

Investigation of the functional dimensions of teats up to mid-lactation in Bulgarian Murrah buffaloes established by ultrasonography

Ivan Fasulkov^{1*} Pencho Penchev² Yordanka Ilieva² Manol Karadaev¹

Abstract

The aim of this study was to monitor the changes in teat measurements from early to mid-lactation in the Bulgarian Murrah buffaloes. The experiment was carried out with twenty (10 primiparous and 10 pluriparous) lactating buffaloes. Five series of ultrasound measurements were performed, each consisting of two sessions for each animal corresponding to the phases of lactation: first lactation month (day 1 to day 30 postpartum) and mid-lactation (day 122 to day 152). The studied parameters were teat canal length (TCL), teat diameter (TD), teat wall thickness (TWT), and teat cistern diameter (TCD). The data were processed using the software product LSMLMW and MIXMDL via the following model used for each of the traits: In Model 1, fixed effects of calving month, parity, lactation phase, milking time, teat position, and milk yield were used. Another linear model was constructed to test the covariance between teat position and lactation phase (Model 2). Only two of the ultrasound teat measurements were significantly affected by lactation phase. While TD of all four teats decreased from the first to the fourth month ($P \leq 0.001$), TCD increased ($P \leq 0.001$). Teat position is a significant source of variation in TWT ($P \leq 0.01$) and TCL ($P \leq 0.001$). All ultrasound measurements have significantly greater values in the pluriparous compared to the primiparous buffaloes. Parity is a significant source of variability for all studied functional traits, best expressed for TL and TCL ($P \leq 0.001$). The present study showed that TCL practically does not change during most productive lactation, despite the period of intensive machine milking, unlike most other teat measurements. This justifies the practice of not changing the milking system parameters and udder prophylactics throughout lactation periods.

Keywords: buffaloes, lactation, teat measurements, ultrasonography

¹Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Stara Zagora 6000, Bulgaria

²Agricultural Academy, Agricultural Institute, Shumen 9700, Bulgaria

*Correspondence: ivan.fasulkov@trakia-uni.bg (I. Fasulkov)

Received February 17, 2026

Accepted August 5, 2026

<https://doi.org/10.56808/2985-1130.4022>

Introduction

Buffalo milk is a delicacy product marked by high density, associated not only with its higher dry matter but also with the higher proportion of high-melting triglycerides (Penchev *et al.*, 2016; Khan *et al.*, 2019), and with a characteristic, appealing odor, attributed to the specific volatile organic compounds and to the high concentrations of short-chain fatty acids (Garau *et al.*, 2021; Wang *et al.*, 2024; Liao *et al.*, 2025). The fat percentage of buffalo milk is twice as high, but cholesterol is lower, and the lipid globules are larger, as compared to cow milk (Islam *et al.*, 2014; Zhou *et al.*, 2018; Abdel-Hamid *et al.*, 2023). Noteworthy is also the presence of gangliosides, which are not found in cow milk and which have anti-inflammatory and anti-toxin effects (Jiang *et al.*, 2022). It is rich in whey proteins, calcium, and, in particular, colloidal Ca (Islam *et al.*, 2014; Vargas-Ramella *et al.*, 2021).

The Bulgarian Murrah was especially developed as a dairy breed with good adaptability. The intensity of galactopoiesis is high up to the middle of lactation – in most cases the peak is in the second month, while some of the buffaloes have the peak yield in the third and fourth months (Penchev *et al.*, 2011; Penchev and Peeva, 2013), but in some particular herds in the first month (Ilieva *et al.*, 2022).

The relationships between udder morphology, teat anatomy, milk production, milkability, and udder health have been extensively documented in the literature (Ambord *et al.*, 2009; Ambord *et al.*, 2010; Boselli *et al.*, 2014; Kaur *et al.*, 2018; Boselli *et al.*, 2020). Mammary gland conformation and type traits are often considered selection criteria in breeding programs of various dairy species (Mirza *et al.*, 2019; Boselli and Borghese, 2024).

In comparison to dairy cows, buffalo teats have fewer muscles (scattered and arranged in 2-3 layers) but more collagen in thicker bundles (Ozenc *et al.*, 2020). Recent research has shown that teat anatomy is typical of each buffalo breed, but can be affected by other factors, like management and milking type (Ambord *et al.*, 2010; Boselli *et al.*, 2014; Costa *et al.*, 2020; Boselli and Borghese, 2024). The anatomical characteristics of the teat, besides being typical for each population, can also differ between specialized (intensively selected for dairy) and non-specialized breeds. A recent multinational study showed the specific differences among the different breeds, including the relatively long canal in the Bulgarian Murrah (Boselli *et al.*, 2024).

A better understanding of the dynamics of teat morphology development is fundamental, as it allows optimization of milking devices and procedures, as well as adaptable control of udder health. Udder morphology is very important for farming practice, for efficient and less stressful milking procedures involving adequate teat cups and vacuum during machine milking (Caria *et al.*, 2012; Pierro *et al.*, 2025). In buffalo, interest in these functional traits is expected to increase in parallel with the demand for milk and dairy foods, especially in parallel with awareness of buffalo dairy's superb qualities. Moreover, in the

Bulgarian Murrah buffaloes, such detailed research has not been done.

The aim of this study was to monitor the changes in teat measurements from early to mid lactation in the Bulgarian Murrah buffaloes, established by ultrasonography.

Materials and Methods

Ethical approval: All procedures were in agreement with the requirements for welfare and animal protection included in Ordinance No.20/1.11.2012 of the Ministry of Agriculture and Food, Republic of Bulgaria (approval no. 18-4678/23.08.2022 from the Bulgarian Food Safety Agency).

Animals and management: The study was carried out from September 2024 to November 2025 on the buffalo farm of the Agricultural Institute – Shumen. The farm applies machine milking in cans at a vacuum level of 44 kPa, 60 cycles per min, and a pulsator ratio of 60:40. Mechanical milking is performed twice a day – approximately at 4 a.m. and 4 p.m. No udder stimulation was done prior to milking – neither manual massage nor hormonal (oxytocin) treatment. The diet involves 20 kg maize silage, 2 kg leguminous hay, 4 kg cereal straw, and 4 kg compound feed per lactating buffalo. The ration was formulated for a milk yield of 9.0 kg/d.

Design and organization of the study: Pluriparous ($n_{pl} = 10$) and primiparous ($n_{pr} = 10$) lactating buffaloes from the main herd were assigned. All animals were selected to be clinically healthy, with 4 functional teats. Five series of measurements were generally performed, each consisting of two sessions for each animal corresponding to the phases of lactation under consideration – first lactation month (around postpartum Day 15 ± 3 days) and mid-lactation between the fourth and fifth month (around postpartum Day 136 ± 3 days):

series I: Session 1 – September, 2024; Session 2 – January, 2025; $n_{pl} = 3$, $n_{pr} = 3$;

series II: October, 2024 – March, 2025) – $n_{pl} = 7$, $n_{pr} = 1$;

series III: (January, 2025 – May, 2025) – $n_{pr} = 2$;

series IV: (June, 2025 – October, 2025) – $n_{pr} = 1$;

series V: (July, 2025 – November, 2025) – $n_{pr} = 3$.

The measurements were taken by two operators, the same for each of the sessions and with the same functions – one of them always and only operating the ultrasound probe (Operator #1) and the other always and only manipulating the image to take on-screen measurements (Operator #2). Each session consisted of two sub-sessions: measurements before the morning milking and before the evening milking of the same animals.

Tools and morphological traits: The ultrasonography was done using an ultrasound device, SonoScape S2 Vet (SonoScape, China), equipped with a multifrequency (5.0–10.0 MHz) linear array transducer. The serial ultrasound examinations were performed via a transcutaneous approach with a vertical position of the probe and a 7.0 MHz frequency (Fasulkov *et al.*, 2014). For better contact between the probe and the teat skin, ultrasound contact gel (Eco-Ultra gel, Milano, Italy) was used. The following measurements were taken to be studied: teat canal length (TCL); teat diameter (TD); teat wall thickness (WT); cistern diameter (CD) – all shown in Figure 1. A manual measurement was also taken – teat length (TL) by ruler as the distance from the teat base to its tip.

Data processing and statistical analysis: Once obtained, the teat measurements of the 20 buffaloes were pooled so that, for each morphological measurement, 320 entry lines were collected – for each of the 4 teat positions, at each of the 2 milking times within each of the 2 lactation phases, for each of the 20 buffaloes. The data were processed using the software product LSMLMW and MIXMDL (Harvey, 1990) via

the following model used for each of the traits (morphological measurements):

$$\text{Model-1: } Y_{fijkq} = \mu + CM_f + P_g + LP_i + MT_j + TP_k + MY_q + e_{fijkq}$$

where μ is the mean value; CM_f – the fixed effect of calving month – calendar month in which the first session of the animal (i.e., first lactation month) was performed whereupon the series start ($f = 1 \dots 5$); P_g – the fixed effect of parity ($g = 1 \dots 6$); LP_i – the fixed effect of lactation phase ($i = 1, 2$); MT_j – the fixed effect of milking time ($j = 1 \dots 2$); TP_k – the fixed effect of teat position ($k = 1 \dots 4$); MY_q – the fixed effect of milk yield, as set in classes of up to 5.0 kg, 5.1–8.0 kg, 8.1–11.0 kg and over 11.0 kg ($q = 1 \dots 4$); and e_{fijkq} – the residual error.

Another linear model was constructed to test the co-effect between teat position and lactation phase – Model-2: including the interaction $[LP \times TP]_{i*k}$ ($i*k = 1 \dots 8$) instead of LP_i and TP_k .

The data were presented as mean $\pm$ standard error of the mean (SEM). The differences in the obtained results were considered statistically significant at $P \leq 0.05$.

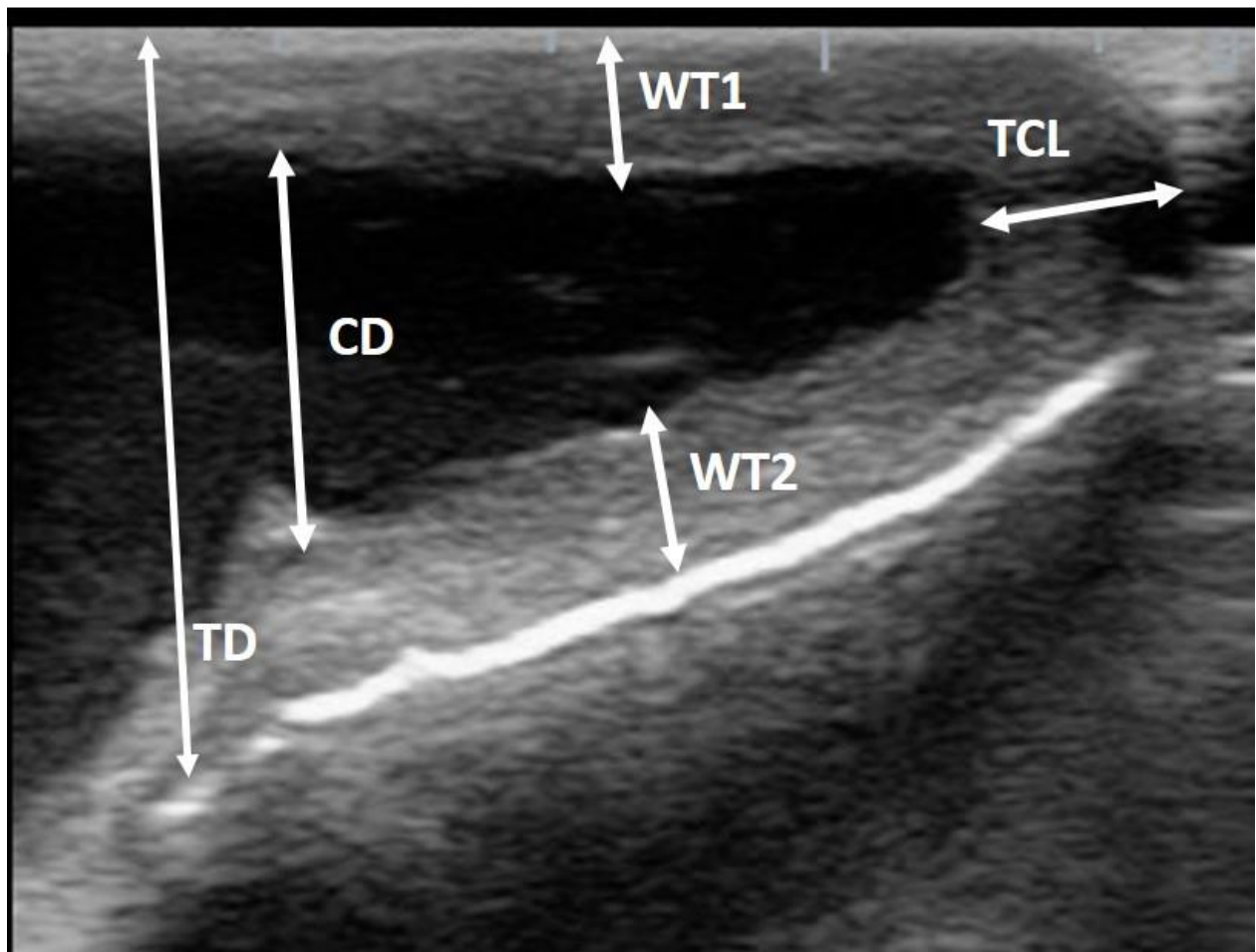


Figure 1 Ultrasound measurements of studied parameters in buffalo teat. Legend: TCL – teat canal length; TD – teat diameter (in the widest middle part of the teat); WT1 – teat wall thickness at a point chosen at random, and WT2 – teat wall thickness at a point opposite to WT1, the arithmetic mean there of considered as an average of teat wall thickness (WT) used in the data processing ($WT = WT1 + WT2$); cistern diameter (CD) at 4 cm above the teat tip.

Results

The results of the conventional statistical procedure are presented in Table 1. They show that the differences between primiparous and pluriparous buffaloes regarding teat length (TL) are significant with respect to caudal teats only, the first-calvers having 8.5% ($P \leq 0.05$) smaller TL than the adult animals. Despite the measurements being performed manually, the standard errors of the means were relatively small, ranging from 2.4% to 2.9%.

The differences in teat canal length (TCL) and thickness of the teat wall (WT) are highly significant, indicating by 13.0 and 13.8% shorter canal for cranial and caudal teats of primiparous compared to pluriparous buffaloes; and a 23.0% ($P \leq 0.001$) thinner wall of the cranial teats and by 18.9% of the caudal.

Also statistically proven is the lower teat diameter (TD) of the primiparous animals – by 9.3% ($P \leq 0.05$) for the cranial teats and 8.4% for the caudal. The between-age-group differences concerning cistern diameter (CD) are non-significant ($P > 0.05$), with a tendency for wider cisterns in the primiparous buffaloes.

The analysis of variance (Table 2) shows that calendar month and parity have a significant effect on all teat morphological dimensions, and teat position is a significant source of specific variation in only teat length, manually measured, and average teat wall thickness. Milk yield and time of milking (a.m. or p.m.) are not statistically significant factors.

The factor lactation phase has a strong effect, but only on teat diameter and cistern diameter. However, the interaction between lactation phase and teat position is a statistically significant factor for all measurements, except teat canal length.

Figure 2 shows that, compared to the cranial teats, in mid-lactation the caudal left teat is longer ($P \leq 0.01$).

Table 1 Conventional statistics of teat traits (measurements, cm) in primiparous (n=10) and pluriparous (n=10) buffaloes by medial position.

Teat trait	Teat position							
	Cranial				Caudal			
	Primiparous		Pluriparous		Primiparous		Pluriparous	
	Mean ± SEM	CV	Mean ± SEM	CV	Mean ± SEM	CV	Mean ± SEM	CV
TL	6.43 ± 0.191	26.6	6.87 ± 0.197	25.3	6.93 ± 0.166	21.4	7.57 ± 0.211	24.9
TCL	2.54 ± 0.052	18.2	2.92 ± 0.059	18.0	2.56 ± 0.057	19.8	2.97 ± 0.068	20.6
TD	2.54 ± 0.065	22.8	2.80 ± 0.090	28.7	2.63 ± 0.063	21.5	2.87 ± 0.083	26.0
CD	2.95 ± 0.082	24.8	2.78 ± 0.082	26.5	3.08 ± 0.079	22.9	2.93 ± 0.085	25.9
WT	0.40 ± 0.094	21.1	0.52 ± 0.012	21.2	0.43 ± 0.012	24.9	0.53 ± 0.009	14.7
	TL ^{NS} , TCL ^{***} , TD*, CD ^{NS} , WT ^{***}				TL*, TCL ^{***} , TD*, CD ^{NS} , WT ^{***}			

Legend: TL – teat length; TCL – teat canal length; TD – teat diameter; CD – teat cistern diameter; WT – teat wall thickness.

Significance of differences between age groups within measurements as per Student-Fisher t-test: NS – not significant ($P > 0.05$); * – $P \leq 0.05$; *** – $P \leq 0.001$.

Discussion

The findings about the morphology of the udder, teats in particular, are important from a very practical standpoint. On the basis of them, the teat cups and the working vacuum level of the milking machine could be designed and set to perform the most efficient and less stressful milking procedure (Caria *et al.*, 2012; Pierro *et al.*, 2025). In this context, the present study shows that teat canal length practically does not change during most productive lactation, despite the period of

The thickest cisternal wall is observed for the right caudal teat, being 10 to 11% thicker compared to the two left teats ($P \leq 0.001$), as shown in Figure 3 based on Model-1.

Figure 2 indicates that, concerning manual teat length measurement, significant differences exist within lactation phase only, but not within teat position between phases. In this way, in the first lactation month there is a difference of 0.61 cm ($P \leq 0.05$) in favor of the caudal left teat compared with the cranial right, while in the second month the caudal teats are longer than the cranial ones by 0.65 to 1.14 cm ($P \leq 0.05$). This outlines the dependency normally observed in practice in buffaloes that the caudal teats are longer, especially the sinistral. Though not statistically proven, the shorter cranial teats have a trend of diminishing in length with the advance of the lactation period, while the caudal teats become longer – the longest sinistral showing a growth of 32 mm. Figure 4 shows that there is no statistically significant difference between lactation phase and teat position (Model-2) on teat canal length.

Figure 3 shows that the diameter of all four teats decreases from the first month postpartum to mid-lactation; the differences from 0.791 to 0.900 cm are significant at $P \leq 0.001$. In parallel with this, as Figure 5 shows, the cisternal diameter increases from 0.748 to 0.873 cm, which, for the caudal dextral teat, is significant at $P \leq 0.01$, and for the rest – at $P \leq 0.001$.

According to Figure 6, the thickest teat wall is observed in the caudal right teat, significantly different ($P \leq 0.05$) from the two cranial teats at mid-lactation. There is a trend, though non-significant, for the walls of the cranial teats to become thinner with the advance of lactation and for that of the caudal teat to become thicker, which is in parallel with the established changes in teat length from Figure 2.

intensive machine milking (twice daily for four months), unlike most other teat measurements. This justifies the practice of not changing the milking system parameters and udder prophylactics throughout the lactation period. We can note that a limitation of our study was the small number of buffaloes used in it, but nevertheless the results of the teat measurements showed reliable differences depending on parity and lactation period of animals. Despite this limitation, the present study provides

valuable data on the ultrasonographic changes of teat parameters in the Bulgarian Murrah breed, a

comparatively less-studied livestock species under practical field conditions.

Table 2 Analysis of variance of teat measurements expressed in F-values.

Sources of variance	df	TL	TCL	TD	CD	WT
Model-1						
R ²		0.419	0.300	0.536	0.483	0.418
Calving month	4	30.00***	7.45***	7.44***	13.52***	9.87***
Parity	5	6.91***	8.53***	3.34**	2.50*	2.61*
Lactation phase (LP)	1	0.25 ^{NS}	0.12 ^{NS}	182.28***	137.39***	0.00 ^{NS}
Milking time	1	0.62 ^{NS}	0.11 ^{NS}	1.01 ^{NS}	0.10 ^{NS}	2.21 ^{NS}
Teat position (TP)	3	6.50***	0.44 ^{NS}	0.80 ^{NS}	2.26 ^{NS}	5.24**
Milk yield	3	1.38 ^{NS}	1.94 ^{NS}	1.68 ^{NS}	2.05 ^{NS}	1.43 ^{NS}
Model-2						
R ²		0.424	0.301	0.537	0.484	0.426
LP*TP	7	3.16 ^{NS}	0.27 ^{NS}	26.27***	20.54***	2.88**

Legend: TL – teat length; TCL – teat canal length; TD – teat diameter; CD – teat cistern diameter; WT – teat wall thickness.

For Model 2, only the added co-effect is presented, as the estimates and their statistical significance remained practically unchanged from Model 1. Significance of F-values: NS – not significant ($P > 0.05$); * – $P \leq 0.05$; ** – $P \leq 0.01$; *** – $P \leq 0.001$.

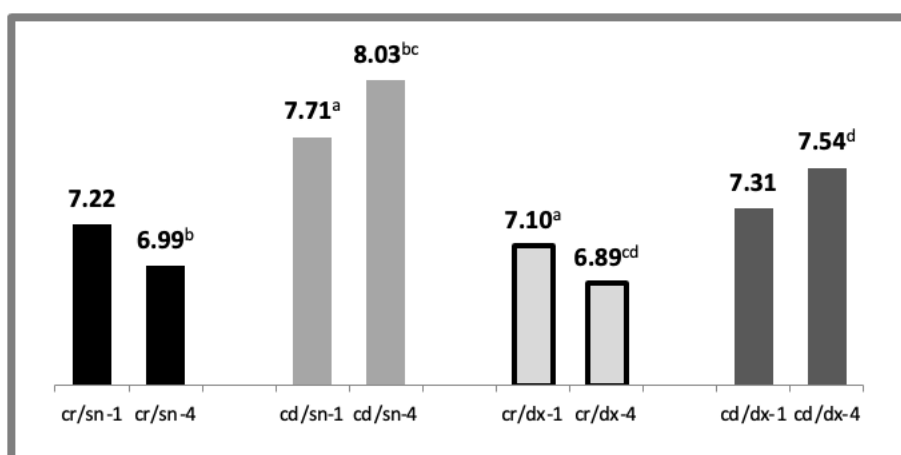


Figure 2 LSM estimates of the co-effect between lactation phase and teat position (Model-2) on teat length, cm (manually). Legend: levels: cr – cranial teat; cd – caudal teat; sn – sinistral teat; dx – dextral teat; -1 – at first lactation month; -4 – at mid-lactation (fourth/fifth month); td (differences of interest only): When symbols superimpose, identical – $P \leq 0.05$.

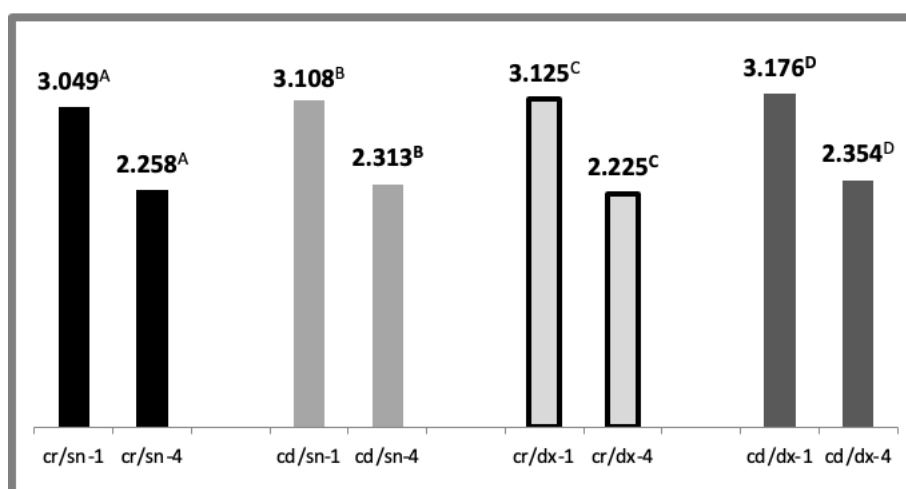


Figure 3 LSM estimates of the co-effect between lactation phase and teat position (Model-2) on teat diameter, cm (ultrasonographically). Legend: levels: cr – cranial teat; cd – caudal teat; sn – sinistral teat; dx – dextral teat; -1 – at first lactation month; -4 – at mid-lactation (fourth/fifth month); td: When superscript symbols are identical – $P \leq 0.001$.

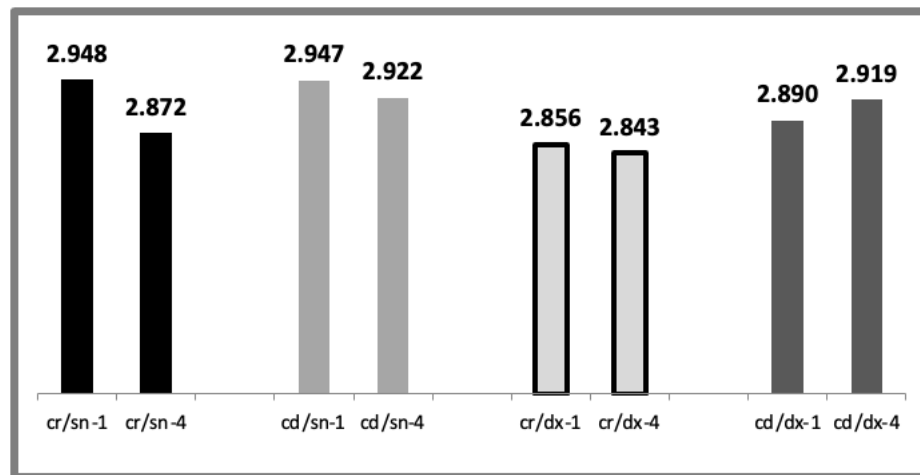


Figure 4 LSM estimates of the co-effect between lactation phase and teat position (Model-2) on teat canal length, cm (ultrasonographically). Legend: levels: cr – cranial teat; cd – caudal teat; sn – sinistral teat; dx – dextral teat; -1 – at first lactation month; -4 – at mid-lactation (fourth/fifth month); td: Differences of interest not significant ($P > 0.05$).

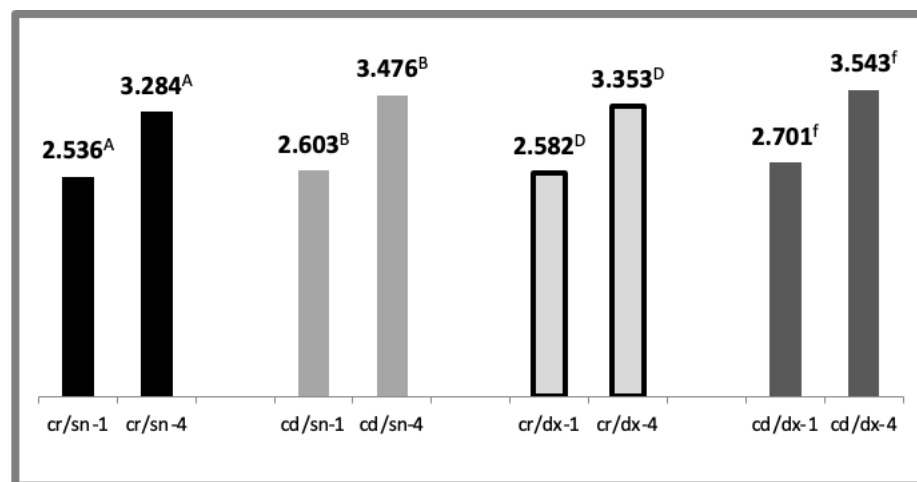


Figure 5 LSM estimates of the co-effect between lactation phase and teat position (Model-2) on teat cistern diameter, cm (ultrasonographically). Legend: levels: cr – cranial teat; cd – caudal teat; sn – sinistral teat; dx – dextral teat; -1 – at first lactation month; -4 – at mid-lactation (fourth/fifth month); td: When superscript symbols are identical, differences are significant as follows: for A, B, D – $P \leq 0.001$; for f – $P \leq 0.01$.

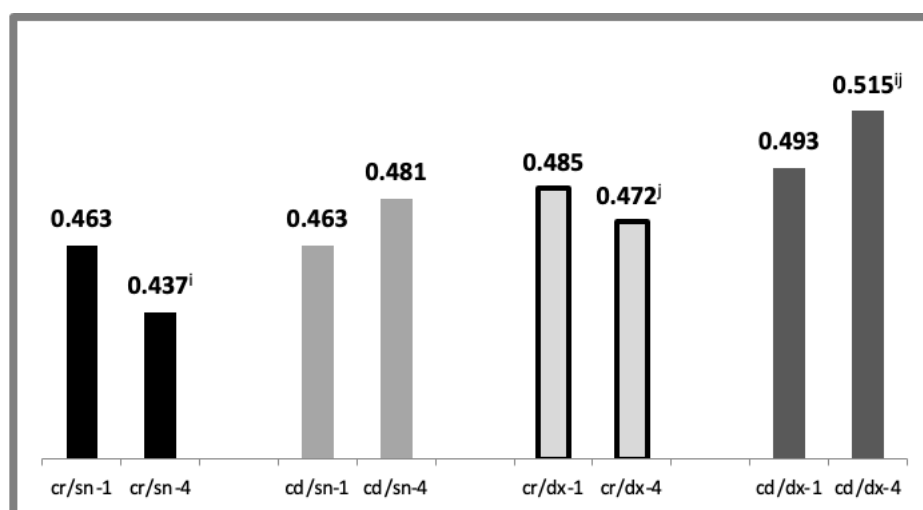


Figure 6 LSM estimates of the co-effect between lactation phase and teat position (Model-2) on teat wall thickness, cm (ultrasonographically). Legend: levels: cr – cranial teat; cd – caudal teat; sn – sinistral teat; dx – dextral teat; -1 – at first lactation month; -4 – at mid-lactation (fourth/fifth month); td: When superscript symbols are identical – $P \leq 0.05$.

According to Ambort *et al.* (2010), pre-stimulation of the udder before milking in the Italian Mediterranean buffaloes affords better visualization of the teat canal during ultrasound check, which is significantly shorter after stimulation than without stimulation (Costa *et al.*, 2020). In our study of buffaloes without pre-stimulation, all the images were detailed enough, as seen in Figure 1. Moreover, according to Boselli and Borghese (2024), the response to stimulation may differ from animal to animal, and among species, as well as, presumably, among breeds. There is large variation among breeds, with the TCL of Bulgarian Murrah and Azeri buffalo being twice as long as that of Nilli-Ravi (Boselli *et al.*, 2024). The same is true for the Murrah breed in India, where hind and fore teats had even longer canals (Thomas *et al.*, 2004), but more importantly, the authors found similarity in TCL within parities and within lactation phases. The differences in canal length among breeds suggest that milkability would be different too, calling for different milking approaches and udder care.

In the buffalo, the longer teat canal, compared to cattle, is associated with greater resistance of the muscle walls of the teat (Thomas *et al.*, 2004; Ambord *et al.*, 2009; Boselli and Borghese, 2024) and hence more difficult milk ejection, which necessitates higher vacuum pressure to open the teat canal. This, on the one hand, is a difficulty for machine milking but, on the other, determines a higher resistance to mastitis (Thomas *et al.*, 2004; Kaur *et al.*, 2018; Sannino *et al.*, 2018). Obviously, the Bulgarian Murrah, except for the other dairy traits, has inherited the relatively long teat canal from the Indian Murrah, not from the other breed involved in the creative cross-breeding process, like Nilli-Ravi, which is marked with a very short TCL (Boselli *et al.*, 2024).

At the same time, Bulgarian Murrah has a relatively high diameter of the cistern and low wall thickness (Boselli *et al.*, 2024). The increased TD and TW with increasing parity in dairy cows is explained as a response to repeated milking, namely to the mechanical stress (Klein *et al.*, 2005). According to Stauffer *et al.* (2021), this is due to the increase in size of the veins in the teat tissue as a result of machine milking, without any evidence of extravasation. In the buffalo, TD and TL also increase with parity – observed for Murrah in India (Bharti *et al.*, 2015) and other breeds (Boselli *et al.*, 2024), confirming the results in the present study.

In buffaloes, the fraction of cisternal milk in the udder, which is directly available, is only 5%, compared to 20% in cattle (Bertoni *et al.*, 2020). To be ejected, the alveolar fraction needs stimulation, expressed in higher levels of oxytocin (exogenous or endogenous), so that the myoepithelial cells contract.

Besides productivity, breeding and selection objectives should consider mammary gland morphology and susceptibility to mastitis in each particular breed. A study by El-Ghousien *et al.* (2004) demonstrates that the teat wall in buffaloes is twice as thick as that of Friesian cows, and also the teat cistern is larger, with several interceptions of large ducts with

elastic thin walls, thus forming numerous pockets instead of a single cavity cistern as in the dairy cows.

Like in dairy cows, the caudal teats are longer than the cranial teats and contain more milk, the ratio being 60:40 (Mota-Rojas *et al.*, 2024), explaining the higher teat diameter in response to the higher milking frequency. In the present study with the Bulgarian Murrah, this difference is much smaller.

The effect of teat position, expressed in different sizes of dextral and sinistral teats, can be attributed to the milking methods used. In dairy cattle, some authors suggested that the differences found between left and right teats may be affected by the position of the milking unit (right or left approach of the milking person), which can cause different pressure on one side (Boselli *et al.*, 2024). In our case, the milker approaches the buffalo cow from the left hind side, closest to the caudal sinistral teat, so that the milking cup of the device applies the best vacuum.

This study established that only two of the ultrasound teat measurements are significantly affected by lactation phase. While the diameter of all four teats decreases from the first to the fourth month ($P \leq 0.001$), their cistern diameter increases ($P \leq 0.001$). As an essential functional trait, the practically unchangeable teat canal length during lactation justifies the also constant parameters of the milking system associated with milkability and udder prophylactics. Teat position is a significant source of variation in two other teat traits – wall thickness ($P \leq 0.01$) and teat length ($P \leq 0.001$). Though not statistically proven, the wall thickness of the cranial teats decreases with the advance of lactation, and that of the caudal teats increases, which renders caudal teats' walls thicker at mid-lactation, well expressed and significant for the dextral teat only. The posterior teats in buffaloes are more productive, and milking operators keep them plugged to the system for a longer time, in this way applying more vacuum, which is well expressed in a direct correlation with teat wall thickness (Ambord *et al.*, 2009). In practice, the anterior teats are less and less milked, while the posterior teats are responsible for the increasing milk yield up to the third month postpartum.

Teat length changes in the same way up to the fourth/fifth lactation month, also non-significantly, resulting in greater length of the caudal teats; the significantly longest is the caudal sinistral teat, which is to be attributed to the approach of the milking operator and the application of the cups of the milking device. Parity is a significant source of variability of all studied functional traits, best expressed for teat length and canal length ($P \leq 0.001$). The explanation for this is due to the repeated impacts on the teats as a result of machine milking, reflected in a change in their morphological characteristics, which has also been proven by other authors (Bharti *et al.*, 2015; Atigui *et al.*, 2024; Boselli *et al.*, 2024).

In conclusion, the analysis of the obtained results indicated that changes in individual teat parameters could be easily demonstrated by ultrasonographic measurements. All teat measurements (cranial and caudal) had significantly greater values in the

pluriparous buffaloes compared to the primiparous, except for the smaller cistern diameter, where the differences were not proven. The present study showed that TCL practically does not change during most productive lactation, despite the period of intensive machine milking, unlike most other teat measurements. Being strongly related to healthcare and milkability, this justifies the practice of not changing the milking system parameters and udder prophylactics throughout lactation periods.

Acknowledgments

The research leading to these results has received funding from the Ministry of Education and Science of the Republic of Bulgaria under the Project № BG-RRP-2.004-0006-C02 “Development of research and innovation at Trakia University in service of health and sustainable well-being”.

Conflicts of interest: The authors declare no conflicts of interest.

Statement of author contributions: **Ivan Fasulkov:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing, Funding acquisition. **Pencho Penchev:** Data curation, Investigation, Methodology, Software, Resources, Writing – original draft, Writing – review and editing. **Yordanka Ilieva:** Data curation, Formal analysis, Investigation, Methodology, Software, Resources, Writing – original draft, Writing – review and editing. **Manol Karadaev:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review and editing. All authors have read and agreed to the published version of the manuscript.

Declaration of artificial intelligence use: During the preparation of this manuscript, the authors did not use any AI sources.

References

- Abdel-Hamid M, Huang L, Huang Z, Romeih E, Yang P, Zeng Q and Li L 2023. Effect of buffalo breed on the detailed milk composition in Guangxi, China. *Foods* 12: 1603.
- Ambord S, Stoffel MH and Bruckmaier RM 2010. Teat anatomy affects requirements for udder preparation in Mediterranean buffaloes. *J Dairy Res.* 77: 468–473.
- Ambord S, Thomas CS, Borghese A, Mazzi M, Boselli C and Bruckmaier RM 2009. Teat anatomy, vacuum to open the teat canal, and fractionized milk composition in Italian buffaloes. *Milchwissenschaft* 64: 351–353.
- Bertoni A, Napolitano F, Mota-Rojas D, Sabia E, Álvarez-Macías A, Mora-Medina P, Morales-Canela A, Berdugo-Gutiérrez J and Guerrero-Legarreta I 2020. Similarities and differences between river buffaloes and cattle: Health, physiological, behavioral and productivity aspects. *J Buffalo Sci.* 9: 92–109.
- Bharti P, Tomar AKS, Verma MR, Bharti PK and Singh BP 2015. Effect of lactation order on morphological traits of teat and udder in Murrah buffaloes. *J Anim Res.* 5: 561–565.
- Boselli C and Borghese A 2024. Different types of milk flow curves and factors affecting milkability in buffalo species. *J Buffalo Sci.* 13: 125–132.
- Boselli C, Campagna MC, Amatiste S, Rosati R and Borghese A 2014. Pre-stimulation effects on teat anatomy and milk flow curves in Mediterranean Italian buffalo cows. *J Anim Vet Adv.* 13: 912–916.
- Boselli C, Costa A, De Marchi M, Zia MA, Shahid MQ, Ahmad N, Fasulkov I, Karadaev M, Ilieva Y, Penchev P, Derisavi F, Nikookar M, Naderfard H, Eid LN, Hegazy MM, Abouelghait HA, Campagna MC and Borghese A 2024. Teat morphology across five buffalo breeds: A multi country collaborative study. *Trop Anim Health Prod.* 56: 61.
- Boselli C, De Marchi M, Costa A and Borghese A 2020. Study of milkability and its relation with milk yield and somatic cell in Mediterranean Italian water buffalo. *Front Vet Sci.* 7: 432.
- Caria M, Boselli C, Murgia L, Rosati R and Pazzona A 2012. Effect of vacuum level on milk flow traits in Mediterranean Italian buffalo cow. *Ital J Anim Sci.* 11: e25.
- Costa A, De Marchi M, Visentin G, Campagna MC, Borghese A and Boselli C 2020. The effect of pre-milking stimulation on teat morphological parameters and milk traits in the Italian water buffalo. *Front Vet Sci.* 7: 572422.
- El-Ghousien SS, Ashour G, Shafie MM and Badreldin AL 2004. Comparative study of the udder structure in buffaloes and cattle: 1- morphological and anatomical characteristics of the mammary gland. *Egyptian J Anim Prod.* 41: 321–333.
- Fasulkov I, Vasilev N, Karadaev M and Dineva G 2014. Visualization and measurement of teat structures in Black-and-White cows trough ultrasonography. *Mac Vet Rev.* 37(1): 89–93.
- Garau V, Manis C, Scano P and Caboni P 2021. Compositional characteristics of Mediterranean buffalo milk and whey. *Dairy* 2: 469–488.
- Harvey WR 1990. User's Guide for LSMLMW and MIXMDL PC-2 Version. Mixed Model Least Squares and Maximum Likelihood Computer Program. Columbus, OH: Ohio State University.
- Jiang M, Meng Z, Cheng Z, Zhan K, Ma X, Yang T, Huang Y, Yan Q, Gong X and Zhao G 2022. Effects of buffalo milk and cow milk on lipid metabolism in obese mice induced by high fat. *Front Nutr.* 9: 841800.
- Kaur G, Bansal BK, Singh RS, Kashyap N and Sharma S 2018. Associations of teat morphometric parameters and subclinical mastitis in riverine buffaloes. *J Dairy Res.* 85: 303–308.
- Khan TI, Nadeem M, Imran M, Asif M, Khan KM, Din A and Ullah R 2019. Triglyceride, fatty acid profile and antioxidant characteristics of low melting point fractions of buffalo milk fat. *Lipids Health Dis.* 18: 1–11.

- Klein D, Flöck M, Khol JL, Franz S, Stüger HP and Baumgartner W 2005. Ultrasonographic measurement of the bovine teat: Breed differences, and the significance of the measurements for udder health. *J Dairy Res.* 72: 296–302.
- Liao J, Yang J, Suo H and Song J 2025. Buffalo milk: Nutritional composition, bioactive properties, and advances in processing technologies-a comprehensive review. *Food Chem.* 29: 199–213.
- Mirza RH, Waheed A, Khan MA, Faraz A, Ishaq HM, Dilshad SMR and Javed K 2019. Genetic and nongenetic factors affecting some udder measurements in Nili Ravi buffaloes of Pakistan. *Buffalo Bull.* 38: 551–560.
- Mota-Rojas D, Napolitano F, Chay-Canul A, Ghezzi M, Braghieri A, Domínguez-Oliva A, Bragaglio A, Álvarez-Macías A, Olmos-Hernández A, De Rosa G, García-Herrera R, Lendez P, Pacelli C, Bertoni A and Barile VL 2024. Anatomy and physiology of water buffalo mammary glands: An anatomofunctional comparison with dairy cattle. *Animals* 14: 1066.
- Ozenc E, Bozkurt MF, Yazici E, Seker E, Bayraktaroglu AG, Ozcinar U and Dogan N 2020. Teat characteristics in relation to animal temperament during milking in buffaloes, and comparison of buffalo and cow teat morphology. *Reprod Domest Anim.* 55: 559–566.
- Penchev P, Boichev M, Ilieva Y and Peeva Tz 2011. Effect of different factors on lactation curve in buffalo cows. *Slovak J Anim Sci.* 44: 103–110.
- Penchev P, Ilieva Y, Ivanova T and Kaley R 2016. Fatty acid composition of buffalo and bovine milk as affected by roughage source – silage versus hay. *Emir J Food Agric.* 28: 264–270.
- Pierro F, Pascarella L, Santinello M, Matera R, Lettieri S, Biffani S and Neglia G 2025. Characterisation of milk ejection dynamics in dairy buffaloes based on Lactocorder® measurements. *Ital J Anim Sci.* 24: 2169–2180.
- Sannino M, Faugno S, Crimaldi M, Di Francia A, Ardito L, Serrapica F and Masucci F 2018. Effects of an automatic milking system on milk yield and quality of Mediterranean buffaloes. *J Dairy Sci.* 101: 8308–8312.
- Stauffer C, Van der Vekens E, Stoffel MH, Schweizer D and Bruckmaier RM 2021. Increased teat wall thickness in response to machine milking. *J Dairy Sci.* 104: 9082–9092.
- Thomas CS, Svennersten-Sjaunja K, Bhosrekar MR and Bruckmaier RM 2004. Mammary cisternal size, cisternal milk and milk ejection in Murrah buffaloes. *J Dairy Res.* 71: 162–168.
- Vargas-Ramella M, Pateiro M, Maggiolino A, Faccia M, Franco D, De Palo P and Lorenzo JM 2021. Buffalo milk as a source of probiotic functional products. *Microorganisms* 9: 2303.
- Zhou L, Tang Q, Wasim Iqbal M, Xia Z, Huang F, Li L, Liang M, Lin B, Qin G and Zou C 2018. A comparison of milk protein, fat, lactose, total solids and amino acid profiles of three different buffalo breeds in Guangxi, China. *Ital J Anim Sci.* 17: 873–878.