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Teat morphology across five buffalo breeds: a multy-country collaborative study

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a) Abstract

The buffalo (*Bubalus bubalis*) is a species of worldwide importance, raised to produce milk, meat, and hides, and often used as a working animal in rural contexts with low access to hi-tech solutions. In the present study 100 lactating buffaloes (50 primiparous and 50 pluriparous) of five popular breeds were recruited to characterize and compare teat morphology. In particular, the focus was put on the Nili Ravi, Mediterranean, Egyptian, Bulgarian Murrah and Azeri buffaloes raised in Pakistan, Italy, Egypt, Bulgaria and Iran, respectively. In all countries, a longitudinal cross-section ultrasound was obtained before the milking to measure teat parameters at individual level: overall, teat canal length (TCL) averaged 24.13 mm, teat diameter (TD) 30.46 mm, cisternal diameter (CD) 17.80 mm and teat wall (TW) 7.12 mm. The most variable trait across breeds was TCL which was positively correlated with CD and TD and negatively with TW, regardless of the teat position (front/rear or left/right). A strong negative correlation was found between TW and CD (-0.43). The analysis of variance revealed that the fixed effect of breed significantly affected all the traits except TD. In fact, Bulgarian Murrah, Azeri, and Egyptian buffaloes presented the greatest estimate of TCL, whereas NR the smallest (14.70 mm). The TW was maximum in Nili Ravi, Egyptian, and Mediterranean buffaloes, with estimates equal to 8.19, 7.59, and 8.74 mm, respectively. Nili Ravi also showed the greatest TL (82.39 mm). In terms of CD, the lowest least square mean was that of Mediterranean buffaloes (12.14 mm). Primiparous and pluriparous buffaloes differed in terms of TD, TW and TL, with older animals presenting the highest least square mean. In terms of position, instead, significant differences were observed for TD, CD and TL when comparing front and rear teats, as left and right teats did not differ. Teat anatomy includes a set of heritable morphological features and is therefore breed-dependent. Differences presented in this study could be attributed to the divergent breeding objective and selective pressure across the five breeds; e.g., in some cases such as Mediterranean buffalo, selection for decades was oriented to improve milk production and milkability and achieve optimal conformation for mechanical milking. A better understanding of the mammary gland anatomical descriptors can be informative of the history of a breed and could provide useful insights to guide possible selection.

Keywords: genetic diversity, dairy buffalo, teat anatomy, mammary gland morphology.

b) Introduction

The water buffalo (*Bubalus bubalis*) is a widespread bovine specie, with a global population estimated at over 204 million heads. The 98% of animals are found in Asia, 0.8% in Africa, mainly Egypt, 0.9% in South America, and 0.2% in Europe, mainly Italy (Minervino et al. 2020). Other than delivering products like milk, meat, and hides, buffaloes have been used for working purpose in some confined/rural contexts with scarce access to developed solutions.

Buffalo milk has a high nutritional value; in fact it has about twice the energy content of cow's milk, is extremely rich in calcium and is a good source of essential minerals, amino acids and vitamins (Vargas-Ramella et al. 2021; El Sabry et al. 2022). Water buffalo is classified into two types with different chromosome number, i.e. the river (2n=50) and the swamp buffalo (2n=48). The river type makes up about 70% of the world's water buffalo population and its milk accounts for a substantial share of total milk production in India and Pakistan. Swamp buffaloes, on the other hand, are morphologically different from the former, being less heavy and less productive in terms of milk (Minervino et al. 2020).

The domestication of the river buffalo likely occurred 6,300 years ago in north-western India, from where the domesticated animals migrated westwards through south-western Asia, Egypt and Anatolia, reaching the Balkans and the Italian peninsula. According to literature, there are 123 breeds, 90 just in Asia, many of which are local and with a limited diffusion (Minervino et al. 2020). Out of the river buffalo breeds farmed globally, the Murrah is the most popular as it has been intensively selected for milk production in the north-western territories of India. Murrah buffaloes' main morphological characteristic is the presence of curled horns (Minervino et al. 2020). In Bulgaria, the Bulgarian Murrah (BM) has been officially recognized as a population and it is a crossbreed between the Indian Murrah and the Mediterranean breed. The second most represented breed in the world is the Nili Ravi (NR), the most important breed in countries of South Asia like Pakistan and India. As an example, the Indian Punjab region hosts over 10,000,000 NR heads in. The Azeri (AZ) – also known as Caucasian - buffalo breed originates from the Indus Valley and descended from the Indian buffalo populations (Minervino et al. 2020). The AZ can be found in Iran, Azerbaijan and along the Caspian Sea, but can be found also in Georgia and Armenia.

The Egyptian breed (EG) was introduced into Egypt from India probably around the 7th century b.C. Animals belonging to the EG breed are pivotal for the Egyptian dairy industry, as they produces 45% of the milk consumed in the country. The Mediterranean (MI) is an Italian breed that descends from the river buffalo introduced to Europe from India during the 8th century during the Arab occupation of Sicily and territories of the southern Italian peninsula. Nowadays, the MI is officially recognized as a specialized dairy breed. Animals in Italy are subjected to official genetic selection towards dairy purposes through the National Association of Buffalo Species Breeders (ANASB). Most of the typical products obtained from MI milk are labelled as Protected Designation of Origin (PDO), e.g. "Mozzarella di Bufala Campana" and "Ricotta di Bufala Campana" (Italian Ministry of Agriculure & Forestry. Disciplinary of Production of Mozzarella di Bufala Campana DOP, 11 February 2008).

In developed countries, particularly in Europe, the milking is becoming more and more mechanical rather than manual. This happens for two main reasons: on one hand, to reduce tiring and expensive labor, and, on the other hand, to improve hygienic conditions, udder health and quality of milk (Thomas, 2008).

The relationships between udder morphology, teat anatomy, milk production, milkability and udder health have been extensively documented in the literature (Ambord et al. 2009, 2010; Boselli et al. 2014, 2020; Kaur et al. 2018). Mammary gland conformation and type traits are often considered as selection criteria in breeding programs of various dairy species (Borghese et al. 2007; Mirza et al. 2018). Recent research has shown that the teat anatomy is typical of each buffalo breed, but can be affected by other factors, like husbandry system, and milking type and routine (Boselli et al. 2014, 2020; Borghese et al. 2007; Klein et al. 2005; Costa et al. 2020).

The anatomical characteristics of the teat, besides being typical for each population, can also differ between specialized (intensively selected for dairy) and not specialized breeds. A better understanding of the differences between breeds is fundamental, as it allow customization of milking devices and procedures. As an example, the teat cup liners and the working vacuum level of milking machine could be adjusted to perform the most appropriate and less stressful milking procedures (Caria et al. 2012, 2013).

The aim of this study was to survey and compare external and internal teat measurements of five buffalo breeds involving animals in early lactation. Teat canal length (TCL), teat length (TL), teat diameter (TD), cisternal diameter (CD), and teat wall (TW) were measured in each teat before milking, covering possible variability associated to effects like parity and teat position.

c) Materials and Methods

Design of the Study

This study was carried out between 2021 and 2022 in commercial or experimental pilot farms located in Asia (n = 2), Europe (n = 2) and Africa (n = 1). One farm per country, i.e. per breed, was involved in order to obtain data from MI, BM, NR, EG, and AZ buffaloes reared in Italy, Bulgaria, Pakistan, Egypt and Iran, respectively. In each farm, 10 primiparous and 10 pluriparous clinically healthy lactating buffaloes in the first month of lactation were randomly selected for the teats measurements collection. In total, 100 animals all with 4 functional teats were enrolled for this study.

The same protocol, preliminarily discussed, was adopted in each country. Briefly, in each farm only 1 operator (veterinarian) was trained and allowed to measure teats parameters on the animals before the evening milking session. Measurements of primiparous and pluriparous took place in 2 different dates.

Farms Involved

The present section contains description of the five farms' main characteristics.

- **Italy**: commercial farm located in the Lazio region, where the herd size was 110 MI lactating buffaloes. Animals were milked in a herringbone milk plant, with vacuum level of 42 kPa, 60 cycles per min, and pulsator ratio at 60:40. Mechanical milking was performed twice a day – approximately at 4 a.m. and 4 p.m. Buffaloes were fed the same total mixed ration made using sorghum silage, alfalfa hay, and a special mix formulated for a milk yield (MY) of 10.0 kg/d.
- **Bulgaria**: experimental farm of the Agricultural Institute – Shumen, located in the North-East of the country. The farm hosted 68 lactating BM animals, which were machine milked in cans at a vacuum level of 44 kPa, 60 cycles per min, and pulsator ratio 60:40. Mechanical milking was performed twice a day – approximately at 4 a.m. and 4 p.m. The lactating group's diet involved per each buffalo 20 kg maize silage, 2 kg leguminous hay, 4 kg cereal straw, and 4 kg compound feed. The ration was formulated for a MY of 9.0 kg/d.
- **Pakistan**: NR herd of the Livestock Experiment Station of Buffalo Research Institute (Pattoki, Pakistan). The farm had approximately 100 lactating buffaloes during the experimental period and milking was performed manually and twice a day, approximately at 5 a.m. and 4 p.m.. The daily ration

for lactating buffaloes consisted of seasonal green fodders (maize, sorghum, oats, and berseem) at a rate of 10% of the body weight per animal, along with 4 kg of concentrate. The ration was formulated for a MY of 10.0 kg/d.

- **Egypt**: the study was carried out at the Mehallet-Mousa Experimental Station located in the North Nile Delta. The farm belongs to the Animal Production Research Institute (APRI, Kafr El-Sheikh Governorate) and account for approximately 90 lactating buffaloes. Buffaloes were manually milked twice a day at 6 a.m. and 4 p.m. throughout the whole lactation and were fed the same total mixed ration: cover, corn silage, rice straw and concentrate, and mix for lactating cow. The ration was design to cover the nutritional requirements of a lactating buffalo with a MY of 9.0 kg/d.
- **Iran**: the AZ buffaloes investigated in this study belonged to the Nikookar buffalo farm located in Gilan Province, in north Iran, along the Caspian Sea. The farm hosted 500 buffaloes under semi-intensive system. Milking was mechanically performed twice a day (6 a.m. and 6 p.m.) under a vacuum level of 38 kPa, 60 cycles per min, and pulsator ratio 60:40 (Nikookar et al., 2021). Animals received mixture of self-produced feedstuff (mainly rice stem, rice bran and wheat bran, soy bean, barley and corn) formulated to satisfy the nutritional requirements, assuming a MY of 8.0 kg/d.

Measurement of Anatomical Traits

In each farm, a trained veterinarian performed the echography to assess the teat morphological measurements before milking, as per protocol. Each teat was put in a cup of hand-warm water, and the ultrasound probe was applied to the outside of the cup by using ultrasound gel (Ambord et al. 2009, 2010; Boselli et al. 2014; Ozenc et al. 2020; Weiss et al. 2004). Longitudinal cross-section b-mode ultrasound images were obtained (Fig. 1) for each teat and were used to measure TCL and, at 4 cm above the teat tip, the TD, the left and the right wall thickness, and the CD. The total wall thickness (TW) was calculated as the average of the left and the right wall as in Fig. 1. Once available, the teats measurements of the 100 buffaloes were inserted into a shared database for data elaboration.

The images were recorded with the following instruments:

- **Italy - MI**: echograph Honda HS 101V (Honda Electronics, Japan) equipped with a 5-MHz linear-array transducer.

- **Bulgaria - BM**: SonoScape S2 Vet (SonoScape, China), equipped with a multi-frequency (5.0-10.0 MHz) linear-array probe applied in vertical position with frequency adjusted at 7.0 MHz.
- **Pakistan - NR:** ultrasonic diagnostic scanner with linear probe of 7.5 MHz (HS 1500V, Honda Electronics, Toyohashi, Japan).
- **Egypt - EG**: ultrasonography performed using an ultrasound SonoScape A5 Vet (Sonoscape Co. LTD, Shenzen, China) supplied with a multifrequency linear transducer (3.0-8.0 MHZ).
- **Iran - AZ**: portable ultrasound machine Draminiski (Model vet mini, ROA 7.0.728605) coupled with ultrasound probe of 5 MHz frequency.

Data Analysis

The SPSS software (version 19) was used for data manipulation, editing, and analysis. Descriptive statistics, including the coefficient of variation (CV, %) and the standard error of the mean (SEM), were calculated. To evaluate association between the traits, Pearson's correlations and their significance were assessed in SPSS and the Shapiro-Wilk normality test was used to evaluate the distribution of the data. For the analysis of variance, the GLM procedure of SPSS was adopted. The model accounted for the fixed effect of breed, parity, and teat position, and two interactions as described below:

$$y_{ijkl}=\mu+P_i+B_j+T_k+ V_l + (P\times B)_{ij}+(P \times T)_{ik}+(P \times V)_{il} + (B\times T)_{jk} + (B\times V)_{jl} + e_{ijkl},$$

where: y_{ijkl} is the vector of phenotypic observations for each morphological trait recorded, μ is the intercept, P is the fixed effect of the i-th parity of the buffalo (i = 1 and >1), B is the fixed effect of the j-th breed (5 levels: MI, NR, BM, EG, and AZ), T is the fixed effect of the k-th position I (front *vs* rear), V is the fixed effect of the l-th position II (left *vs* rigth), $(P \times B)$ is the first-order interaction between parity and breed, $(P \times T)$ is the first-order interaction between parity and teat position I, $(P \times V)$ is the first-order interaction between parity and teat position II, $(B \times T)$ k is the first-order interaction between breed and teat position I, $(B \times V)$ is the first-order interaction between breed and teat position II, and e_{ijkl} is the random residual. A multiple comparison of least square means (LSM) was performed using the Bonferroni's test considering as significant P-values < 0.05.

d) Results

Descriptive Statistics and Correlations

A total of 400 teats belonging to 100 buffaloes were measured in 5 countries on animals belonging to the local breed farmed and used for dairy purpose. The MY, which was recorded at evening milking after teat anatomical trait measurements, ranged from a minimum of 3.31 kg to 4.15 kg in NR and BM (data not shown). Overall and breed-specific descriptive statistics of the teat morphological traits are shown in Table 1. The CV ranged from 18.45 to 37.93 % for TD and TCL, respectively. This indicated that the trait with the largest variability across all the breeds was TCL. Within breed, the standard deviation achieved also values greater than 9, with 9.13 and 10.97 mm in AZ and EG. The lowest standard deviation of TCL was calculated in NR (3.62 mm).

As regards the data distribution, data points were plotted and visually inspected before the normality test (Fig. 2). The traits presented a normal distribution with the only eception of TD (P = 0.341; Fig. 2). Looking at raw means (Table 1), it was evident how TCL was on average lower in NR than in AZ (15.36 vs 29.13 mm), whereas TL showed an opposite trend (82.38 vs 54.87 mm). In general, BM showed the lowest average TW and the highest average CD (Table 1).

Pearson's correlation coefficients of the anatomical features studied are reported in Table 2; the same were calculated also for front and rear teats separately and for the right and left lateral position separately (Table 2). In some cases, the correlation assessed on the front teats differed from that of rear teats. As an example, TCL was significantly correlated with TD, TW, and CD in front teats, however in rear teats the TCL was not in significant association with TD (-0.003). In front teats the correlation between TD and TW was significant, unlike rear teats. The same can be said for the correlation between TW and TL which was significant in posterior but not in anterior position. Overall, the strongest associations were similar in front and rear teats, and were calculated between TD and CD and between TW and CD.

The correlations between left and right teats, mirror those observed for the front and rear comparison, with minor exceptions. TCL correlates with CD, TW and TD on the left side, whereas on the right side the correlation with TW is not significant. TL correlated significantly with TD on the left side, but with CD and TW on the right side.

Fixed Effects

The analysis of variance revealed that only part of the fixed effects significantly influenced the teat traits investigated and that none of the interactions was significant, except for that between breed and parity. The LSM of breed, parity, and teat position I and II are shown in Table 4. The TCL differed among the breeds, with AZ, BM and EG showing a significantly higher estimate compared to MI and NR. NR showed the lowest TCL (14.70 mm), which was 15.49 mm far from the greatest LSM (Table 3). A clear difference between breed is evident also from Fig. 3, where two sample images of NR and BM teats are shown. Out of all the anatomical traits measured, only TD was not affected by breed. The LSM were close to each other and statistically similar, falling within the range from 29.62 and 31.22 mm (Table 3). The cistern with the lowest diameter was found in MI, followed by NR and EG whose LSM were similar, i.e. 15.93 and 15.84 mm, respectively. As regards the TW, results indicated that thickness is a variable feature in buffalo (Table 3). In fact, MI, NR and EG showed higher LSM compared to AZ (6.30 mm) and BM (4.77 mm). Considering the TL, the LSM belonged – in descending order – to NR, EG, BM, MI and AZ (Table 3), but LSM of EG and BM were similar (67.85 vs 72.50 mm, respectively).

Parity affected the variability of 3 out of the 5 traits investigated. In fact, only TD, TW an TL were significantly different in the two categories, with pluriparous buffaloes generally showing greater LSM than primiparous. While positon I affected TD, TL and CD (Table 3) - with the rear quarters presenting a greater LSM than front ones - lateral position (position II) did not significantly affect the traits.

Regarding the interaction between breed and parity, the analysis revealed that TCL was greater in pluriparous than primiparous of AZ and BM, while in EG, MI and NR there was an opposite trend (data not shown). Both TD and TW were higher in pluriparous BM, EG and NR, while in MI and AZ breed the LSM of primiparous was greater compared to that of pluriparous (data not shown). The highest CD was found in the primiparous of BM, EG and MI and in the pluriparous of AZ and NR. Finally, TL of primiparous was superior only in the case of EG buffaloes; in the other breeds, in fact, older cows always showed longer teats.

e) Discussion

Breed-related Differences

Ultrasound examination on teat anatomy in dairy species is a powerful tool to characterize udder anatomy in a given breed or population and therefore having reference values to identify potential anomalous teats (Ambord et al. 2009, 2010; Boselli et al. 2014; Thomas et al., 2004; Weiss et al. 2004). The method used in the present study was standardized across the five countries to allow for an undisturbed and fair comparison of the breeds investigated. Results of this study should be considered as preliminary. Data, in fact, refer to only five farms (one per breed), therefore findings should be interpreted with caution as it was not possible to account for the effect of farm, which, however, should be investigated in further studies.

The teat canal is the only orifice between the internal milk secretory system of the mammary gland and the external environment. The anatomical characteristics of the teat reported in literature are different, because there is large variability due to breed. In India, for the Murrah breed, Thomas et al. (2004) reported an average TCL of 3.1 cm, with average values of 3.7 ± 0.2 and 3.0 ± 0.1 cm for the hind and fore teats, respectively. In that study, authors found that TCL was similar in animals at different parities and stages of lactation. To reduce distubances, however, the protocol adopted in the present study was intended to get rid of the potential effect of lactation stage and only animals in the first month of lactation were selected.

Pushkin Raj (2010) measured teats on both healthy and mastitic animals of the same breed raised in Chennai (India), reporting an average TCL of 17.7 (front teats) and 20.3 mm (rear teats). For the crossbred Murrah and Mediterranean × Murrah, Bittner et al. (2010) observed TCL values between 14.0 and 15.0 mm in front and rear teats, respectively which is close to the TCL of NR (15.36 mm; Table 1). Average TCL (Table 1) was intermediate between the value of Thomas et al. (2004) and those of Bittner et al. (2018) and Pushkin Raj (2010). The TW was scarcely variable in the study of Bittner et al. (2010), falling between 0.71 to 0.72 cm. In this study, TW had a CV of 32.16% and there were differences among the breeds. Considering the raw means in Table 1, the lowest and greatest average were found in BM and MI, respectively. The average TD of all breeds (Table 1) was lower than that reported by Pushkin Raj (2010), whose values fell between 36.4 and 37.0 mm. CD recorded in BM was lower than that detected by Pushkin Raj (2010) whose range was: 2.72 - 3.14 cm.

In some studies carried out on MI buffaloes, the TCL was measured before application of a pre-milking treatment: not stimulation, pre-stimulation, or oxytocin injection. Observed values fell between 23.6 and 27.8 mm (Ambord et al. 2009: Boselli et al. 2010). In the same breed, studies have demonstrated that there is a reduction in the TCL with a 3 min pre-stimulation, i.e. with a decrease ranging from 25.5 to 19.5 mm (Ambord et al. 2010) and from 23.6 to 14.8 mm (Boselli et al. 2014).

Changes in other anatomical parameters have been observed before and after the treatments (no stimulation, manual pre-stimulation, or oxytocin injection). For instance, studies reported a reduction in TW (from 97 to 84 mm), and an increase in both CD (from 13.1 to 17.1 mm) and TD (from 32.7 to 32.9 mm; Boselli et al. 2014). Similarly, Ambord et al. (2009, 2010) reported TW to reduce (approximately 1 cm) and observed increased CD (from 39 to 89 mm) and TD (29.2 to 29.6 cm). For the Anatolian-Mediterranean type breed, a recent study conducted by Ozenc et al. (2020), showed a large variability of teat traits in relation to animal temperament (docile or nervous), pre-stimulation length (0, 3 and 6 min), and teat position. In absence of stimulation, TCL of buffaloes fell between 18.5 to 23.5 mm; however, after a few min of pre-stimulation in the same animals the TCL reduced up to 22.0 mm (Ozenc et al., 2020). El-Ghousien et al. (2022) reported an average TCL of about 13.0 mm in EG buffaloes, which is very low compared to the present study (Table 1). Nevertheless, it should be pointed out that El-Ghousien et al. (2022) measured the teats *post-mortem* and with different instrumental methods compared to the present study. The same authors reported TD in EG buffaloes to vary from 22.0 to 23.0 mm, while in this study TD averaged 31.01 mm in the same breed. Al-Galil ASA (2016) for the same breed, found the normal teat walls appeared as high reflective structure with three discrete layers; the outer hyperechoic layer, the middle thicker hypoechoic layer and the inner hyperechoic layer. The teat cistern appeared as a dilated anechoic area with few hypoechogenic dotes corresponding to the milk contents. In subclinical mastitis, the teat canal and cistern appeared with irregular contour lining, homogenous hypoechogenic contents, narrower lumen, slightly thickened wall and loss the characteristic three layered appearances. In this way the authors measured teat TW thickness from 0.63 to 1.05 cm for healthy and mastitic buffaloes respectively, walues similar to how found in our study.

In this study, clinically healthy buffaloes were enrolled to avoid bias results due to diseases such as mastitis. In this regard, Hussain et al. (2013) carried out a *post-mortem* study on healthy and mastitic NR buffaloes and analyzed teat traits like TD, TL, and TCL in infected and healthy quarters. The analysis of variance revealed that mastitis can affect the teat anatomy. Overall, the TCL measured by Hussain et al. (2013) was about 3 times greater than the TCL of this study which was measured using echography images. In fact, TCL averaged 47.7 and 52.1 mm in healthy and mastitic quarters, respectively.

In terms of LSM, the NR breed showed the lowest TCL. This difference suggests that the breed needs specific adaptations of milking devices and that milkability can be dramatically different compared to other breeds. Peculiar NR anatomical characteristic should be taken into account when developing any mastitis control program or designing specific milking equipment for this breed.

Finally, only partial information on teat anatomy is available for the AZ breed (Alkhateeb et al. 2021). For example, no information is published for TCL. The average TL in AZ buffaloes has been reported to be 48.2 and 78.8 mm in front and rear teats, whereas TD averaged 30.8 mm in front teats and 42.0 mm in the rear ones (Alkhateeb et al. 2021). This paper reports for the first time TCL, TW and CD measurements of the AZ buffaloes and represents a new and important information for the characterization of the breed.

Other Fixed Effects

In dairy cows, Klein et al. (2004) observed an increase in TD and TW with increasing parity, likely due to the cumulative effect of repeated milking events which include exposure to mechanical stress that, in turn, may increase thickness of the teat wall (i.e. TD; Hebel et al. 1979).

In a recent study, data of 59 Murrah buffaloes were used to evaluate the effect of parity on the teat and udder morphological traits (Bharti Praveen et al., 2015). Authors observed that the traits differ according to the buffaloes parity and therefore age. In general, TD and TL increased with parity, confirming the results observed in the present study (Table 3).

Regarding the frontal teat position, i.e. position I, in our study significant differences were found only for TD, CD and TL. Rear teats had greater LSM compared to front ones (Table 3) and, although several explanations can be proposed for this trend, the most important reason is related to the teat capacity. Like in dairy cows, rear teats, in fact, are slightly larger than the front ones and contain more milk. The approximate ratio in buffalo as well as in cows is 60:40; this explains why milking the hind quarters takes longer in general (Thomas, 2008).

Lateral position of the teats did not affect the anatomical characteristics of the teats. Although there is scarce literature available to discuss this result, in Indian dromedary Kumar et al. (2023) found that the TD of left posterior teats was greater compared to the other teats and that the right anterior teat was the one with the lowest TD. It is important to consider that the effect of teat position can also depends on the milking methods used. In dairy cattle, some authors suggested that the differences found between left and right teats may be affected by the milking position (right- or left-side milking), which can cause a greater pressure on the rear teats of one side with a progressively consequent increase in the teat size.

In this study, two breed were manually milked (NR, EG) and the procedure adopted did not include the so called 'knuckling' or 'stripping' (Tamil Nadu Agricultural University, 2009), an intentional actions performed with the fingers in the past to reduce resistance of the teat sphincter. This manual stress can cause stretching and damage to the teats, with different effects on the internal and external anatomical traits of the left and right teats. Although both manually milked, NR and EG presented different averages and LSM in our study for TCL and TL, suggesting that the milking type does not mask other effects and does not overlap with the effect of breed. Authors of this study recommend future studies comparing teat anatomy in buffaloes of different breeds subjected to both mechanical and manual milking.

General Considerations

Several studies conducted on large and small ruminants demonstrate that teats morphology and milkability traits are connected. In fact, most of the udder health selection indexes of dairy cows put emphasis on mammary gland morphological characteristics. Apart from Italy, where genetic selection is currently active and monitored by ANASB, in other buffalo populations, selection towards milk yield and/or mastitis resistance is not officially pursued on a large scale level. Accurate phenotypes and pedigree data are fundamental for making breeding decision and for establishing robust mating plans, but the collection is difficult to implement, especially in some areas and contexts.

Correlated traits are useful indicators for breeders, as they can be used for genetic improvement. Correlations between teat anatomical traits and milkability and between teat anatomical traits and mastitis/udder health are reported in literature for different dairy species. In dairy cows, for example, Weiss et al. (2004) observed that while the flow rate of milk was positively correlated with TD, the same was negatively correlated with TCL. Bobìc et al. (2014) recorded data on both Simmental and Holstein Friesian cows and found significant differences between teat anatomy and milking traits in the two breeds and calculated significant correlations between TCL and milk flow rate (negative) and between TCL and milking time (positive), indicating that an higher milk flow is associated to a shorter milking session.

In dairy camel, Atigui et al. (2014) reported positive correlations among udder characteristics, teat measurements and milkability traits. In particular, a significant positive association was observed between TD and udder depth and between TD and several milkability traits. Findings of Atigui et al. (2014) indicate that camels with larger teats are milked more easily, which could be due to larger teat canals or lower resistance of the teat sphincters and higher intra-mammary pressure due to higher milk in the udder.

In buffaloes, several authors (Boselli et al. 2010, 2014; Costa et al. 2020; Thomas et al 2004; Napolitano et al. 2022) studied the association between the main teat anatomical traits and milk flow. In buffalo, there is a great resistance of the muscle walls of the teat (Ambord et al. 2009; Borghese et al. 2007: Thomas et al. 2004), meaning that higher vacuum pressure is required during mechanical milking to open the teat canal and reach the plateau phase compared to cattle. This peculiar characteristic is important to consider when preparing milking protocols and machineries. Ambord et al. (2009) reports that buffaloes adapt more difficultly to mechanical milking compared to cows, likely due to the presence of longer teat canals. A possible approach to investigate the influence of teat anatomy on milkability is to measure the vacuum required to open the teat canal (VO) which is 20 kPa in dairy cows. In dairy cows, the correlation between VO and TCL was positive and significant (0.82) as well as the one between VO and TW (0.62); on the other hand, negative was the association between VO and CD (-0.65) (Weiss et al., 2004). Given these associations, it is reasonable to assume that TCL and other teat anatomical traits, can have an influence on milk ejection consequently affecting the milkability.

Teat anatomy, especially TCL, is crucial for the defense mechanisms against bacterial colonization of the teat canal. Cobirka et al. (2020) showed how the teat end's level of defense against pathogens depends upon several specific physical and physicochemical factors including, among others, TCL, amount of keratin present, and milk flow rate. It is authors' belief that the long TCL of buffaloes (more than double compared to dairy cows) has an indirect beneficial effect on udder health, mastitis resistance, and milk somatic cell count.

In conclusion, a better understanding of the main anatomical teat parameters of the most popular buffalo breeds raised in the world (MI, AZ, BM, EG and NR) is useful to characterize the population, define reference values, and therefore easily identify anomalies. Moreover, studying the effect of parity and teat position is also pivotal for proper evaluation of anatomical characteristics and for decision-making. Mammary gland morphology is heritable and associated with udder health in dairy breeds/types, therefore, a deeper knowledge of phenotypic variability can support breeders to guide possible selection plans. Although this work represents a preliminary investigation due to the small number of animals and herds involved, it is recommended to validate findings in future improving representativeness of farm management and systems by including a larger number of herds.

Authors of the present study expect that in the coming decade these findings would be useful to breeders: the focus on functional traits like udder health is expected to increase in buffalo, in parallel with the demand of milk and dairy foods, especially in African and Asian countries where the population is dramatically increasing along with the food demand. Other than accounting for productivity level, the genetic selection and improvement of buffaloes should aim to boost milk quality, fitness and health, relying on easy-to-measure proxies - like teat measurements and milk composition. Findings advise that any breeding objective or indicator trait/proxy for selection programs to improve udder health of buffaloes should be tailored according to the breed-specific mammary gland morphology, productivity level, and susceptibility to clinical or subclinical mastitis.

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g) Statements & Declarations

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Author contributions

Conceived of or designed study: the experimental design and data collection protocol were conceived, planned and developed by Antonio Borghese, Angela Costa and Carlo Boselli.

Performed research: all authors contributed to the measurement and data collection in the field.

Analyzed data: Antonio Borghese, Angela Costa and Carlo Boselli performed the statistical analysis and interpretation of the results.

Wrote the paper: the primary research draft was written by Antonio Borghese, Angela Costa and Carlo Boselli. Angela Costa and Carlo Boselli improved and corrected the manuscript. All authors discussed the results and commented on the manuscript. The final version of the manuscript was read, improved and approved by all authors.

Data availability

Data will be made available on reasonable request.

Declarations

Ethics approval

The ultrasound teat measurements were performed by veterinarians, in accordance with internationally accepted standard ethical guidelines for the use and care of animals.

h) Conflict of Interest Statement

No conflicts of interest.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

g) References

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