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On the Relationship Among Linear Type Traits and Functional Longevity in the Italian Mediterranean Buffalo Using a Weibull Proportional Hazards Model

M. Gómez-Carpio,[1] D. Rossi,[1] R. Cimmino,[1] Y. Gombia,[1] D. Altieri,[2] R. Di Palo,[2] G. Campanile,[2] S. Biffani,[3,*] and G. Neglia[2]

[1]Italian National Association of Buffalo Breeders, Caserta, Italy
[2]Department of Veterinary Medicine and Animal Production, Federico II University, 80137 Naples, Italy
[3]Consiglio Nazionale delle Ricerche (CNR), Istituto di biologia e biotecnologia agraria (IBBA), Via Edoardo Bassini, 15 - 20133 Milano, Italy

ABSTRACT

Weibull proportional hazards model was used to identify the non-genetic effects that affect length of productive life (LPL) of the Italian Mediterranean Buffalo (IMB). Data were provided by the Italian National Association of Buffalo Breeders (ANASB) and included records of reproductive, productive, and linear type traits from 59,943 buffalo with first calving from 2002 to 2019. Data were divided into 4 geographical regions to determine if the relationship between the investigated effects and LPL varied by region. LPL was defined as the number of days from the first calving to culling for those buffaloes that were culled (uncensored) or to the date of the last test-day for those that are still alive (censored). The Weibull model included time-dependent effects of herd-year-season of calving, parity and stage of lactation, production as within-herd deviations, as well as time-independent effects of age at first calving, year of birth, type of reproduction (natural mating vs artificial insemination), classifier and ten linear type traits. The average duration of productive life was 1,604 days. Both year of birth and production level had a significant effect on culling risk. Moreover, culling risk decreased linearly across parities. The reference point for age at first calving relative to other classes was set at 35 months. A significant relationship between linear type traits and relative culling risk was also observed, being highest for buffaloes with the lowest linear scores. The results show that the Weibull model provides consistent and robust risk estimates. Therefore, this model would be recommended for future implementation of the first genetic evaluation of LPL in the Italian Mediterranean Buffalo.

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*Corresponding author: Stefano Biffani, Consiglio Nazionale delle Ricerche (CNR), Istituto di biologia e biotecnologia agraria (IBBA), Via Edoardo Bassini, 15 - 20133 Milano, Italy. Telephone, +39- 347 948 3725. Email, stefano.biffani@cnr.it

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INTRODUCTION

Length of productive life (LPL) is an important trait to be considered when dealing with livestock productivity, profitability and welfare. Indeed, sustainability of any production system depends on several factors and increasing LPL, i.e., increasing the proportion of animals with optimal physiological conditions, is a first step forward to guarantee a steady increase of herd profits. The Italian Mediterranean Buffalo (IMB), a river buffalo breed that makes up more than 90% of the European buffalo population and accounts for over 95% of buffalo milk production in Europe (EUROSTAT, 2023), is a significant financial resource due to the production of Mozzarella di Bufala Campana, which holds Protected Denomination of Origin (PDO) status in Southern Italy. From 2012 to 2021, the production of Mozzarella di Bufala Campana PDO has increased by 40% (CLAL, 2023) and in 2021 its production has reached a value of 459 million euros, with a 7.8% increase over 2020 (ISMEA, 2023). If coupled with the high genetic potential for milk production and the higher price per liter when compared with dairy cattle, buffalo has turned into a species of great economic interest among farmers (Minervino et al., 2020, Gómez et al., 2021). This aspect has led to a substantial increase in the purchase price of heifers or pregnant buffaloes. Therefore, interest has grown among breeders to improve LPL and to consider it as a new and innovative selection criterion in current breeding programs. In dairy cattle, increasing productive lifespan not only reduces involuntary culling (Zavadilová and Štípková, 2012) and improves voluntary culling of cows (Sewalem et al., 2008), but also helps dairy companies increasing market profits (De Vries, 2020) to satisfy consumers' needs, improving animal welfare, addressing climate change and promoting environmental sustainability (Grandl et al., 2019).

Several studies have investigated the effect of non-genetic factors on LPL in different populations. Milk production, stage of lactation, age at first calving, parity, variation in herd size, and geographical region are factors that contribute significantly to culling decisions (Roxström et al., 2003, Ducrocq, 2005, Rostellato et al., 2021). When dealing with LPL, an additional and important issue is to first understand its relationship with linear type traits and then if such a relationship differs across geographic regions. This possible relationship has a relevant weight when these traits will be used, for example, in a breeding program or when a possible genotype by environment interaction is going to be considered. Indeed, morphological conformation can be important in mountainous areas or at low temperature but not in humid and swampy areas. Moreover, if the ultimate objective is to develop a genetic evaluation for LPL, understanding which factors contributes to its variation will facilitate the development of a model for breeding value estimation (Caraviello et al., 2004).

Survival analysis through a proportional hazard model represents the most widely used methodological approach for evaluating LPL. Indeed, it is a method that has been widely adopted and validated in different dairy cattle (Vukasinovic et al., 2001, Caraviello et al., 2004, Rostellato et al., 2021), beef cattle (Forabosco et al., 2004, Rogers et al., 2004) and sheep and goat breeds (Mukasa-Mugerwa et al., 2000, Sawalha et al., 2007, Getachew et al., 2015). One of the main advantages of this approach is that it allows the inclusion of time-dependent covariates, where the variables can change their value during the lifetime of an individual until the occurrence of an episode such as culling or death (Lee and Wang, 2003, Schneider Mdel et al., 2005). When life data are studied, survival analysis has several advantages with respect to other statistical methods. Indeed, this approach can combine information on alive (censored) and culled (uncensored) individuals, eventually exploiting all available information (Larroque and Ducrocq, 2001, Vukasinovic et al., 2001, Mészáros et al., 2013b). Additionally, survival analysis can both handle the nonlinear nature of longevity data and accommodate time-dependent variables, i.e., effects influencing productive life that vary with time, such as milk production (Rostellato et al., 2021). Despite these advantages, there are currently no studies that have applied this methodology to the buffalo populations. Therefore, the purpose of this study was to use a Weibull proportional hazards model to investigate non-genetic effects which influence LPL of the Italian Mediterranean Buffalo.

MATERIALS AND METHODS

Ethics Statement

Animal welfare and use committee approval was not needed for this study as data sets were obtained from pre-existing databases based on routine animal recording procedures.

Data

Data were provided by the Italian National Association of Buffalo Breeders (ANASB) and consisted of 2 data sets containing reproductive and productive and linear type traits records, respectively. The production data set included a total of 6,576,041 milk test-days (average number of test-days per buffalo ~5) collected between 1982 and 2019 from 262,254 buffalo cows. The morphological data set included 83,169 linear type traits records collected from 2004 to 2020. Then, the 2 data sets were merged by animal and date of calving, ending up with 230,703 records, from 59,943 buffalo cows with first calving from 2002 to 2019. Buffalo farming in Italy depends on some environmental and socioeconomic factors which vary across regions. For this reason, the complete data set (ALL) was then divided into 4 subsets based on geographic origin and buffalo population density by region: Campania (CAM), Lazio (LAZ), Northern regions (NOR) and Southern regions and Islands (SOU) (**Table 1**).

Campania, with its fertile plains, mild climate and humid areas, is the leading region for buffalo production. This is driven not only by a strong market demand for mozzarella and by a well-developed dairy industry but also by the effect of the Protected Designation of Origin (PDO) status for Buffalo Mozzarella, which adds economic value and incentivizes high production standards. Lazio, also benefits from favorable environmental and socioeconomic conditions – e.g., proximity to major markets like Rome - which have boosted the increase of a significant buffalo population. In the Northern regions, buffalo density is lower, partly due to less suitable environmental conditions such as a colder climate and drier terrain. The focus on other types of livestock and different farming traditions also makes buffalo farming less prevalent here. In contrast, the Southern regions and Islands, including Sicily, are characterized by warmer climates and more traditional, smaller-scale farming systems. Although the warmer temperatures are suitable for buffaloes, these regions lack the humid environments found in CAM and LAZ. Socioeconomic factors, such as lower infrastructure development and less access to a competitive market, influence buffalo production, though it remains an important part of the local agricultural economy (**Figure 1**).

LPL was defined as the number of days from first calving to death/culling (animals with known elimination date) or as the number of days until the date of the last test-day (censored, therefore alive or dated death / elimination not known). The editing criteria included restrictions on the age at first calving (between 24 and 48 mo of age) and parity (until parity 12).

Ten linear traits were retained, namely, stature (STAT), body depth (BD), body length (BL), foot angle (FA), fore udder attachment (FUA), rear udder width (RUW), udder depth (UD), teat placement (TP), teat length (TL), and body condition score (BCS) (**Figure 2**). The scale used for scoring varied according to the set of observed traits, linear traits were scored on a 1–50 scale, while BCS was scored on a 50–85 scale. Each score was transformed into classes with a range of 5 and 10 points for the linear trait and BCS, respectively (**Supplementary Table 1**). Overall, 12 official classifiers were enrolled in the scoring procedures. Buffaloes are evaluated after calving only once in their lifetime, and the time of evaluation is when the animal is between 3 and 4 years old.

Several additional effects can affect LPL. These include contemporary groups (herd-year-season of calving), which might account for important changes in the herd management that occur during an animal's lifetime. For example, stable expansion or facility modernization can influence the risk of a buffalo being culled during a particular period. While expansion and modernization generally aim to improve productivity and animal welfare, they can also introduce new dynamics that impact culling decisions. Stage of lactation can also affect PLP and days (d) in milk (DIM) was divided into 4 classes: 1 to 60 d (1), 61 to 114 d (2), 115 to 174 d (3), 175 to 305 d (4). Another factor that can cause elimination is if the buffalo cow is daughter of a bull authorized for artificial insemination, this effect has been classified into 2 classes, yes (1) or not (2).

To avoid having only censored animals in recent years, year of birth was used to group individuals based on a 5-year interval. Four classes were created: ≤ 2003, 2004–2008, 2009–2013 and ≥2014.

LPL is defined as the ability to delay involuntary elimination and can be approximated by correcting the real survival time for the production level by herd-year. Indeed, low production performances are among the main causes of voluntary elimination. To consider both milk kg and cheese yield (i.e., fat and protein contents) each buffalo cow was assigned to a quintile group based on average milk and cheese yield by a 2-year interval within herd. The average milk and cheese were obtained based on the total production per lactation, with a truncation at 270 d. The cheese yield is calculated according to the following formula: 116.615 + 2.015 * (P % * F %) + 2.929 * $(P\%)^2$.

Model

The model for the functional survival analysis was the following:

$$h_{ijklmnopqr}(t) = h_0(t)\, exp[P_i(t) + D_j(t) + QM_k(t) + QY_i(t) + c_m(t) + Y_n + A_o + F_p + I_q + T_r],$$

where

- $h_{ijklmnopqr}(t)$ = risk function of a particular animal in time t;
- $h_0(t)$ = Weibull baseline hazard function;
- $P_i(t)$ = time-dependent effect of parity (12 classes);
- $D_j(t)$ = time-dependent effect of stage of lactation, class DIM (4 classes);
- $QM_k(t)$ = time-dependent effect of milk kg, quintile group (5 classes);
- $QY_i(t)$ = time-dependent effect of cheese yield, quintile group (5 classes);
- $c_m(t)$ = time-dependent random effect of contemporary (heard-year-season) group (12768 classes);
- Y_n = time-independent effect of years of birth (4 classes);
- A_o = effect-independent of the time of age at the first calving (25 classes);
- F_p = time-independent effect of daughter of artificial insemination (AI) sires (1,2);

Table 1. Distribution of the numbers of buffalo cows included in the analysis for the complete data set and for each of the four geographic regions (acronym)

Data set[1]	Records	Buffalo	Censored	%	Uncensored	%
ALL	230703	59943	20845	34.77	39098	65.23
CAM	113027	30411	11675	38.39	18736	61.61
LAZ	65504	15200	4855	31.94	10345	68.06
SOU	27876	7698	2392	31.07	5306	68.93
NOR	24296	6634	1923	28.99	4711	71.01

[1]CAM = Campania; LAZ = Lazio; SOU = Southern regions and Islands; NOR = Northern regions.

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Figure 1. Provinces comprising each of the 4 standard regions of Italy by buffalo population density. CAM = Campania; LAZ = Lazio; SOU = Southern regions and Islands; NOR = Northern regions

- I_q = time-independent effect of official classifiers (12 classes);
- T_r = time-independent effect of the linear trait (9 classes) and BCS (4 classes).

All linear type and BCS traits were analyzed jointly in the same model. The impact of each non-genetic effects on LPL was assessed on the complete data set and separately in each region.

To obtain accurate estimates of ρ (the shape parameter of the Weibull distribution) and γ (the log-gamma distribution parameter of contemporary group effects) a reduced data set was used, which was obtained by retaining only the largest herds (90,394 records from 24,592 buffalo cows). Once the parameters were obtained, they were used for risk estimates on both the full data set and the 4 subsets.

Software

Data editing and all statistical analysis were carried out using the R v.3.6.1 programming environment (R Core Team, 2019). Survival Kit version 6.12, a series of FORTRAN programs written by Mészáros et al.,(2013b), was used to estimate the parameters and estimate the risk of elimination.

RESULTS AND DISCUSSION

Overview

The *Bubalus bubalis* or Water Buffalo is one of the most rustic domestic species, able to survive in extreme wet or swampy conditions. Buffaloes have higher longevity than cattle and they can surpass 30 years of age, while maintaining their reproductive capacity until they are 18–25 years old (Minervino et al., 2020). However, in the IMB this parameter has been reducing in recent years, with an average LPL of 4.39 years (**Table 2, Figure 3**). By regions, LAZ showed the longest LPL (LPL ~1,839 d), while the CAM region with the largest number of buffaloes showed an LPL of ~1,505 d (**Supplementary Table 2**). LPL in censored and uncensored buffalo cows was 1,392 d (d) and 1,716 d, respectively. The average age at death (from birth to known culling date) was 2,778 d (~7.61 years) with the longest-lived buffalo dead at 7,413 d (~20.31 years) (**Table 2**).

The decrease in LPL (**Figure 3**) is related to various factors. These include voluntary selection based on some production threshold, or simply the implementation of diseases eradication plans (such as tuberculosis, brucellosis), which may lead to a significant and drastic reduction of IMB productive life. Nowadays, breeders are interested in increasing LPL especially because of the high price of a liter of milk but also because it is extremely expensive to buy a heifer or a first calving female to replace a culled female. Indeed, buffalo breeding is economically important, especially for small-scale producers (Minervino et al., 2020). The role of the mozzarella market in Italy has been increasing over the last years and such a trend is reflected in the number of buffalos registered each year at ANASB herd book. For example, since 2020 almost 35,000 females were registered at ANASB herd book, representing approximately 30% of the total population of Italian dairy buffalo with an average milk production of 2,356 kg in 270 d of lactation, 8% fat and 4.63% protein (Minervino et al., 2020, Gómez et al., 2021, ANASB, 2024).

Weibull parameters

Regarding the estimated Weibull parameters, ρ and intercept log (λ) describe the baseline hazard function and the baseline survival function S (t) = exp (- (λ t) ρ), respectively. The parameter ρ indicates whether the phenotypic expression of a trait decreases, remains con-

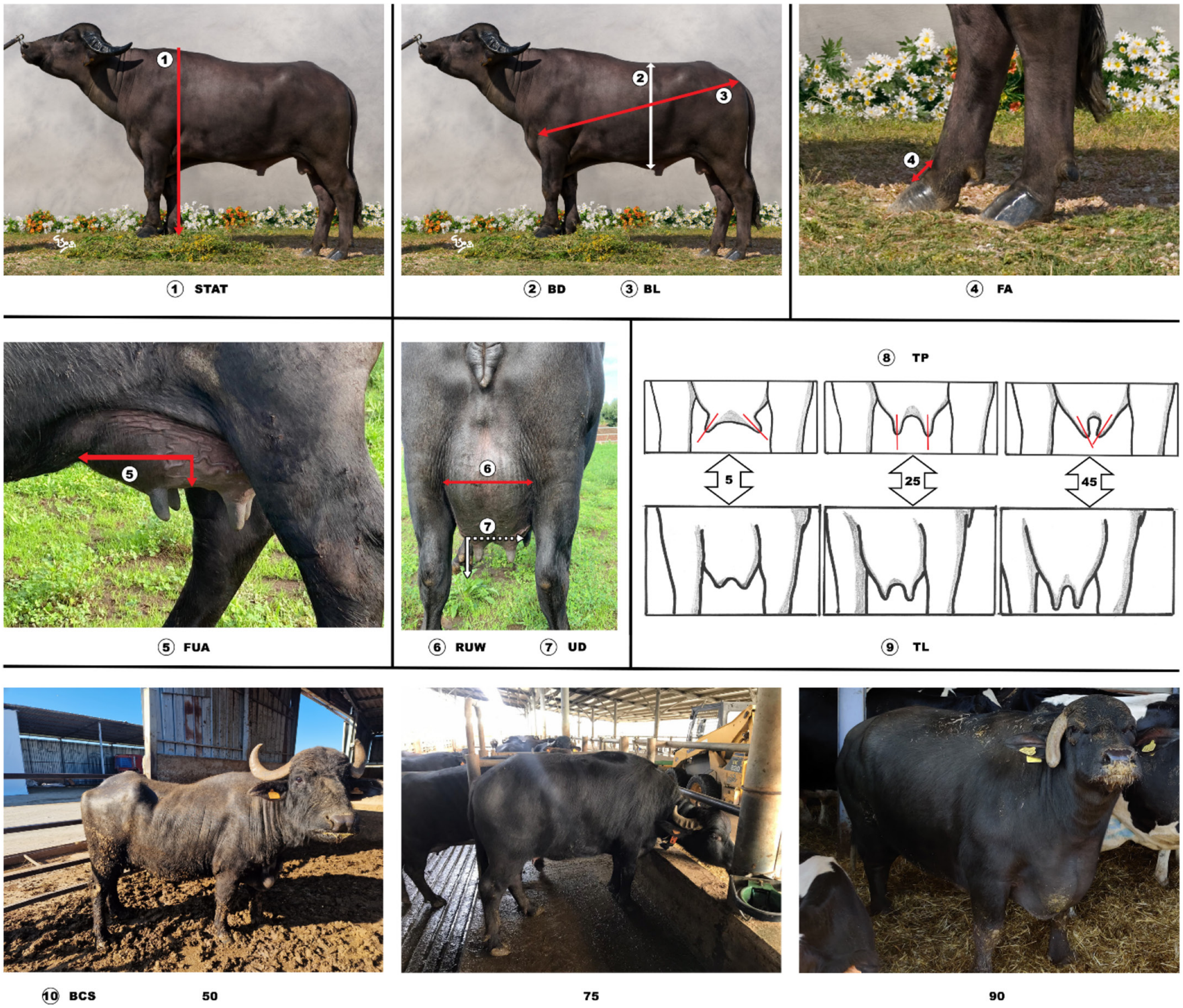

Figure 2. Scoring criteria used for the linear and BCS traits in the IMB. STAT = Stature; BD = Body depth; BL = Body length; FA = Foot angle; FUA = Fore udder attachment; RUW = Rear udder width; UD = Udder depth; TP = Teat placement; TL = Teat length; BCS = Body condition score

Table 2. Length of productive life (LPL) and other reproductive parameters in the IMB

Parameters	Buffalo (n)	Mean	SD[1]	Min[1]	Max[1]
LPL all records (d[2])	59943	1604	998	30	6519
LPL censored (d[2])	20845	1392	915	30	5387
LPL uncensored (d[2])	39098	1716	1022	30	6519
Age at the first calving (month)	59943	35	5	24	48
Parity (n)	59943	3	2	1	12
Distance between first and last calving (d[2])	59943	2031	955	244	5346
Age at death (d[2])	39098	2778	1029	800	7413

[1]SD = Standard deviation. Min = Minimum. Max = Maximum.

[2]d = days.

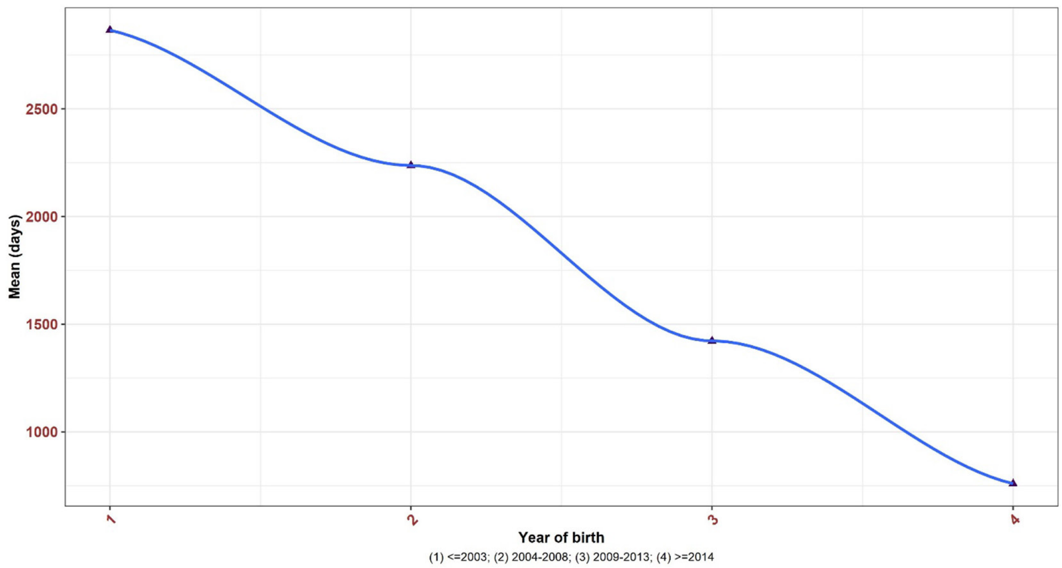

Figure 3. Phenotypic trend of LPL (days) in the IMB

stant or increases over time, when ρ < 1, ρ = 1 and ρ > 1, respectively (Garcia et al., 2017). Indeed, the baseline hazard function λ0 (t) shows the risk of a buffalo being eliminated at time t, considering that it is still alive at time t - 1.

Table 3 shows the Weibull parameters for ALL population and the 4 geographic regions. In the present study, the estimated shape parameter of the Weibull distribution (ρ = 2.28) was similar to that reported in a study of the longevity of Nellore with values of ρ between 2.45 and 2.63 (Caetano, 2011, Garcia et al., 2017), or to a previous study in Chianina beef cattle where Forabosco et al. (2004) reported a Weibull distribution of 2.00. As suggested by Garcia et al. (2017), an ρ > 1 shows that the risk of culling increases with increasing age of the buffalo cows (**Figure 4**). Across regions, this parameter varied between 2.22 in the SOU region to 2.88 in the LAZ region, values higher than what reported in other studies (Vollema et al., 2000, Vukasinovic et al., 2001, Caraviello et al., 2004, Sasaki et al., 2012, Morek-Kopeć and Zarnecki, 2017). The heterogeneity of the parameter ρ by regions probably reflects the differences in the mean survival curve between regions.

The estimates of γ, the parameter of the log-gamma distribution of contemporary group effects were stable at 1.16 by regions and 1.17 for ALL populations. Similar results were observed in other studies, where gamma parameters of the herd-year-season effect ranged from 1.12 to 1.94 (Strandberg and Roxström, 2000, Vollema et al., 2000, Forabosco et al., 2004). However, different values were observed by Caraviello et al. (2004) in various regions of the US, where herd-year-season gamma parameters effect ranged from 3.5 to 13.9. The same authors concluded that the gamma parameter was strongly affected by the environment. However, in the IMB, this parameter suggests that among regions there are no large differences in the survival of the buffalo due to the management of contemporary groups. One possible explanation can be related to the buffalo reproductive seasonality. As Neglia et al. (2020) pointed out, buffalo have a 10-mo gestation period. If they are bred during times of decreasing daylight, their milk production for mozzarella

Table 3. Number of buffalo cows included in the analysis, mean failure time (days from first calving until the known elimination date), estimated scale parameter (ρ), estimated parameter of the distribution of the contemporary herd-year-season groups (γ) and the intercept (ρlog (λ) for the complete data set and for each of the geographic regions

Data sets[1]	Buffalo	Mean failure time, d	Rho (ρ)	Gamma (γ)	Intercept [ρlog(λ)]
ALL	59943	1716	2.28	1.17	−16.47
CAM	30411	1627	2.27	1.16	−16.76
LAZ	15200	1975	2.88	1.16	−21.71
SOU	7698	1556	2.22	1.16	−16.01
NOR	6634	1684	2.41	1.16	−17.42

[1]CAM = Campania; LAZ = Lazio; SOU = Southern regions and Islands; NOR = Northern regions.

would primarily occur in the autumn and winter months (November to March), when demand for mozzarella cheese is low. To align milk production with the peak demand for mozzarella (March to August), buffalo are bred using estrus synchronization and artificial insemination from February/March to September. This approach might average out possible differences across regions.

Survival Analysis

The proportion of total variation explained by the fitted model was 0.61. This proportion is obtained according to Maddala (1983) and depends on the total number of records and on maximum likelihoods of restricted and full model. The results for fixed effects are expressed as risk ratio (RR). RR describes the incremental risk of death associated with a particular level of a variable relative to the risk for the reference class, which is usually set to 1. Values greater than 1 indicate a higher risk of elimination associated with that particular level of an environmental factor (e.g., calving in winter season), while an RR < 1 means a lower risk compared with the reference class (Morek-Kopeć and Zarnecki, 2017).

Age at the first calving (AFC)

Among the fixed environmental factors included in the model, AFC had a strong impact on the risk of culling, both in ALL population and the 4 geographic regions (**Figure 5**). The AFC is a trait that is strongly influenced by the herd-management and strategic choices of the breeders (Van Eetvelde et al., 2020), suggesting that when AFC is delayed, the risk of elimination increases significantly ($P < 0.01$). This agrees with previous studies investigating the influence of AFC on longevity (Vollema et al., 2000, Fernàndez de Sevilla et al., 2009). However, other authors found that it had a slight effect on the risk of culling (Vukasinovic et al., 2001, Getachew et al., 2015, Morek-Kopeć and Zarnecki, 2017).When a female calves for the first time at older age her productive life is expected to be shorter, and it has been suggested that late calving is related to poor fertility or other health problems that also affect the permanence of the female in the livestock farm (Vukasinovic et al., 2001, Páchová et al., 2005, Strapáková et al., 2013).

Class of year of birth

The effect of the class of year of birth on IMB productive life is shown in **Figure 6**. This effect was considered in the model to reflect both different culling policies over the years and the extreme peculiarity of buffalo population dynamics. For instance, the class of year of birth is included to account for generational differences and changes in management practices that affect the initial conditions of life, which can eventually have a lasting impact on the health and productivity of the animals. In our study, these effects showed an important influence on the LPL of the IMB. The interval 2004–2008 was considered as the reference class. As it can be observed in **Figure 6**, RR has been increasing with a remarkable increase in recent years (class 4). The same pattern can be observed in each region even if with different magnitudes. The upward trend, which translates into a constant

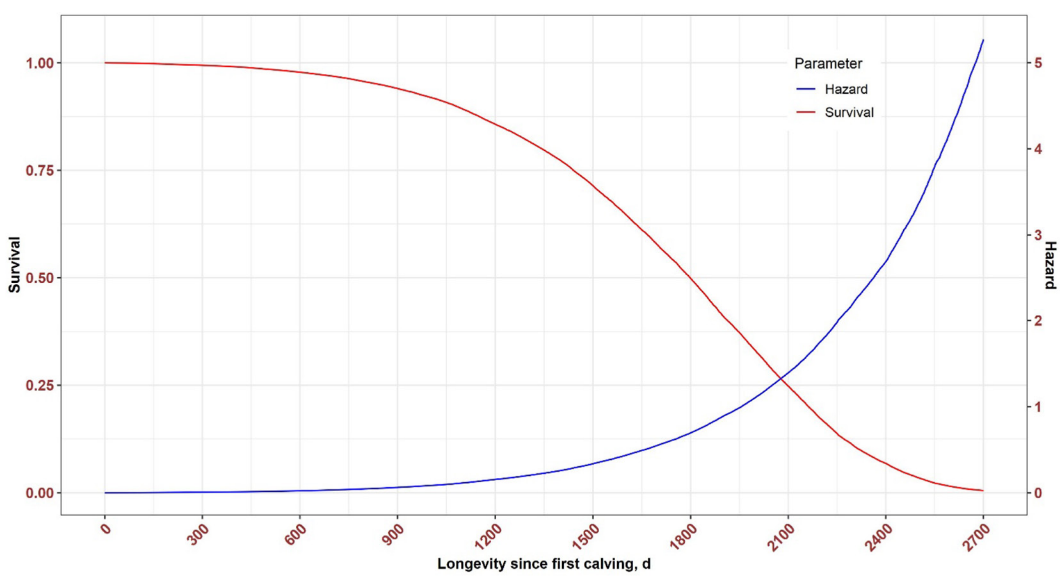

Figure 4. Baseline survival and baseline hazard function. Ratio between mean survival and mean risk of culling

increase in the RR of culling, can be attributed to several factors. Enhanced management practices and diseases control measures, which have become more rigorous and frequent since 2010, contribute significantly to this trend. However, other factors are also influential but harder to identify than management. Economic conditions, including fluctuations in milk prices and shifts in market demand, play a pivotal role in farm economics and thereby could influence culling decisions over time. In **Figure 6** differences among regions are evident, and the RR increase since 2009 (class 3 and 4) is larger in NOR region than the other regions. If we compare buffalo farming in the north of Italy to buffalo farming in CAM, LAZ or SOU regions there are 2 main differences. The first one is the lack of the PDO status which causes larger milk price fluctuations, eventually forcing the farmer to adjust herd size – i.e., increasing or decreasing it. The additional difference is because buffalo farming in the north used to replace dairy cattle farming during the quota era. Indeed, buffalo milk production had no restrictions, and some farmers switched to dairy buffaloes. On 1 April 2015, 31 years after being put into place, dairy quotas were abolished and not all buffalo breeders decided to keep buffaloes. Moreover, environmental situations, such as extreme weather events like prolonged droughts or floods, affect feed availability and quality, potentially heightening the risk of culling.

Daughter of an AI bull

The risk of elimination for being the daughter of an AI bull has never been considered in previous survival studies, however, in the IMB this issue may be relevant, due to the small number of bulls authorized for artificial insemination and their high genetic potential. AI in IMB is around 40% (ANASB, 2024) and because of being an extra cost it might influence the farmers' decision to cull or not. The expected outcome would be a lower risk of culling for an AI bull's daughter than a natural mating bull's daughter.

The results show that except for the SOU region, in general, the risk of culling from being a daughter or not of an AI bull had a slight impact on the permanence of the female in the livestock farm **(Table 4)**. For example, in CAM, the region with the largest buffalo population, the risk of culling was the same, that is, a buffalo cow is eliminated regardless of whether it is the daughter of an AI bull and most likely it will be culled due to low production. The result observed in the SOU region (RR = 0.680) was interesting, showing a lower risk of elimination for those buffalo daughters of an AI bull. This could

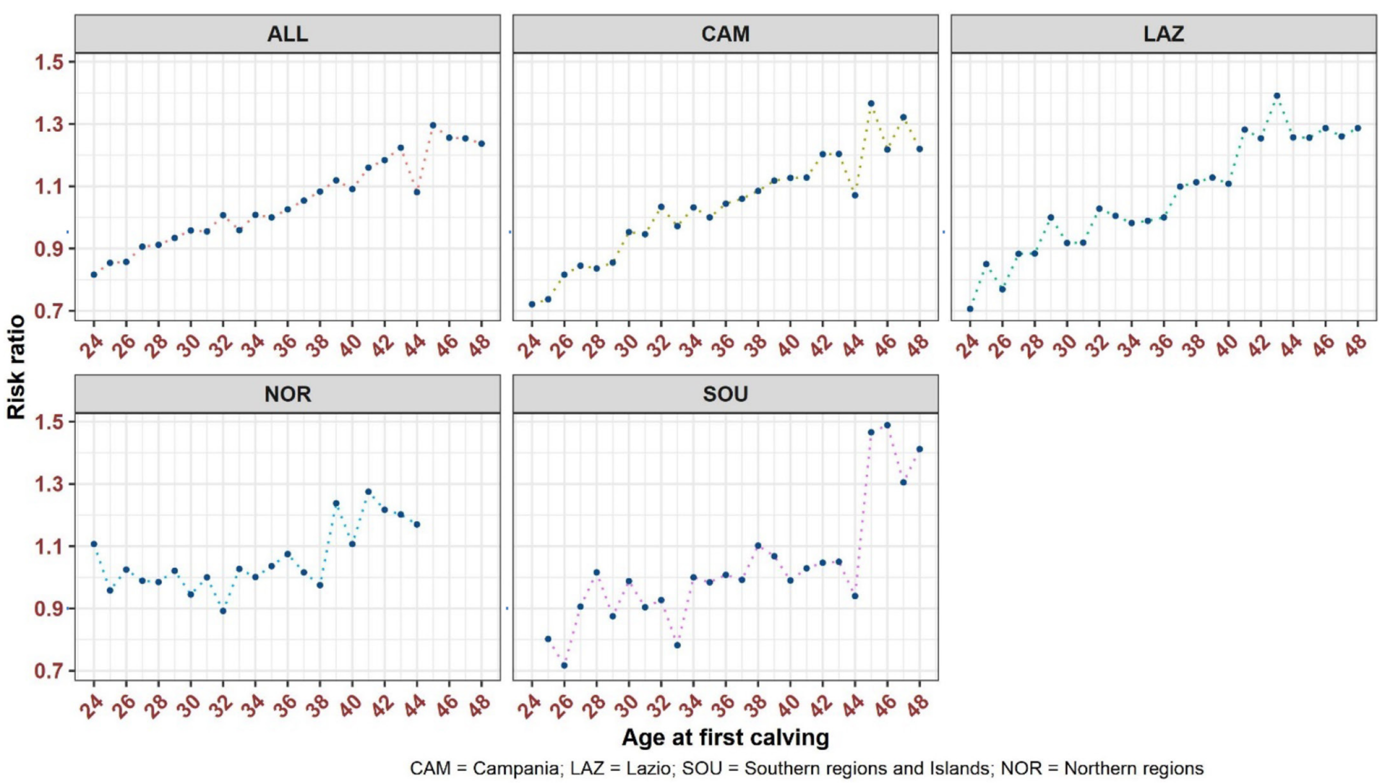

Figure 5. Relative risk of involuntary culling for age at first calving in the complete data set and for each of the geographic regions in the IMB

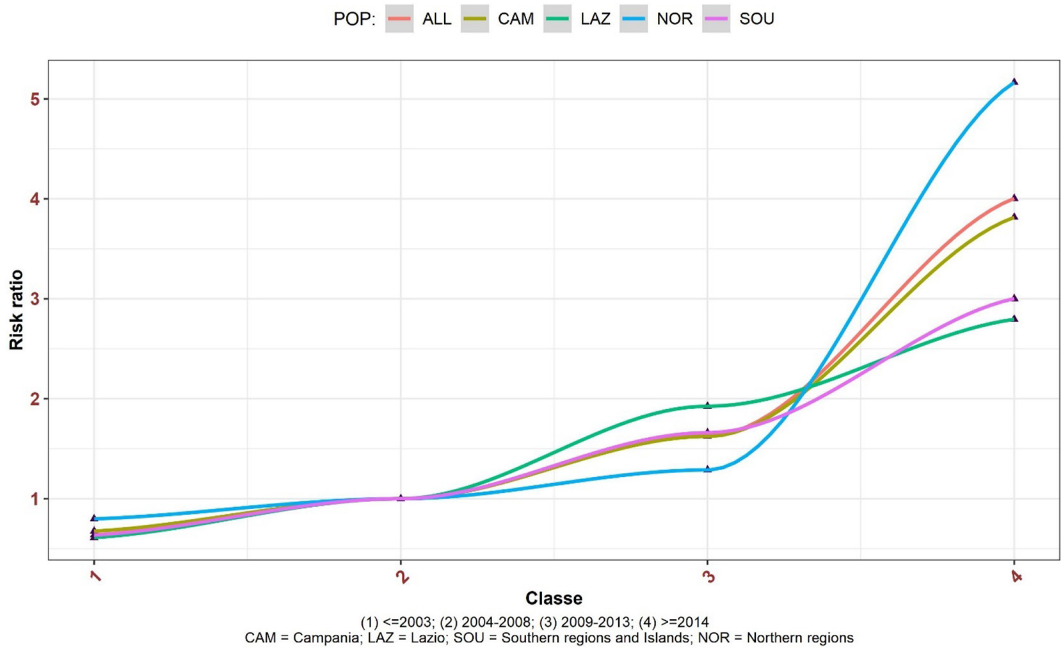

Figure 6. Relative risk of involuntary culling for different classes of year of birth

be due to several factors specific to the southern region. The farm sizes in this region compared with central Italy may influence farmers to be more selective and careful with their livestock management, leading to less frequent culling. Additionally, the economic conditions in this region, where farmers often face tighter budgets and lower financial resources, may increase reliance on AI bulls to improve herd genetics. AI is a more cost-effective option compared with purchasing new breeding stock. Consequently, these economic and structural factors likely contribute to the lower culling risk for buffalo daughters of AI bulls in the SOU region.

DIM class

The risk of culling according to the stage of lactation is shown in **Table 4**. The RR changed slightly across lactation ranging from a minimum of 0.917 (DIM 4 in NOR region) to a maximum of 1.075 (DIM 1 in SOU region). These results disagree with what reported, mainly in dairy cattle, by other authors (Páchová et al., 2005, Sasaki et al., 2012, Mészáros et al., 2013a), where lactation stage have a strong impact on LPL. A possible explanation may be the concomitant effect of milk price and milk production level. Production level in buffaloes is lower than dairy cattle, especially Holstein, but its economic value is much higher, 1.77 €/kg buffalo milk vs 0.47 €/kg dairy cattle milk (ISMEA, 2024). This means that a farmer is probably not going to cull a buffalo cow if its production level at a particular lactation stage is below the average. By region, perhaps CAM shows a higher risk of culling at the end of lactation, but the results reflect the selection strategy exercised especially in this region. While in the SOU region the result was the opposite (beginning of lac-

Table 4. Estimates of risk ratio of involuntary culling in function is daughter of an AI bull, DIM class and qualifier for the complete data set and for each of the geographic regions

	Regions[1]				
Effect	CAM	LAZ	NOR	SOU	ALL
Daughter of AI sire					
Not (2)	1	1	1	1	1
Yes (1)	1.014	0.929	0.833	0.680	0.895
DIM					
1	0.993	1.011	0.965	1.075	1.012
2	1	1	1	1	1
3	1.020	0.981	1.005	1.018	1.007
4	1.023	0.959	0.917	0.977	0.992
Classifiers					
Q1	0.896	0.977	0.962	0.890	0.921
Q2	1	1	1	1	1
Q3	1.018	1.050	1.079	1.211	1.024
Q4	—	—	1.149	—	1.165
Q5	0.956	1.078	1.437	0.924	0.989
Q6	1.045	1.373	—	1.069	1.094
Q7	1.472	0.940	0.792	—	0.895
Q8	0.831	—	—	0.999	0.999
Q9	0.652	—	—	—	0.587
Q10	0.434	—	—	—	0.377
Q11	0.774	—	—	—	0.680
Q12	0.527	0.459	0.395	0.512	0.467

[1]CAM = Campania; LAZ = Lazio; SOU = Southern regions and Islands; NOR = Northern regions.

tation high risk, end of lactation less risk). The high risk at the end of a lactation has been explained by the practice of postponing slaughter until production decreases or when it is known if the female is pregnant, while the high risk of slaughter in the first stage of the first lactation could reflect specific selection criteria for first lactation females (Vukasinovic et al., 2001, Meszaros et al., 2008) or pathological conditions after calving, such as uterine or vaginal prolapses.

Classifiers

Regarding the effect of the classifiers on LPL, the results are observed in **Table 4**. Expert Q2 was considered the reference class. Analyzing the results in the ALL data set, it is observed that expert Q4 presented the highest risk (1.165). By region, the risk ranged between 0.395 (Q12) and 1.472 (Q7). This variation in risk across different classifiers suggest that subjective evaluations by different experts can significantly influence culling decisions. This is not a positive result because all classifiers are supposed to objectively score all individuals or at least to use the same rationale. The highest risk associated with expert Q4 suggests that animals evaluated by this expert had a higher likelihood of being culled, potentially due to stricter or more rigorous evaluation criteria. Conversely, the lower risk associated with expert Q10 could imply a more lenient evaluation approach. These findings highlight the importance of standardizing classification criteria to ensure consistency and fairness in culling decisions across different regions and experts.

Parity

Another factor considered as risk of culling is parity, as shown in **Figure 7**, where lactation 3 was considered as the reference class. Our results indicated that primiparous buffaloes had the highest culling (1.708 for ALL). However, the RR was not the same across regions and LAZ showed the highest culling risk, with a relative risk equal to 2.778. It is not easy to explain the origin of this 2-fold increase in LAZ RR. In LAZ region there are some historical buffalo farms which use to sell females for reproduction, especially primiparous or secundiparous. In some cases, farmers who bought these females are not registered to ANASB and tracking the actual animal life is hampered or partially incomplete. However, these findings highlight the strong impact of parity on LPL and are consistent with previous studies in both cattle (Vukasinovic et al., 2001, Mészáros et al., 2013a, Strapáková et al., 2013) and other livestock species (Kern et al., 2010, Mészáros et al., 2010, Getachew et al., 2015). The highest risk of culling during the first lactation can be attributed to both biological and economic factors. Biologically, first-lactation buffaloes are still in the process of proving their productive and reproductive capabilities. If they show signs of poor health, low milk yield, or reproductive issues early on, they are more likely to be culled (Hedlund and Løvlie, 2015). Economically, farmers often need to optimize the productivity of their herd. If a first-lactation buffalo does not meet the expected production standards, it is more likely to be culled early on, rather than investing further resources into an animal that may not provide adequate returns in the long run. Therefore, a buffalo cow that survives beyond the first lactation has demonstrated the ability to overcome initial challenges (Páchová et al., 2005).

Regarding buffaloes that reach their 12th lactation, they have already demonstrated exceptional longevity and productivity, indicating superior genetic and phenotypic traits that have allowed them to avoid culling for many years. Consequently, factors such as different management practices, the high price of a replacement heifer, and the increasing value of milk may favor this trend in the IMB.

Production level

The production level is still one of the main reasons for voluntary culling and, consequently, one of the most important factors affecting the permanence of an animal in the herd (Vukasinovic et al., 2001, Sewalem et al., 2005, Meszaros et al., 2008, Morek-Kopeć and Zarnecki, 2017). In the models used for genetic evaluations of functional longevity the effect of production is expressed as classes of relative milk, fat or protein production within the herd (Caraviello et al., 2004, Sasaki et al., 2012, Morek-Kopeć and Zarnecki, 2017). In fact, researchers generally agree that the risk of culling increases as production decreases (Rostellato et al., 2021).

Our results confirm this trend also in buffalo. The effect of the production level, in terms of kilos of milk and cheese yield, clearly showed a strong impact on the LPL of the IMB (**Figure 8**). The RR for milk decreased steadily from the first quintile (Q0) (worst 20% based on milk or cheese yields herd averages) to fifth quintile (Q4) (best 20% based on milk or cheese yields herd averages). As for the regions, the risk of elimination was highly significant ($P < 0.01$) and ranged from 0.315 (Q4, SOU) to 0.639 (Q1, LAZ) (**Figure 8A**). Regarding the elimination rate of cheese yield (**Figure 8B**), the trend was similar to milk production, this means, the less production, the greater the risk of elimination. It is important to note that the range of relative risk of culling differs between both traits. This result can be attributed to the fact that farmers prioritize quantity over quality, that is, their main elimination criterion is kilos of milk.

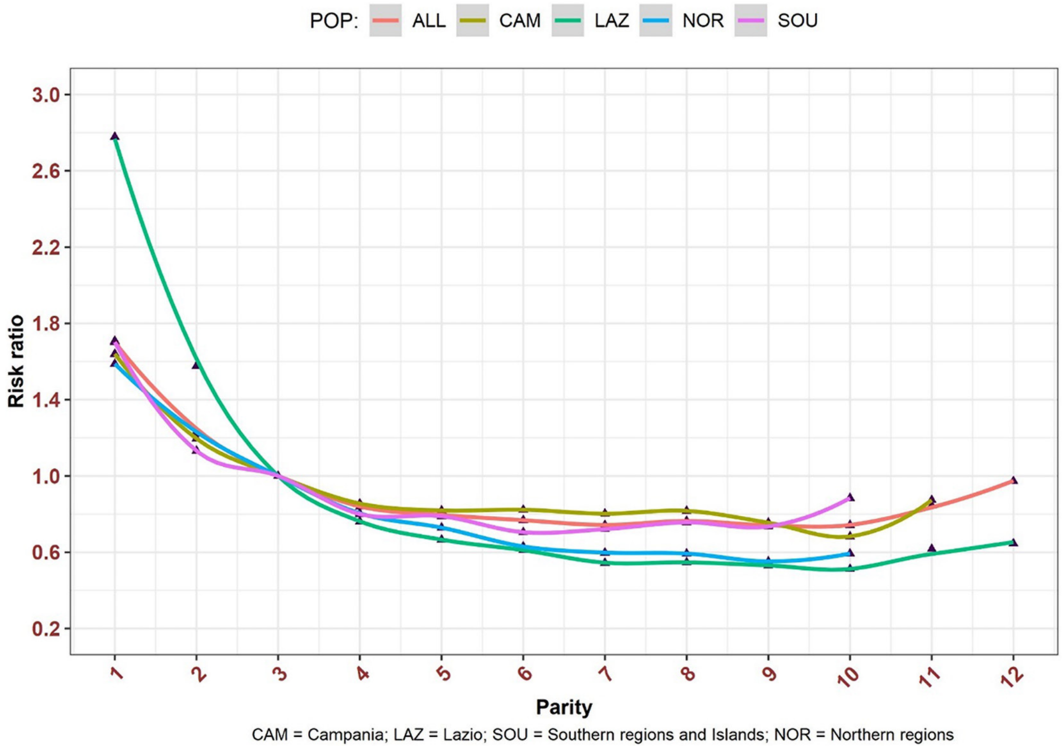

Figure 7. Relative risk of involuntary culling in function of the number of lactations in the IMB

Linear type traits

As shown in **Figure 2**, 9 linear and BCS traits were also included in the model. The morphological conformation plays an important role in the survival of a buffalo in the herd. For example, an extremely lean and / or fat animal has a high risk of culling compared with those in optimal body condition. Similarly, if an animal is outside the IMB breed standard tends to be eliminated at an early age (**Figure 2**, e.g., BCS).

The body condition of an animal is strongly correlated with optimal production (Berry et al., 2003, Pryce and Harris, 2006) and, consequently, with favorable survival in the herd. In the IMB, the BCS is the trait that measures fat deposition, and a score of ≥ 75 was found to be optimal for promoting high functional longevity (**Figure 9**). Small differences were also observed between regions, being an indication that the environmental conditions are affecting the stay of an animal in the farm for a longer time. Body condition is crucial for survival on the herd, as confirmed by a study on French Holstein cows (Rostellato et al., 2021), where the risk of culling was higher for fat cows. Lastly, several studies have reported both positive and negative genetic correlations between BCS and production or fertility, suggesting that these correlations depend on the management system, breed, or selection objectives (Berry et al., 2003, Pryce and Harris, 2004).

Among size traits (**Figure 10**), body depth and body length showed a detrimental effect on LPL (i.e., lower scores correlate with a higher risk of elimination). Within the IMB breed standard, both traits are related to body capacity, which in turn is related to productive potential. Body depth indicates a larger digestive system for efficient nutrient absorption, essential for high milk yield. In cattle, body conformation traits such as body depth affect feed intake and thus milk production (Veerkamp, 1998, Hiendleder et al., 2003). Body length provides more space for internal organs, including the udder, supporting sustained milk production. These traits express the functionality of a subject in terms of dairy character, body capacity, health, and resilience. Our results showed that in the CAM region (geographical area with the highest concentration of buffaloes), the highest risk of elimination is observed when the buffalo has a low score for both body depth and body length. For the breeder, a buffalo cow must have good body structure and robust skeletal evidence. This physical robustness, combined with vitality and strength, will allow the animal to efficiently metabolize large quantities of feed, highlighting the interconnection between morphological traits and overall performance.

Interestingly, stature did not have a strong influence on the permanence of an animal in the livestock farm, as shown in **Figure 10**. A similar result was described

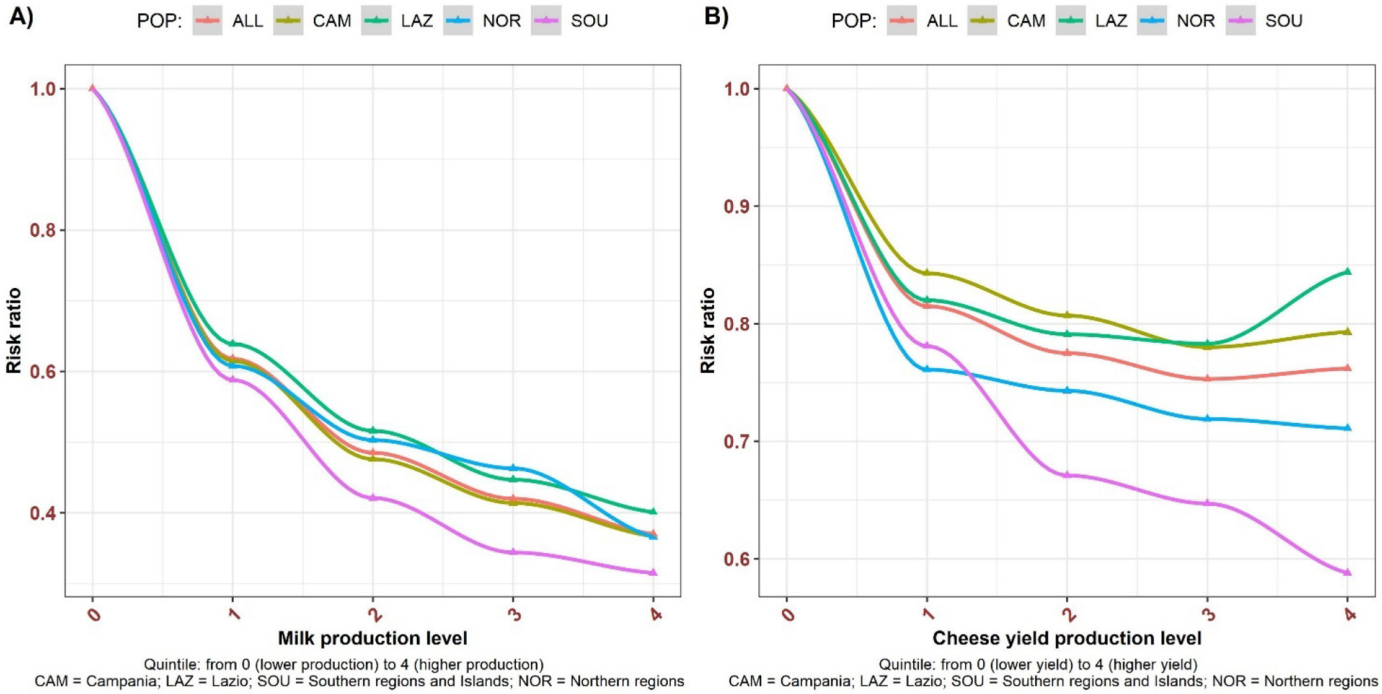

Figure 8. Relative risk of involuntary culling as a function of milk production level (A) and cheese yield (B) in the IMB

in previous studies (Larroque and Ducrocq, 2001, Caraviello et al., 2004), nevertheless, this result differed with Buenger et al. (2001) or Forabosco et al. (2004), who found a strong impact of the stature on longevity. Anyway, this result suggests that buffalo farmers do not prioritize stature in their culling policy, unlike other production related traits (Forabosco et al., 2004).

Foot angle is considered one of the most important linear traits impacting LPL (Larroque and Ducrocq, 2001, Sawa et al., 2013). Surprisingly, we observed no significant effect on LPL (**Figure 10**). This finding aligns with previous research, which has shown the impact of foot angle to range from moderately important (Buenger et al., 2001) to relatively unimportant (Larroque and Ducrocq, 2001). Caraviello et al. (2004) suggested that this result may be due to the difficulty and subjectivity in scoring this trait, leading to an unappreciated risk of elimination. Indeed, foot angle is considered one of the most complex traits to evaluate within the IMB, and as a result, it exhibits low heritability (Gómez et al., 2021).

Estimated risk ratios for udder traits are shown in **Figure 11**. In a dairy breed such as the IMB, the mammary system scores are considered the most important for survival. An ideal udder in IMB should be extended forward, high and wide at the back, firmly attached to the abdomen, well balanced and with perpendicular teat of the right length and diameter (**Figure 2**). Scores that deviate from the optimus are usually associated with an increased risk of culling. Regarding this, for almost all traits, class 25 was set as the reference class (**Figure 11**). The fore udder attachment showed that the risk of elimination decreases with scores above 20, a trend observed in CAM, LAZ, NOR region and in the ALL data set. Regarding rear udder width, a high score means large udder and according to our results it was associated with a lower risk of culling in the LAZ, SOU and ALL data set (**Figure 11B, 11D, 11E**), while intermediate scores were favorable for LPL in the CAM region and NOR (**Figure 11A, 11C**). Reduced survival for low scores related to udder insertion was also found in previous studies (Short and Lawlor, 1992, Larroque and Ducrocq, 2001).

The udder depth trait also shows a higher risk of culling with a low score (score ≤10), especially in LAZ, SOU, NOR and ALL with values ranging from 1.060 to 1.115 (**Figure 11B, 11C, 11D, 11E**). In contrast, buffalo cows from CAM, LAZ and SOU region having udder floor below hock level (udder depth ≥40) had a higher risk of being culled (**Figure 11A, 11B, 11D**). Once again, extreme scores point to a greater risk of culling, as they likely reflect altered production levels. In response, the breeder looks at udder depth score when making culling decision (Rostellato et al., 2021). While udder depth can influence milk production, the relationship is not straightforward and must be evaluated alongside other factors like overall udder health, conformation, and breed specific production standards.

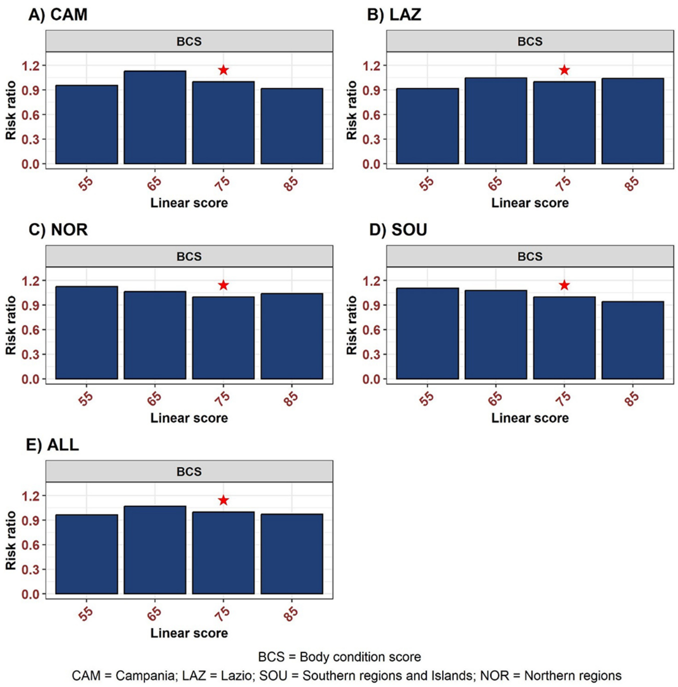

Figure 9. Relative risk of involuntary culling (★ reference class) due to BCS trait for the complete data set and for each of the geographic regions

Within the entire complexity of the mammary system, teat related features are important indicators of longevity and an optimal productive life. An optimal conformation plays a fundamental role in milking-related activities. For example, an optimal direction and length of the teat facilitates milking, while a teat that is too long can lead to infections such as mastitis. Regarding this, low or high scores in the direction of the teat (divergent or convergent) and teat length (long or short) increase the risk of culling (**Figure 11**). The dairy-type traits clearly showed an intermediate optimum with respect to survival, similar results found by Caraviello et al. (2004).

A final comment on the relationship between linear traits and LPL regards the way we have model their effect in the survival analysis. Linear type evaluation occurs after calving only once during the life of a buffalo cow. It means that the age of the buffalo cow and her stage of lactation can affect the final score. Even if age at calving and stage of lactation are included in the model, the actual age at evaluation or the actual stage of lactation at evaluation are not. From a statistical point of view their inclusion sounds reasonable. However, in this case, we think that "not adjusting" the score for age or stage of lactation better reflects what we are going to try to model: the voluntary decision of the farmer to cull a particular buffalo because of a "unfavourable score" on linear traits. If we adjust for age or stage of lactation, we think that we can possibly reduce the voluntary farmer effect.

CONCLUSIONS

Our study unveils the first findings regarding the factors influencing LPL in the IMB. Conformation, production and reproduction have been identified as the most important factors determining culling risk in all analyzed geographic areas. Regardless of the region, risk of culling was moderately to highly related to the mammary system, confirming the importance of udder health as breeding objective. Furthermore, high culling risk in relation to age at first calving, milk production or parity are useful indicators informing selection policies adopted by farmers. Lastly, our research not only highlights the critical factors affecting survival in the herd, but also paves the way for breeding strategies aimed at strengthening the resilience and sustainability of buffalo farming. Increasing the functional longevity of buffaloes entails reducing the number of young animals that would replace culled buffaloes, which in turn improves both economic and environmental sustainability.

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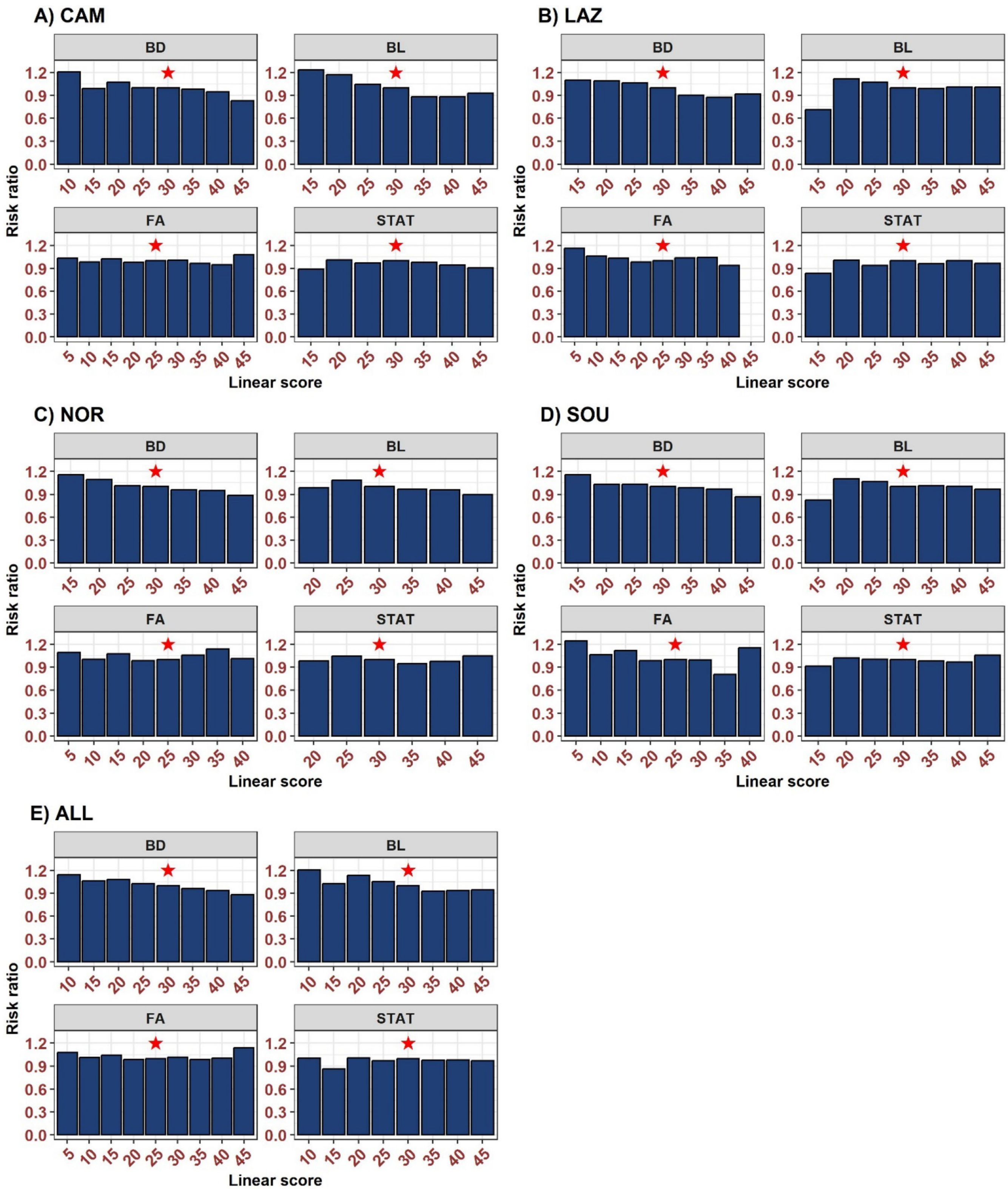

CAM = Campania; LAZ = Lazio; SOU = Southern regions and Islands; NOR = Northern regions

Figure 10. Relative risk of involuntary culling (★ reference class) due to body depth (BD), body length (BL), foot angle (FA) and stature (STAT) score for the complete data set and for each of the geographic regions

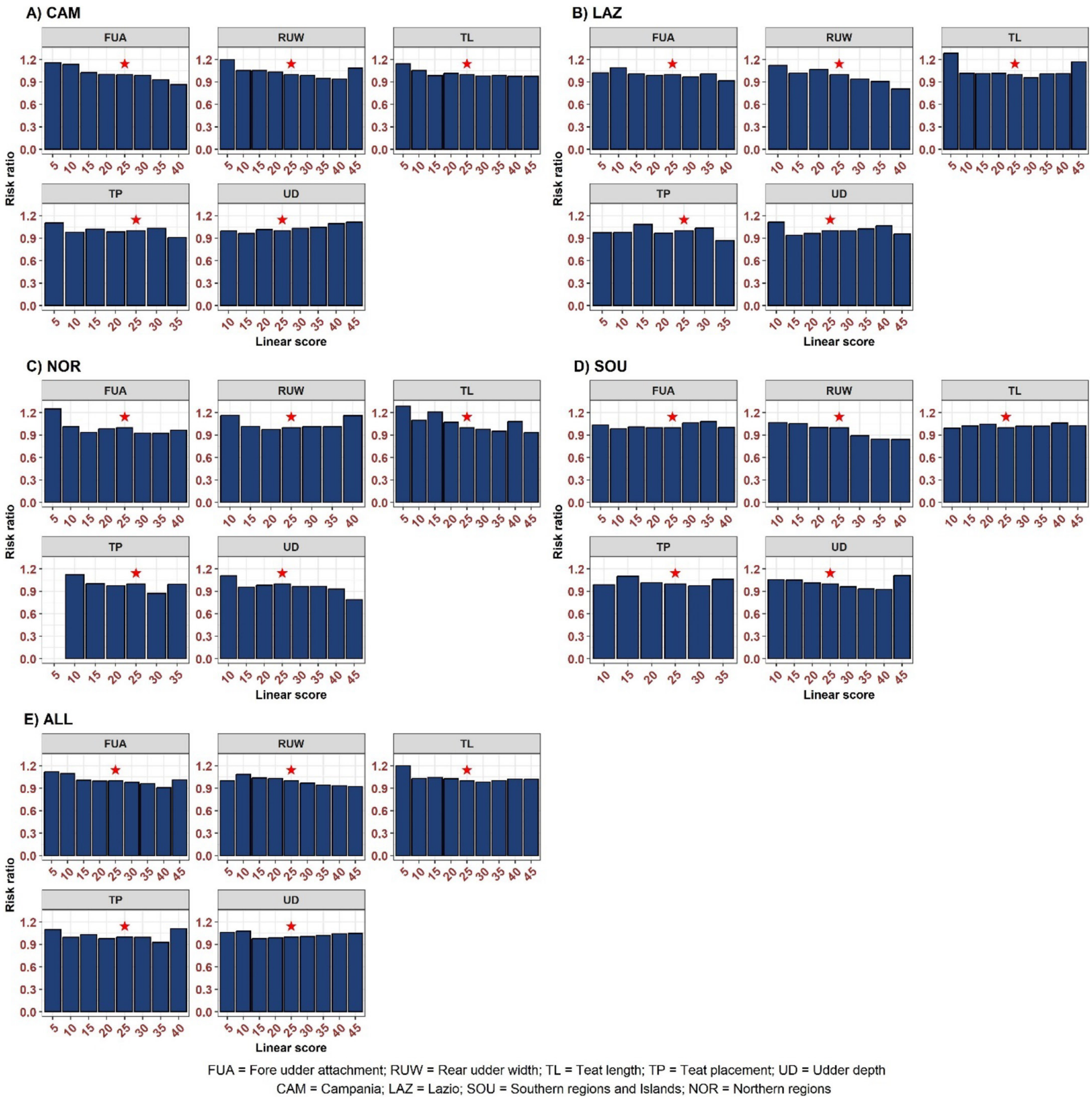

Figure 11. Relative risk of involuntary culling (★ reference class) due traits related to the udder for the complete data set and for each of the geographic regions

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ORCIDS

M. Gómez-Carpio, https://orcid.org/0000-0002-3426-2231
R. Cimmino, https://orcid.org/0000-0002-0146-1787
R. Di Palo, https://orcid.org/0000-0003-4468-0928
G. Campanile, https://orcid.org/0000-0002-3242-7274
S. Biffani, https://orcid.org/0000-0001-5559-3630
G. Neglia https://orcid.org/0000-0002-0989-6072