H. Moon, H.-G. Kang, and I.-H. Kim. 2016. Effect of age at first calving on productive and reproductive performance in dairy cattle. *J. Vet. Clin.* 33:93–96. <https://doi.org/10.17555/jvc.2016.04.33.2.93>.
- Terzano, G. M., S. Allegrini, M. G. D’Elisi, M. Mazzi, M. Razzano, and A. Borghese. 2007a. Effect of intensive or extensive systems on buffalo heifers performances: Blood metabolite values. *Ital. J. Anim. Sci.* 6(sup2):1268–1272. <https://doi.org/10.4081/ijas.2007.s2.1268>.
- Terzano, G. M., G. Neglia, M. Maschio, V. L. Barile, M. Razzano, P. Martiniello, I. Cannone, and A. Borghese. 2007b. Effect of intensive or extensive systems on buffalo heifers performances: Onset of puberty and ovarian size. *Ital. J. Anim. Sci.* 6(sup2):1273–1276. <https://doi.org/10.4081/ijas.2007.s2.1273>.
- Wathes, D. C., J. S. Brickell, N. E. Bourne, A. Swali, and Z. Cheng. 2008. Factors influencing heifer survival and fertility on commercial dairy farms. *Animal* 2:1135–1143. <https://doi.org/10.1017/S1751731108002322>.
- Weigel, K. A., P. M. VanRaden, H. D. Norman, and H. Grosu. 2017. A 100-Year review: Methods and impact of genetic selection in dairy cattle—From daughter–dam comparisons to deep learning algorithms. *J. Dairy Sci.* 100:10234–10250. <https://doi.org/10.3168/jds.2017-12954>.
- Zicarelli, L. 1997. Reproductive seasonality in buffalo. *Bubalus bubalis* 1:29–52. Accessed Dec. 7, 2024. <https://www.cabidigitallibrary.org/doi/full/10.5555/20013056485>.

ORCIDS

- M. Santinello, <https://orcid.org/0000-0001-9418-9710>
A. Calanni Macchio, <https://orcid.org/0009-0001-2926-8292>
A. Lombardi, <https://orcid.org/0000-0002-5862-0283>
R. Matera, <https://orcid.org/0000-0003-2204-0022>
A. Paparella, <https://orcid.org/0000-0001-5428-4107>
S. Biffani, <https://orcid.org/0000-0001-5559-3630>
G. Neglia, <https://orcid.org/0000-0002-0989-6072>
G. Campanile <https://orcid.org/0000-0002-3242-7274>