



Physiological and age-related neck morphometric variability in Holstein dairy cows: A pilot study

M. Lamanna^{a,*}, R. Colletuori^a, M. Canova^a, A. Grandis^a, M. Bovo^b, F. Petretto^c, A. Formigoni^a, D. Cavallini^a

^a Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, BO, Italy

^b Department of Agricultural and Food Sciences, University of Bologna, Bologna, BO, Italy

^c Freelance Engineer, Sassari, SS, Italy

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ABSTRACT

The neck region of dairy cows remains poorly characterised despite its relevance for conformation assessment, phenotyping and precision livestock farming (PLF) applications. This pilot study aimed to describe regional cervical morphometric variability in Holstein dairy cows and to evaluate associations with lactation stage and chronological age. Thirty-five Italian Holstein cows (heifers, lactating and dry) were measured twice under commercial-like conditions ($n = 70$ observations). Minimum, mid and maximum neck girths, thoracic girth and body condition score (BCS) were recorded using a standardised manual protocol. Third-degree polynomial regression models and Pearson correlations were used to assess relationships with days in milk (DIM) and age. Minimum and mid-neck girths showed significant non-linear associations with DIM ($R^2 = 0.322$ and 0.382 ; $P < 0.01$), characterised by early-lactation reductions and late-lactation increases. Maximum neck girth showed a weak and non-significant association with DIM ($R^2 = 0.197$; $P = 0.068$) but was strongly age-dependent ($R^2 = 0.407$; $P < 0.0001$). Thoracic girth was primarily related to age ($R^2 = 0.701$; $P < 0.0001$) and moderately associated with DIM ($R^2 = 0.242$; $P = 0.029$). Cervical measures showed only weak correlations with body weight. This study provides a preliminary anatomically differentiated description of cervical morphometry in dairy cows, highlighting region-specific morphometric variation associated with physiological stage and age and establishing an initial reference framework for future studies on bovine morphometric phenotyping and PLF applications.

1. Introduction

Morphometry is a central aspect in the study of dairy cow conformation, as it provides valuable information for evaluating growth, health, functionality, and productivity (Sabbadin et al., 2025). Parameters such as withers height, trunk length, pelvic width, and especially thoracic girth are widely reported in the literature and commonly used in both scientific and practical contexts (König and Liebich, 2020; Singh, 2017). These measurements allow estimation of body weight, assessment of skeletal development, and identification of morphological anomalies (Costa et al., 2021).

However, despite the overall importance of body measurements in cattle, one anatomical region remains surprisingly neglected in scientific research: the neck. In dairy cows, the neck represents a complex anatomical region comprising skin, muscles, connective tissues,

subcutaneous fat, cervical vertebrae, neurovascular structures, and visceral organs, including the trachea and oesophagus, and shows marked posture-dependent morphological variations (König and Liebich, 2020; Singh, 2017). It is involved in numerous biomechanical functions, including head support, feeding movements, social interactions, and thermoregulation (Sjaastad et al., 2016). Nevertheless, none of the standard morphometric protocols commonly used in veterinary science or animal production systematically include measurements of cervical circumference, and no consolidated reference values exist for this region (Putra and Mekonnen, 2021).

This gap is particularly relevant in the current context of dairy farming, where the increasing introduction of wearable technologies and sensor-based behavioural monitoring systems has led to widespread use of smart collars (Lamanna et al., 2026a). For these devices to be effective and safe, they must be able to fit properly to the anatomical

* Corresponding author at: Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna, Ozzano dell'Emilia, 40064 Bologna, Italy.
E-mail address: martina.lamanna5@unibo.it (M. Lamanna).

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region on which they are applied. In the absence of specific morphometric data, designing ergonomic and reliable devices becomes more complex and more prone to error (Lamanna et al., 2026b). In addition, variations in neck morphology may reflect physiological aspects such as BCS, regional fat deposition, or changes linked to the productive stage, potentially offering an additional parameter of interest for future studies (Costa et al., 2021; König and Liebich, 2020; Singh, 2017).

Beyond technological applications, investigating neck morphology may contribute to a better understanding of individual differences among cows and phenotypic variability within the Holstein breed. It has been hypothesised that cervical morphology could also reflect physiological processes, including changes in body condition, muscle development, or energy balance, although these potential relationships have not yet been investigated and were not assessed in the present study (Klein, 2019; König and Liebich, 2020; Magro et al., 2024; Sjaastad et al., 2016).

In light of this knowledge gap, the present pilot study aimed to provide a first characterisation of neck morphometry in Holstein dairy cows kept under commercial-like management conditions. Three distinct cervical circumference measurements (minimum, medium, and maximum) were recorded using a standardised manual protocol to describe regional cervical morphometric variability. Alongside cervical measurements, thoracic girth and BCS were also recorded to explore potential relationships between neck morphology and other body parameters representative of the animal's physical condition. In addition, milk yield and milk fat percentage were evaluated to characterise the physiological status of the study population across lactation and to provide a biological context for interpreting the observed morphometric variation.

2. Materials and methods

2.1. Ethical compliance

This research did not involve any invasive procedures, experimental treatments, or modifications to the usual handling routines of dairy cows. All measurements were collected during normal herd management activities, without altering feeding, housing, or milking protocols. In accordance with European legislation, all procedures adhered to Directive 2010/63/EU on the protection of animals used for scientific purposes and Directive 98/58/EC concerning the welfare of animals kept for farming purposes. Because the study was purely observational and integrated into routine management, no additional ethical approval was required under national regulations.

2.2. Study design and study site

The study was designed as a descriptive observational investigation aimed at characterizing cervical and thoracic morphometry in dairy cows under commercial-like conditions.

All observations were conducted at the Teaching and Experimental Dairy Barn of the Department of Veterinary Medical Sciences (University of Bologna), located in Ozzano dell'Emilia (BO), Italy. This facility functions as both a research unit and a teaching environment for veterinary and animal science students. Built in 2003, it hosts structured activities ranging from reproductive management to PLF trials.

The barn houses approximately 90 lactating cows and 80 dry cows or replacement heifers at any given time. All animals are purebred Italian Holstein, officially registered in the national herd books. The herd is managed in a free-stall system with deep-bedded stalls, central feed alleys, and well-defined functional zones including: feeding area along both sides of the main alley, resting area equipped with standardised cubicles, outdoor exercise yard accessible to selected groups, robotic milking area housing two Merlin2 milking robots (Fullwood®, UK) installed in 2021.

Milking, feeding, reproduction, and health protocols follow standard farm operating procedures and were not modified for study purposes.

This ensured that all collected measurements reflect realistic physiological and behavioural conditions typical of commercial dairy operations (Lamanna et al., 2025).

2.3. Measurement schedule

Overall, 35 individual Italian Holstein dairy cows were included in the study. All animals were measured on two different periods, resulting in a total of 70 measurements. Data collection was carried out during two standardised measurement periods in spring 2025, approximately one month apart, under comparable management and physiological conditions. The repeated measurements were intended to increase the representation of different physiological states while maintaining consistency in housing, feeding and handling conditions across sampling events.

The time elapsed between consecutive measurements was calculated as the exact number of days between sampling sessions and ranged from 28 to 51 days, with a mean interval of 33.0 ± 6.3 days and a median of 31 days. This interval is consistent with the assessment of short-term physiological variation, such as changes in body condition, stage of lactation, or gestation, while limiting the influence of long-term structural or age-related changes.

Although each cow was measured twice, the statistical analyses treated each measurement as an independent observational unit; therefore, the study was not designed to assess within-animal changes over time.

2.4. Animal-level variables

A comprehensive set of animal-level variables was recorded or derived for each measurement event to provide biological and productive context for the morphometric data.

2.4.1. Identification and demographics

Each cow was identified using its numeric farm code. Date of birth was recorded and used to calculate age at the time of measurement. Age averaged 3.47 ± 1.83 years, from 1.3 to 6.5 years.

2.4.2. Reproductive status

Parity number ranged from 0 (heifers) to ≥ 4 , with a mean of 1.90 ± 1.73 parities. When insemination data were available, days of pregnancy were calculated as the difference between measurement date and insemination date. Among records for which pregnancy could be determined, cows averaged 132.5 ± 95.2 days of gestation, illustrating wide variability in reproductive stage. When insemination data were unavailable or unreliable, pregnancy days were recorded as "ND".

2.4.3. Lactation stage

For lactating cows, DIM were recorded and ranged from 0 days (fresh cows) to 336 days (late lactation), with a herd-level mean of 73.8 ± 95.9 days at the time of measurement.

2.5. Morphometric and body condition measurements

2.5.1. Morphometric variables

Four morphometric variables were recorded at each measurement event:

- Minimum neck circumference (cm)
- Mid-neck circumference (cm)
- Maximum neck circumference (cm)
- Thoracic circumference (cm)

2.5.2. Body condition score

Body Condition Score (BCS) was assessed using the 1–5 scale with 0.25 increments, based on visual inspection and palpation of the lumbar

region, pelvis, and tail head. All evaluations were performed by experienced veterinarians familiar with the herd and standard dairy cattle scoring procedures.

2.6. Additional productive and behavioural variables

For lactating cows, additional productive and behavioural variables were retrieved from the farm's herd management software and automated milking systems when available. These included:

- Milk yield,
- Milk composition parameters (fat, protein, lactose, fat/protein ratio, electrical conductivity),
- Milking duration,
- Rumination time and weekly rumination mean.

These variables were linked to each measurement by animal identification number and date and were treated as contextual information.

2.7. Personnel and standardisation of procedures

All measurements were performed by the same team of four trained operators, each with prior experience handling dairy cows in the study herd. The four operators worked together throughout the data collection period, measuring all study animals according to a standardised protocol. Before data collection, they participated in a calibration session to ensure consistent application of the measurement protocol. During data collection, whenever uncertainty arose regarding a measurement, the operators discussed the assessment and reached a consensus before

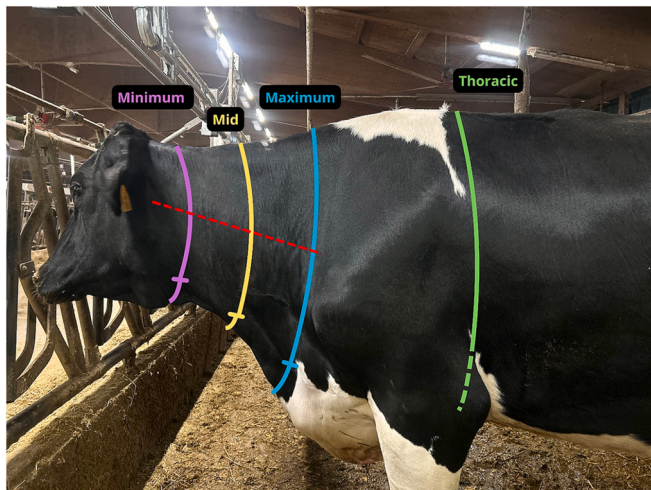


Fig. 1. Schematic representation of cervical and thoracic circumference measurements in a Holstein dairy cow. The red dashed line represents the longitudinal axis of the neck used as the reference line for identifying measurement sites. The minimum neck girth (violet) was measured at the narrowest point near the head-neck junction; the mid-neck girth (yellow) was measured at the midpoint between the minimum and maximum locations identified along this axis; and the maximum neck girth (blue) was measured at the most caudal portion of the neck corresponding to maximal muscular development. The thoracic girth (green) was measured immediately caudal to the caudal angle of the scapula at the point of maximal thoracic expansion. Dashed segments indicate portions of the measuring cord passing posterior to the olecranon, where direct visualisation was not possible. Short transverse marks indicate points where the cord was gently lifted to ensure close adherence to the dewlap (i.e., the loose skin and subcutaneous tissue of the ventral neck) without compressing underlying structures. All measurements were performed with the cow standing in a natural, relaxed posture, without forced neck extension, flexion, or rotation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recording the final value. The calibration session included the standardisation of:

- Identification of anatomical landmarks,
- Acceptable neck posture during measurement,
- Cord placement and tension,
- Criteria for repeating measurements in case of animal movement.

2.8. Measuring instruments

Cervical measurements were performed using:

1. A non-elastic, PVC-coated steel cable (4.5 mm diameter), used to closely follow the anatomical contour of the neck without stretching;
2. A measuring tape, used to measure cord length after removal from the animal, reducing distortion compared with flexible tapes.

2.9. Cervical and thoracic measurement protocols

2.9.1. Cervical circumference

Three cervical circumferences were recorded sequentially along the longitudinal axis of the neck, perpendicular to its main axis, at the anatomical sites described below:

1. Minimum circumference, measured at the narrowest point near the head-neck junction, immediately caudal to the angle of the mandible (*angulus mandibulae*), near the atlanto-occipital articulation;
2. This midpoint was identified along the reference line traced on the neck surface following the longitudinal axis of the neck between the two reference points, and the circumference was then recorded at this intermediate location;
3. Maximum neck circumference, measured at the most caudal portion of the neck, immediately cranial to the cranial margin of the scapula, corresponding to the transition between the cervical region and the shoulder girdle.

All measurements were performed during routine herd management while cows were restrained in the self-locking feed fence at the feed bunk, without any additional restraint specifically for the purposes of the study. Measurements were taken only when cows naturally maintained a relaxed head and neck position, without forced extension, flexion, or rotation (Fig. 1). Measurements affected by movement or postural changes were repeated.

2.9.2. Thoracic circumference

Thoracic girth was measured immediately caudal to the caudal angle of the scapula, at the point of maximal thoracic expansion, keeping the tape level and flush with the body surface without compressive pressure (Fig. 1).

2.10. Data management and statistical analysis

After data collection and quality control, all measurements were compiled into a single Microsoft Excel spreadsheet, in which each row represented one measurement event (unique animal identification number $\times$ measurement date). Variables were checked for internal consistency, completeness, and biological plausibility before being imported into JMP Pro (version 19) for statistical analyses and graphical visualisation. No data transformation or imputation was performed.

Each measurement event was treated as an independent observational unit. Although each cow was measured twice, the dataset was intended to increase the representation of different physiological states rather than to evaluate within-animal temporal changes. Therefore, no formal longitudinal or mixed-effects modelling was applied.

Descriptive statistics (mean, standard deviation, minimum, and maximum) were calculated for all animal-level, productive, and

Table 1

Descriptive statistics of morphometric, physiological, productive, and behavioural variables stratified by production group (dry cows, heifers, and lactating cows). Values are reported as mean $\pm$ standard deviation, with the 25th and 75th percentiles. Missing values indicate variables not available for the corresponding group.

Parameter		Group		
		Dry	Heifers	Lact
Minimum girth, cm	Mean	94.00	82.35	88.40
	Std Dev	5.00	5.61	4.94
	Quantiles25	91.00	78.50	85.00
	Quantiles75	97.00	87.50	92.00
Medium girth, cm	Mean	101.33	91.25	97.06
	Std Dev	5.96	4.30	5.68
	Quantiles25	98.25	90.25	93.00
	Quantiles75	104.00	94.00	100.00
Maximum girth, cm	Mean	160.29	123.00	145.18
	Std Dev	15.32	12.88	15.70
	Quantiles25	146.00	110.50	133.25
	Quantiles75	173.00	134.50	154.75
Thoracic girth, cm	Mean	218.14	191.47	216.73
	Std Dev	11.39	7.95	11.55
	Quantiles25	206.00	184.50	210.70
	Quantiles75	227.00	198.00	224.00
BCS	Mean	3.14	3.79	3.16
	Std Dev	0.20	0.44	0.39
	Quantiles25	3.00	3.38	2.75
	Quantiles75	3.25	4.25	3.50
Weight, daN	Mean	685.33		720.27
	Std Dev	54.12		67.42
	Quantiles25	626.00		692.25
	Quantiles75	732.00		754.50
MY, kg	Mean			40.12
	Std Dev			14.04
	Quantiles25			28.30
	Quantiles75			49.80
Fat, %	Mean			3.98
	Std Dev			0.46
	Quantiles25			3.60
	Quantiles75			4.41
Protein, %	Mean			3.46
	Std Dev			0.14
	Quantiles25			3.36
	Quantiles75			3.54
Lactose	Mean			4.80
	Std Dev			0.18
	Quantiles25			4.75
	Quantiles75			4.91
Weekly rumination average	Mean	537.14	517.41	547.83
	Std Dev	62.86	44.54	69.05
	Quantiles25	508.00	485.50	523.00
	Quantiles75	588.00	554.00	592.25

morphometric variables. Associations between morphometric traits (minimum, medium, and maximum neck girths; thoracic girth) and explanatory variables were initially explored using bivariate scatterplots. Third-degree polynomial regression models were fitted using DIM or chronological age as continuous predictors to account for non-linear relationships. Following the default JMP procedure, predictor variables were mean-centred before polynomial expansion to reduce multicollinearity and improve numerical stability.

Model performance was evaluated using the coefficient of determination (R^2), root mean square error (RMSE), and overall model significance ($\text{Prob} > F$). Fitted curves with confidence intervals were superimposed on scatterplots to facilitate visual interpretation.

Relationships among morphometric traits were examined using a correlation matrix estimated by restricted maximum likelihood (REML), accounting for repeated measurements within animals. Pearson correlation coefficients (r) were derived from the estimated covariance structure, and pairwise relationships were further visualised using a scatterplot matrix.

All statistical tests were two-sided, with statistical significance set at $P < 0.05$.

3. Results

The final dataset comprised 70 measurements collected from 35 individual Holstein cows, with each observation corresponding to a unique combination of animal identification number and measurement date.

Table 1 summarises the descriptive statistics of morphometric, physiological, productive, and behavioural variables according to production group. Overall, dry cows exhibited the greatest neck and thoracic girths, heifers the smallest, and lactating cows intermediate values. Maximum neck girth showed the greatest absolute values and variability among the cervical measurements. Thoracic girth followed a similar distribution, being greater in dry and lactating cows than in heifers. Conversely, heifers presented the highest BCS, whereas dry and lactating cows showed comparable values. Body weight was available only for dry and lactating cows, while milk production, milk composition, and rumination variables were recorded exclusively for lactating animals (Table 1).

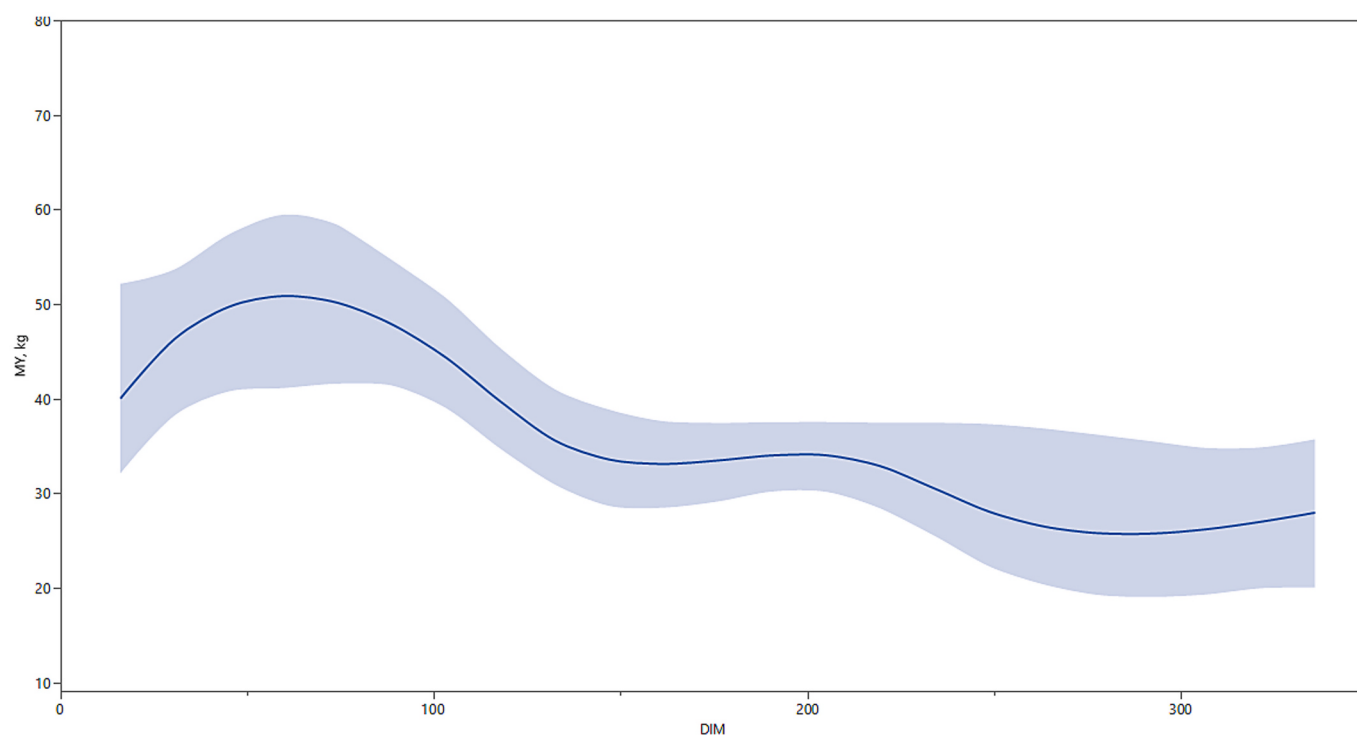


Fig. 2. Smoothed relationship between milk yield (MY, kg) and Days in Milk (DIM), with confidence band.

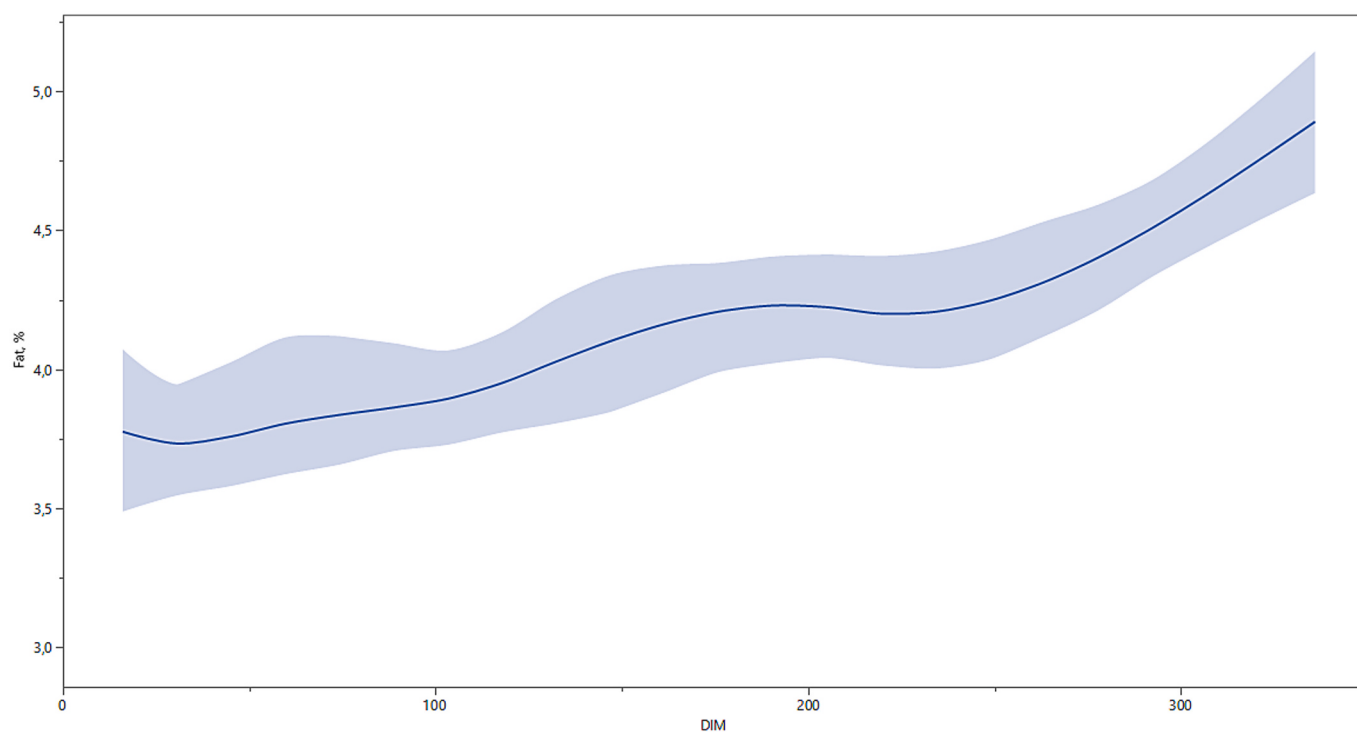


Fig. 3. Smoothed relationship between milk fat percentage (%) and Days in Milk (DIM), with confidence band.

3.1. Physiological and morphometric variables across DIM

Relationships between DIM and productive, morphometric, and body condition variables were explored using third-degree polynomial regression models and smoothed regression curves. Overall, several variables displayed non-linear trajectories consistent with physiological changes occurring throughout lactation.

3.1.1. Lactation curve as physiological context for morphometric measurements

Milk yield followed the expected lactation curve (Fig. 2), increasing rapidly during early lactation to peak values at approximately 40–70 DIM before progressively declining towards late lactation. Variability was greater during early and late lactation, as indicated by the wider confidence bands.

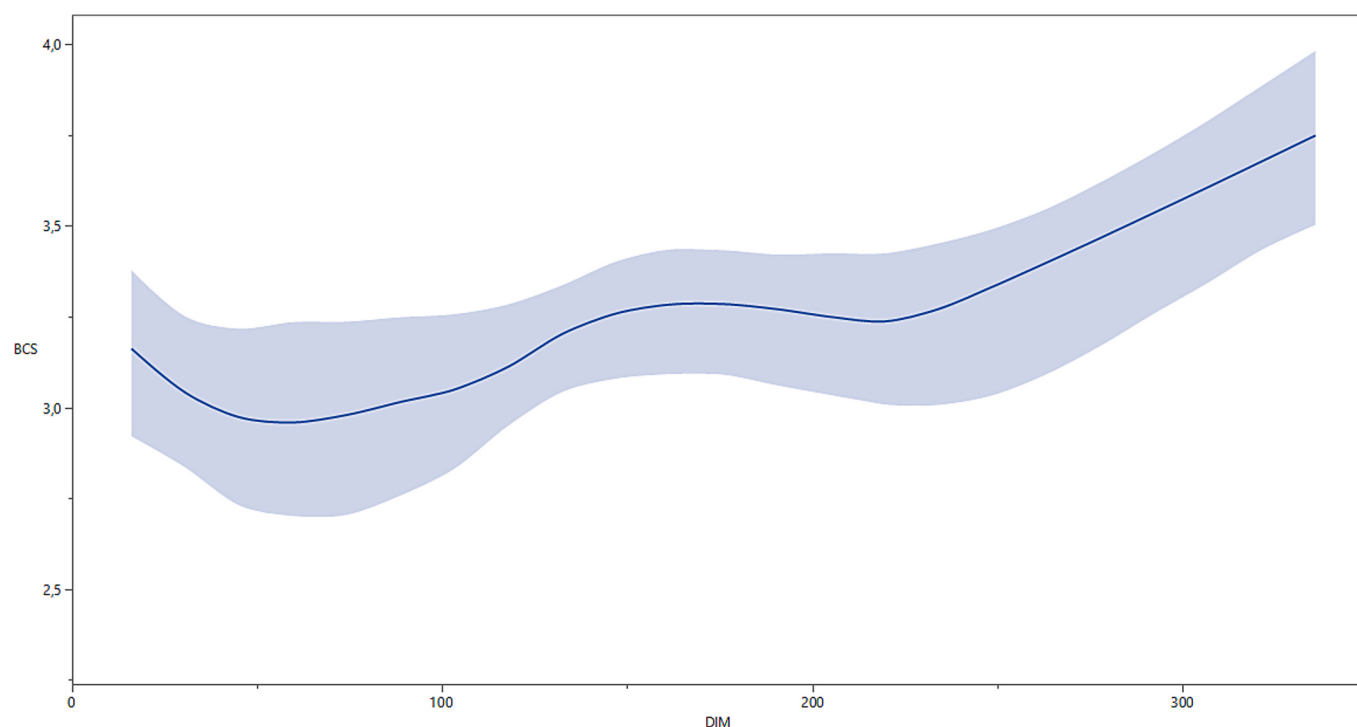


Fig. 4. Smoothed relationship between Body Condition Score (BCS) and Days in Milk (DIM), with confidence band.

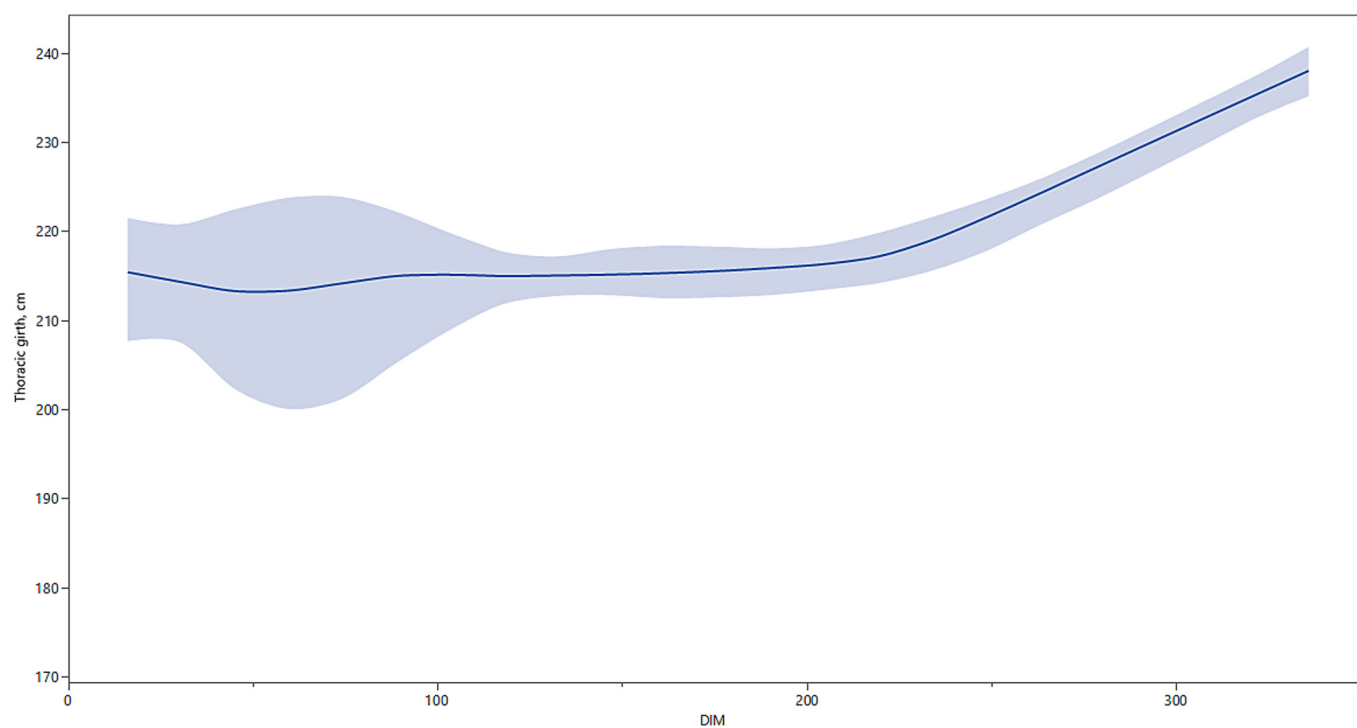


Fig. 5. Smoothed relationship between thoracic girth (cm) and Days in Milk (DIM), with confidence band.

3.1.2. Milk fat profile as physiological context for morphometric measurements

Milk fat percentage increased progressively throughout lactation (Fig. 3). After a slight reduction during early lactation, fat concentration increased gradually during mid-lactation and more markedly towards the end of lactation. Confidence bands indicated greater variability at the beginning and end of the lactation period.

3.1.3. Body condition score in relation to DIM

BCS exhibited the expected physiological pattern across lactation (Fig. 4), decreasing during early lactation before progressively recovering throughout mid- and late lactation. Variability was greater during the early and late stages of lactation than during mid-lactation.

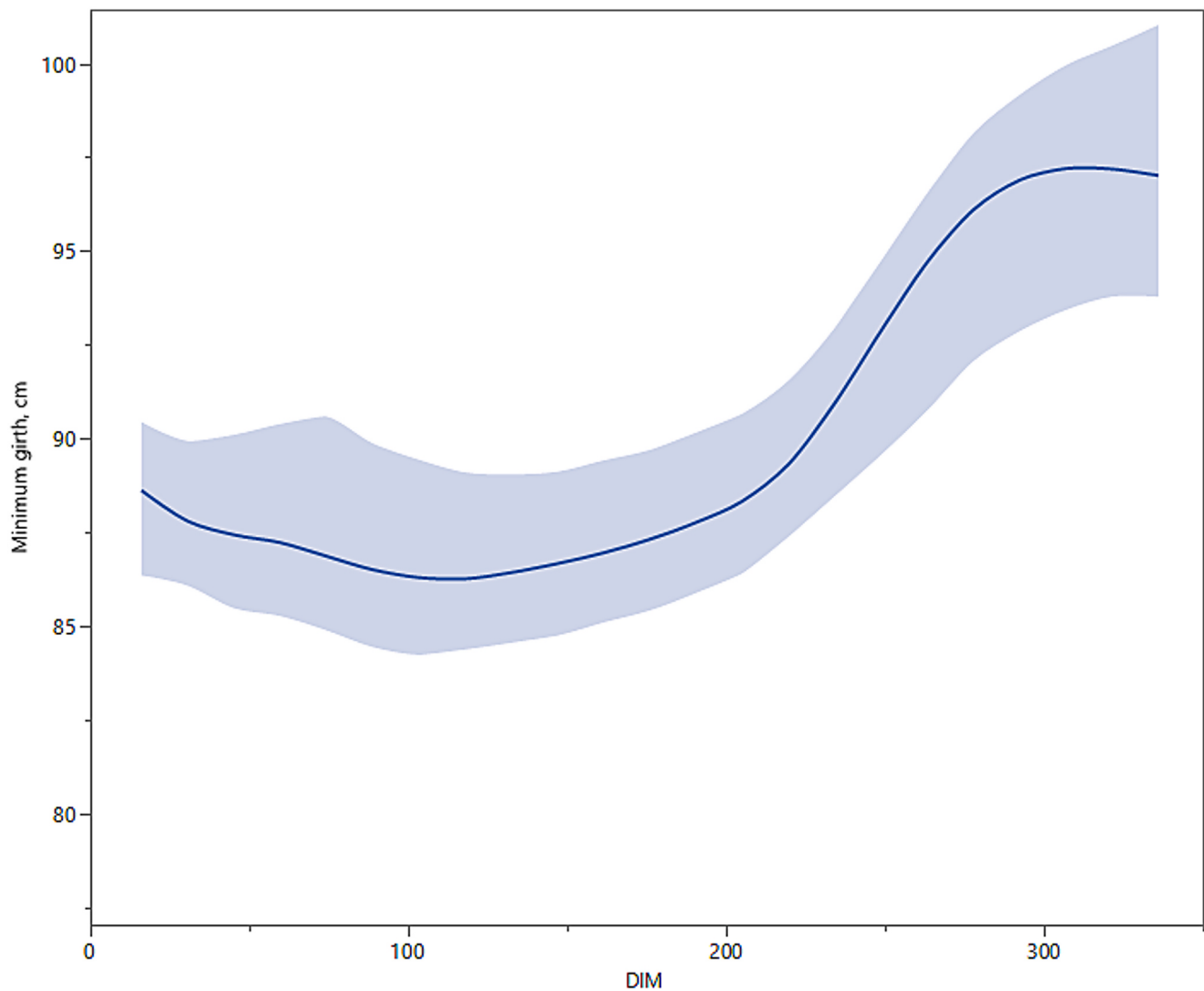


Fig. 6. Smoothed relationship between minimum neck girth (cm) and Days in Milk (DIM), with confidence band.

3.1.4. Thoracic girth in relation to DIM

Thoracic girth showed a modest non-linear association with DIM (Fig. 5). Values remained relatively stable during early and mid-lactation before increasing towards late lactation. The polynomial model explained 24.2% of the observed variability ($R^2 = 0.2425$; RMSE = 10.52 cm) and was statistically significant ($P = 0.0290$).

The fitted equation was:

$$\begin{aligned} \text{thoracic girth (cm)} = & 214.06758 + 0.0027113 \cdot \text{DIM} \\ & + 0.000181 \cdot (\text{DIM} - 116.778)^2 \\ & + 1.3794 \times 10^{-6} \cdot (\text{DIM} - 116.778)^3 \end{aligned}$$

3.1.5. Minimum neck girth in relation to DIM

Minimum neck girth showed a significant non-linear relationship with DIM (Fig. 6). Values decreased during early lactation, reached their minimum at approximately 90–120 DIM, and subsequently increased progressively towards late lactation. The polynomial model explained 32.2% of the observed variability ($R^2 = 0.322$; RMSE = 4.26 cm; $P = 0.0066$).

The fitted equation was:

$$\begin{aligned} \text{minimum girth (cm)} = & 84.592625 + 0.0128239 \cdot \text{DIM} \\ & + 0.0003166 \cdot (\text{DIM} - 119.543)^2 - 6.2902 \times 10^{-7} \\ & \cdot (\text{DIM} - 119.543)^3 \end{aligned}$$

3.1.6. Medium neck girth in relation to DIM

Medium neck girth displayed a similar but more pronounced non-linear trajectory (Fig. 7), declining during early lactation before increasing steadily throughout the remainder of lactation. The polynomial model explained 38.2% of the observed variability ($R^2 = 0.382$; RMSE = 4.68 cm) and was statistically significant ($P = 0.0017$).

The fitted equation was:

$$\begin{aligned} \text{medium girth (cm)} = & 91.497794 + 0.0216293 \cdot \text{DIM} + 0.0004361 \\ & \cdot (\text{DIM} - 116.686)^2 - 1.1517 \times 10^{-6} \\ & \cdot (\text{DIM} - 116.686)^3 \end{aligned}$$

3.1.7. Maximum neck girth in relation to DIM

Maximum neck girth showed only a weak association with DIM (Fig. 8). Although the fitted curve suggested a gradual increase towards

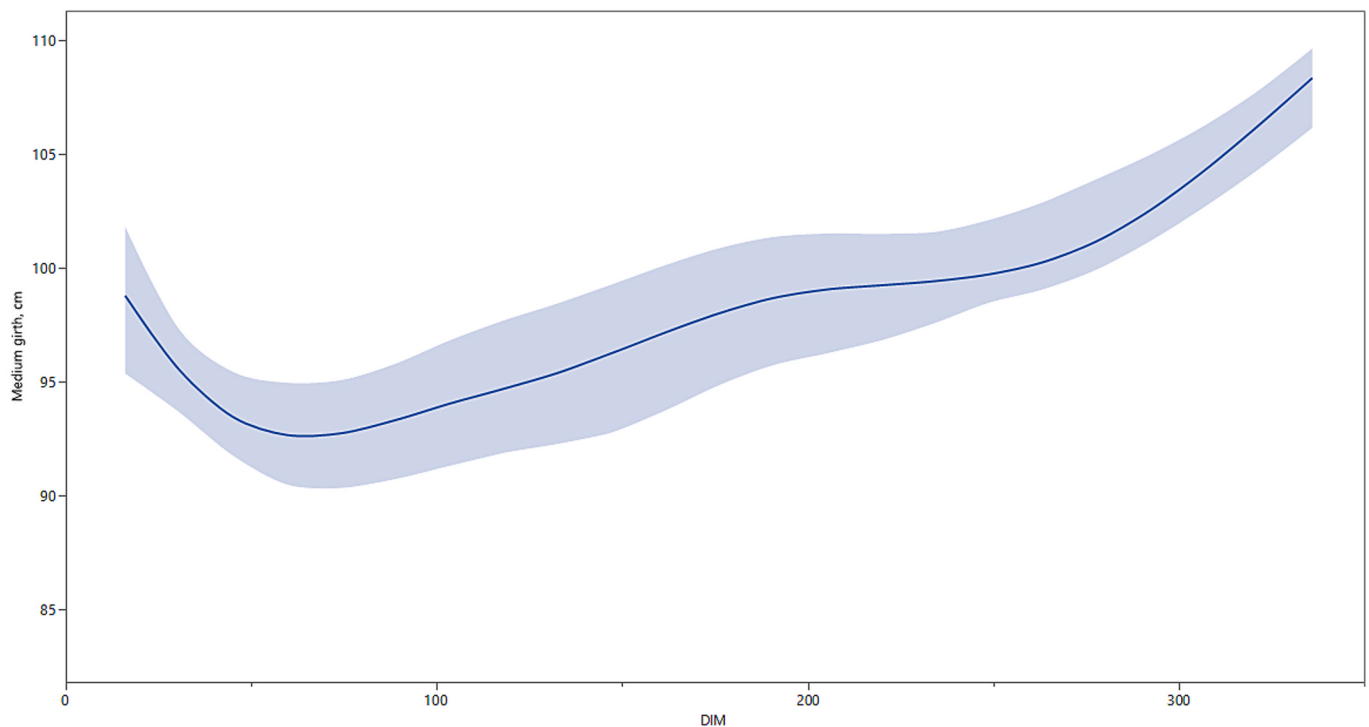


Fig. 7. Smoothed relationship between medium neck girth (cm) and Days in Milk (DIM), with confidence band.

late lactation, the polynomial model did not reach statistical significance ($R^2 = 0.197$; RMSE = 14.71 cm; $P = 0.0685$).

The fitted equation was:

$$\begin{aligned} \text{maximum girth (cm)} = & 140.30426 + 0.0205681 \cdot \text{DIM} + 0.0002055 \\ & \cdot (\text{DIM} - 116.778)^2 + 1.2511 \times 10^{-6} \\ & \cdot (\text{DIM} - 116.778)^3 \end{aligned}$$

3.2. Relationships between morphometric traits and age

All morphometric traits exhibited non-linear relationships with age (Figs. 9–12), although the strength of the associations differed among variables. Thoracic girth showed the strongest age-related relationship, whereas the three cervical measurements displayed more moderate but significant associations. In general, all morphometric traits increased rapidly during the first years of life, followed by a progressive stabilization in adult animals.

3.2.1. Thoracic girth in relation to age

Thoracic girth increased markedly between approximately 1 and 3 years of age before reaching a plateau in older cows (Fig. 9). The third-degree polynomial model showed a strong and highly significant relationship with age, explaining 70.1% of the observed variability ($R^2 = 0.701$; RMSE = 8.76 cm; $P < 0.0001$).

The fitted equation was:

$$\begin{aligned} \text{thoracic girth (cm)} = & 200.27952 + 5.11243 \cdot \text{Age} - 2.88614 \\ & \cdot (\text{Age} - 3.42033)^2 + 0.58646 \cdot (\text{Age} - 3.42033)^3 \end{aligned}$$

3.2.2. Minimum neck girth in relation to age

Minimum neck girth showed a significant non-linear association with age (Fig. 10), characterised by a rapid increase during early growth followed by relatively stable values in adulthood. The polynomial model explained 29.3% of the observed variability ($R^2 = 0.293$; RMSE = 5.38 cm; $P = 0.0002$).

The fitted equation was:

$$\begin{aligned} \text{minimum girth (cm)} = & 86.73944 + 0.66302 \cdot \text{Age} - 0.68919 \\ & \cdot (\text{Age} - 3.44271)^2 + 0.26522 \cdot (\text{Age} - 3.44271)^3 \end{aligned}$$

3.2.3. Medium neck girth in relation to age

Medium neck girth also displayed a significant non-linear relationship with age (Fig. 11), increasing during early growth and showing only minor fluctuations thereafter. The model explained 23.4% of the observed variability ($R^2 = 0.234$; RMSE = 5.54 cm; $P = 0.0026$).

The fitted equation was:

$$\begin{aligned} \text{medium girth (cm)} = & 94.31286 + 0.87846 \cdot \text{Age} - 0.55735 \\ & \cdot (\text{Age} - 3.44772)^2 + 0.17801 \cdot (\text{Age} - 3.44772)^3 \end{aligned}$$

3.2.4. Maximum neck girth in relation to age

Maximum neck girth exhibited the strongest age-related association among the cervical measurements (Fig. 12). Values increased rapidly during early growth and remained relatively stable in adult cows, with a slight increase in older animals. The polynomial model explained 40.7% of the observed variability ($R^2 = 0.407$; RMSE = 15.06 cm) and was highly significant ($P < 0.0001$).

The fitted equation was:

$$\begin{aligned} \text{maximum girth (cm)} = & 156.90552 - 2.30619 \cdot \text{Age} - 3.83260 \\ & \cdot (\text{Age} - 3.42033)^2 + 1.83926 \cdot (\text{Age} - 3.42033)^3 \end{aligned}$$

Fig. 13 illustrates the pairwise relationships among minimum, medium, and maximum neck girths and thoracic girth. Overall, all morphometric variables showed positive and approximately linear associations, with stronger relationships observed between adjacent cervical measurements and between cervical and thoracic girths. No distinct clusters or obvious outliers were evident, indicating continuous morphometric variation across the study population.

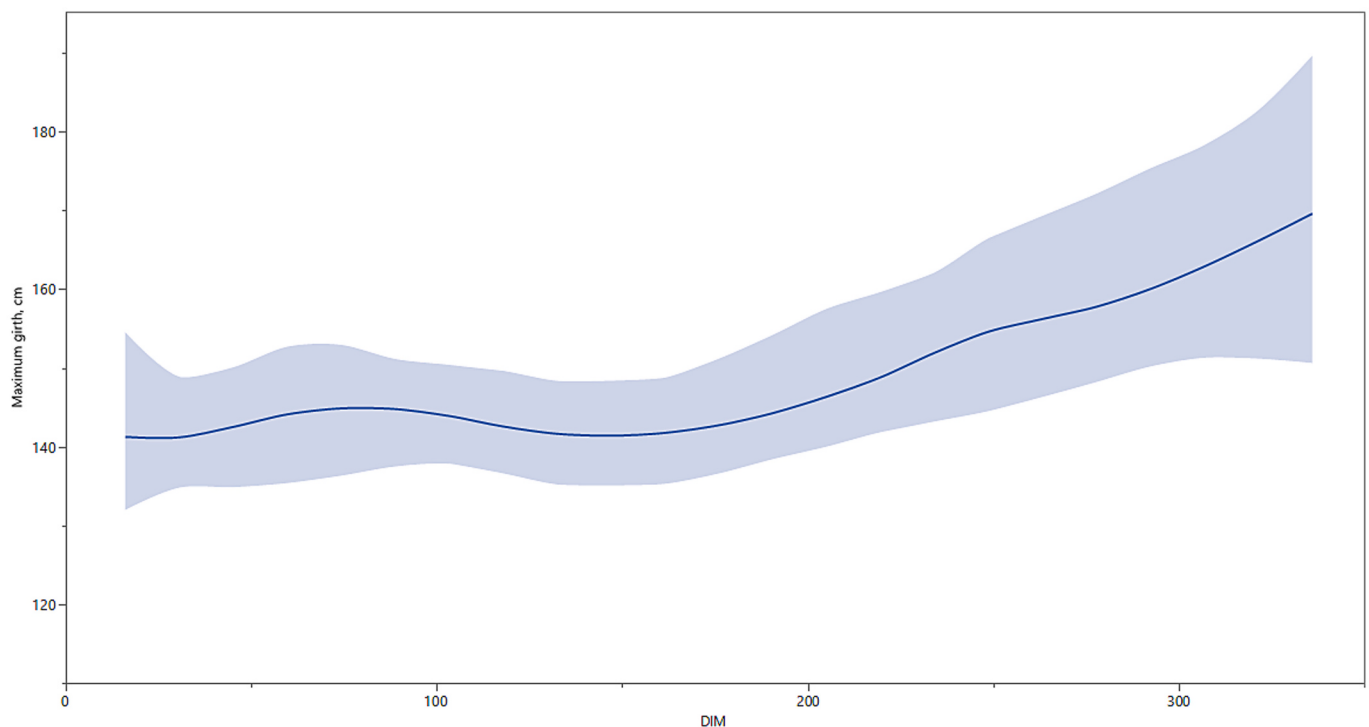


Fig. 8. Smoothed relationship between maximum neck girth (cm) and Days in Milk (DIM), with confidence band.

4. Discussion

The present pilot study contributes preliminary evidence on regional cervical morphometry in Holstein dairy cows by providing an anatomically differentiated description of neck circumference and its relationships with physiological stage and chronological age.

4.1. Morphometric variation across DIM

Lactation is characterised by deep physiological adaptations involving changes in energy balance, endocrine regulation, nutrient partitioning and body reserve mobilisation (Castillo et al., 2025a, 2025b; Sjaastad et al., 2016). Consistent with this well-established physiology, milk yield, milk fat percentage and BCS followed the expected trajectories across DIM, providing physiological context for interpreting the observed morphometric variation (Magro et al., 2024; Roche et al., 2009).

Within this context, minimum and medium neck girths exhibited the clearest associations with DIM, whereas maximum neck girth showed only a weak, non-significant relationship and thoracic girth displayed a more moderate response. These findings suggest that lactation-associated morphometric variation is not uniformly distributed along the cervical axis, supporting the concept that different neck regions respond differently to physiological changes occurring throughout the production cycle.

Anatomically, the cranial and middle portions of the neck contain a greater proportion of superficial soft tissues, whereas the caudal region is characterised by larger muscle groups responsible for head support and locomotion (König and Liebich, 2020; Singh, 2017). Such anatomical differences provide a plausible explanation for the stronger DIM-related associations observed in the cranial and middle cervical regions. However, because the present study relied exclusively on external morphometric measurements, it was not possible to distinguish the relative contribution of muscle, adipose tissue or connective tissue to the observed changes. Consequently, the proposed biological interpretation should be regarded as hypothesis-generating.

Interestingly, the temporal pattern observed for minimum and medium neck girths qualitatively resembled the expected evolution of body condition throughout lactation, with lower values during early lactation followed by progressive recovery towards late lactation. Similar trajectories have been extensively described for body reserve mobilisation and replenishment in dairy cows (Magro et al., 2024; Roche et al., 2009). Nevertheless, neither metabolic indicators nor direct relationships between neck morphometry and BCS were evaluated in the present study. Therefore, although the observed patterns are consistent with current physiological knowledge, any association between cervical morphometry and metabolic status requires dedicated investigation.

Among the cervical measurements, medium neck girth showed the strongest relationship with DIM. This intermediate region may be influenced by both structural characteristics and physiological variability because it represents the transition between the cranial neck, where superficial tissues are relatively abundant, and the more muscular caudal region (König and Liebich, 2020; Singh, 2017). Although this interpretation is anatomically plausible, further studies integrating morphometric measurements with imaging or body composition techniques are required to clarify the tissue compartments underlying these regional differences.

By contrast, maximum neck girth was only weakly associated with DIM despite its greater absolute variability. This observation is consistent with the predominantly structural role of the caudal cervical musculature, which is expected to change more gradually than superficial tissues during the lactation cycle (Klein, 2019; Sehested et al., 2019; Singh, 2017). Similarly, thoracic girth showed only a moderate relationship with DIM, supporting its interpretation as a measure primarily reflecting structural body size rather than short-term physiological variation (Costa et al., 2021). Although the increase observed towards late lactation may partly reflect recovery of body reserves or advancing pregnancy (Sehested et al., 2019), reproductive stage was not explicitly incorporated into the statistical models. Consequently, these mechanisms cannot be distinguished within the present dataset.

The DIM-related analyses suggest that cervical morphometry should not be considered a uniform anatomical trait. Instead, different neck

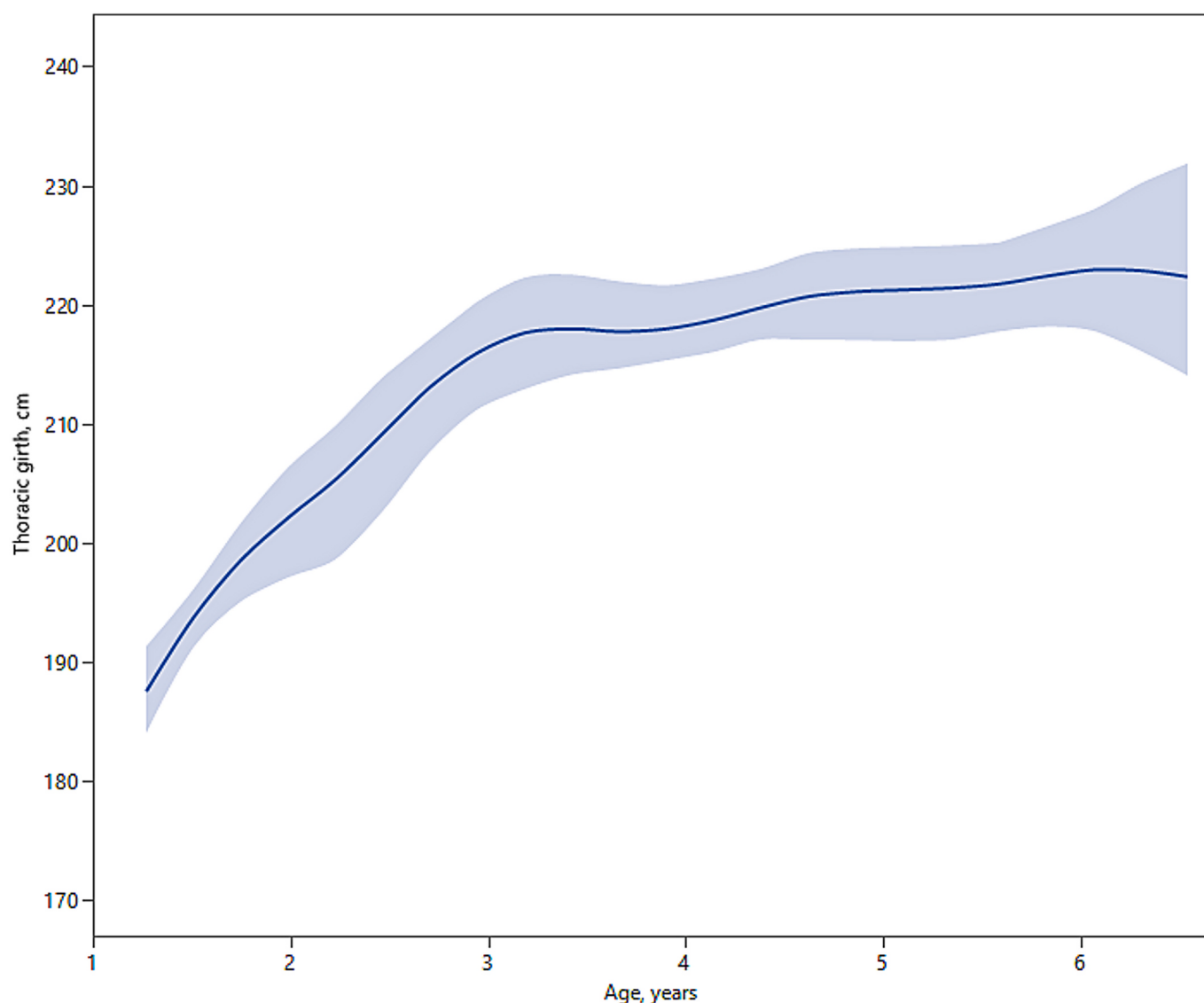


Fig. 9. Smoothed relationship between thoracic girth (cm) and age (years), with confidence band.

regions appear to capture complementary information, with cranial and middle measurements showing greater responsiveness to physiological stage, whereas the caudal neck and thoracic region mainly reflect more stable structural characteristics.

4.2. Morphometric variation in relation to age

In contrast to the DIM-related analyses, chronological age emerged as the principal determinant of thoracic girth and, to a lesser extent, maximum neck girth, indicating that these measurements predominantly reflect long-term structural development. The strong association between thoracic girth and age agrees with its well-established relationship with skeletal growth and overall body size in cattle (Costa et al., 2021). Likewise, the greater age dependency of maximum neck girth is consistent with the progressive development of the caudal cervical musculature and the cumulative effects of maturation, parity and repeated production cycles (Klein, 2019; Roche et al., 2009; Sehested et al., 2019; Singh, 2017).

Conversely, minimum and medium neck girths exhibited weaker associations with age despite their significant relationships with DIM. This contrast suggests that these cervical regions retain greater phenotypic plasticity after skeletal maturity than the caudal neck. From an

anatomical perspective, the cranial and middle portions of the neck contain a higher proportion of superficial soft tissues relative to the caudal region, where muscular structures predominate (König and Liebich, 2020; Singh, 2017). Such regional differences may partly explain why minimum and medium neck girths appeared more responsive to physiological variation, whereas maximum neck girth was more closely associated with structural development. Nevertheless, because tissue composition was not directly evaluated, the anatomical basis of these morphometric differences remains to be confirmed.

The analysis of body weight further supports this distinction between structural and regional morphometric traits. As expected, thoracic girth showed the strongest association with body weight, consistent with its widespread use as an indirect estimator of live weight in cattle (Costa et al., 2021). In contrast, the absence of significant relationships between body weight and the cervical measurements suggests that neck circumference cannot be considered a simple surrogate of body mass. Rather, cervical morphometry appears to provide information that is at least partly independent of overall body size.

This interpretation should, however, be considered in light of the characteristics of the study population. Body weight measurements were available only for adult cows, whereas heifers were not included in this analysis. Consequently, the relatively narrow body weight range may

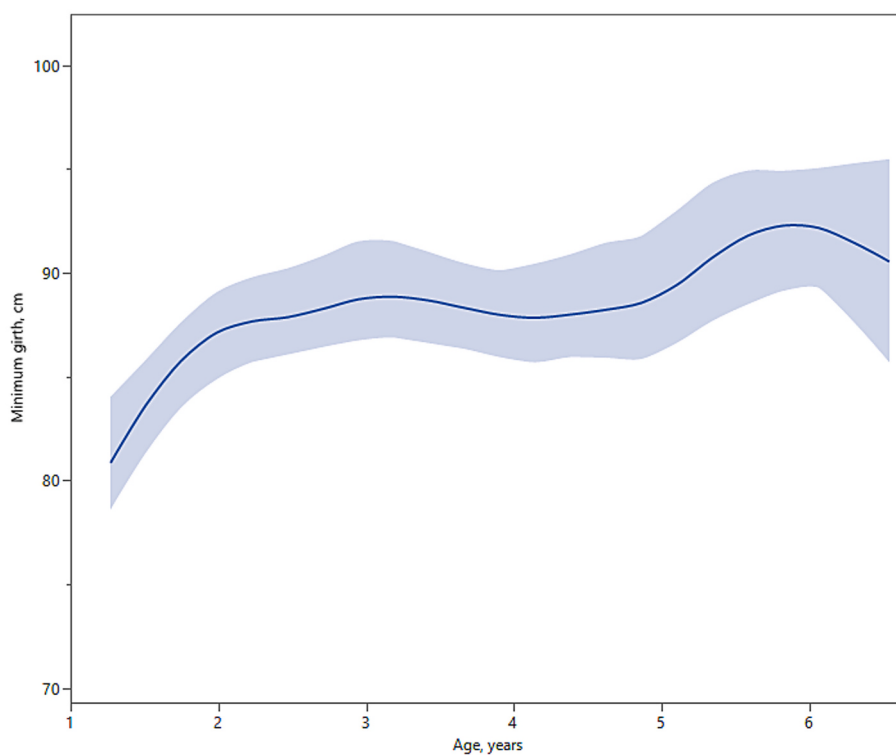


Fig. 10. Smoothed relationship between minimum neck girth (cm) and age (years), with confidence band.

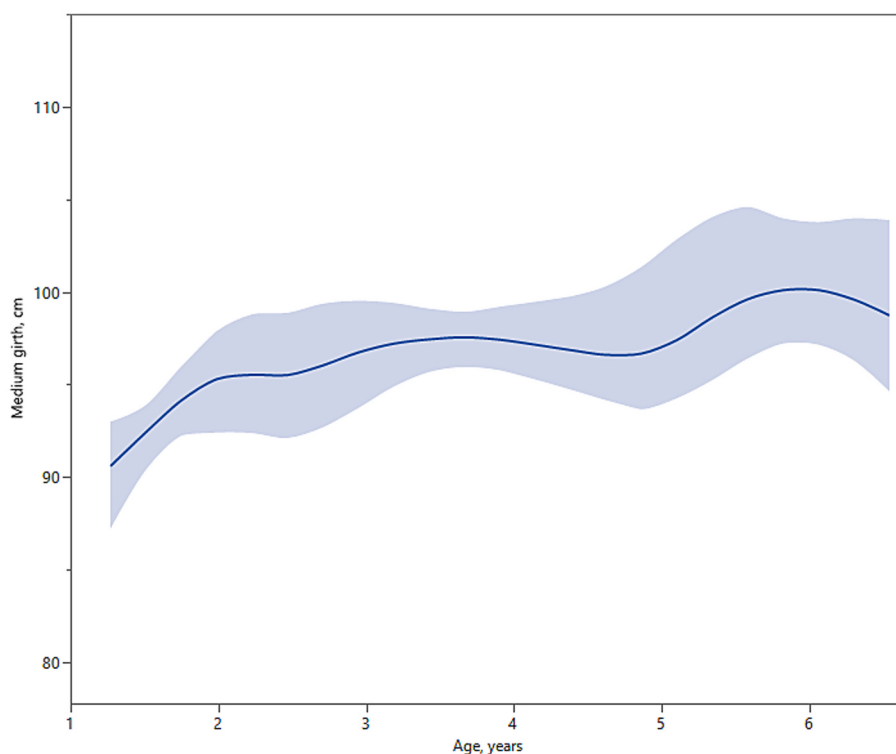


Fig. 11. Smoothed relationship between medium neck girth (cm) and age (years), with confidence band.

have reduced the ability to detect associations between cervical measurements and live weight. Future studies including animals across a wider range of ages and body sizes will be required to determine whether these relationships remain consistent at the population level.

Taken together, the age- and DIM-related findings indicate that

different cervical regions capture complementary aspects of bovine morphology. Whereas thoracic girth and maximum neck girth mainly reflect structural development and body conformation, minimum and medium neck girths appear to be more responsive to physiological variation. These observations reinforce the importance of considering

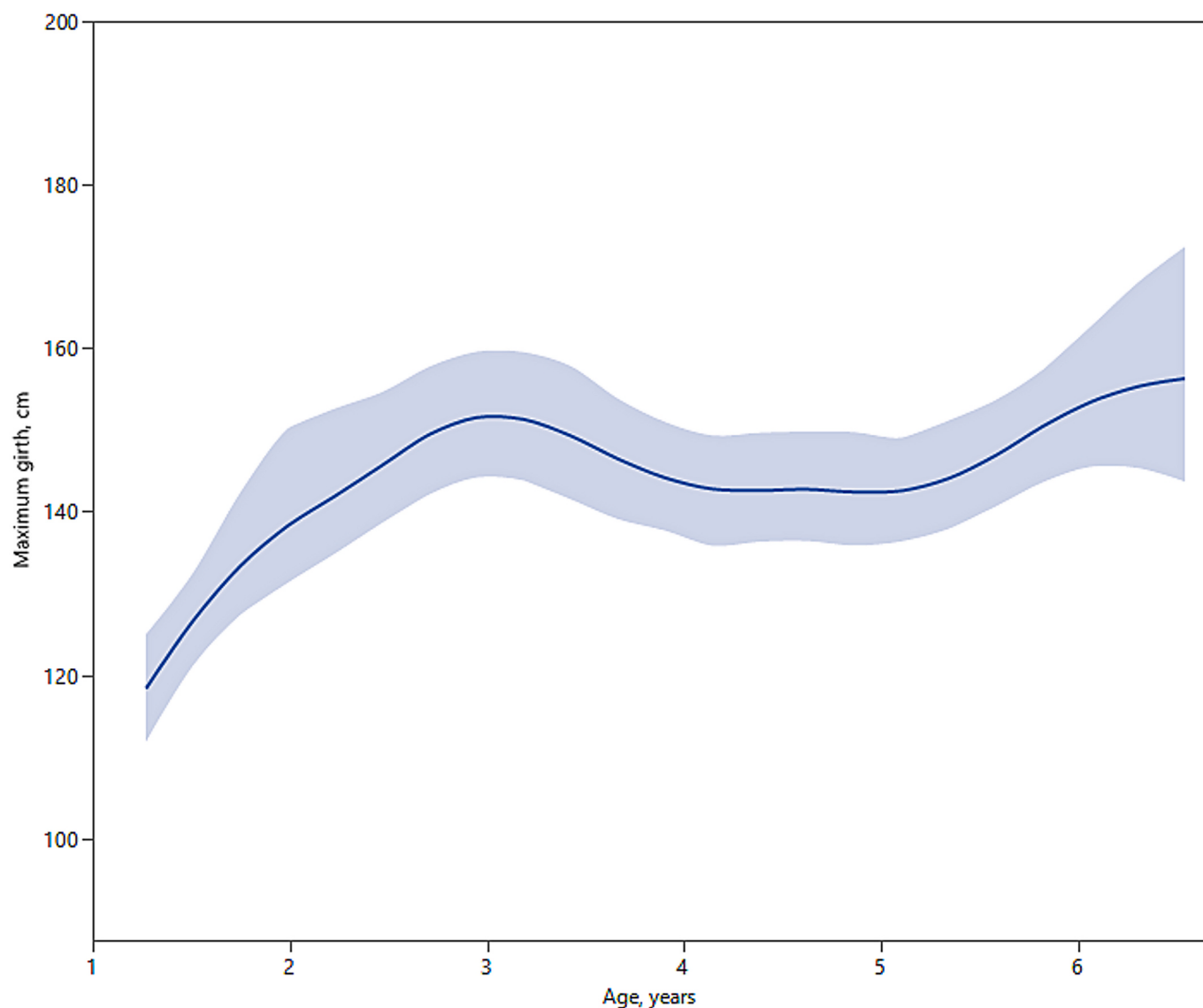


Fig. 12. Smoothed relationship between maximum neck girth (cm) and age (years), with confidence band.

the neck as a heterogeneous anatomical region when describing morphometric variation.

4.3. Limitations

Several limitations should be considered when interpreting the present findings. First, this pilot study was conducted in a single herd of Italian Holstein cows managed under commercial-like conditions. Although this ensured a relatively homogeneous management environment, it limits the generalisability of the absolute morphometric values to other breeds, production systems and management conditions.

Second, although each cow was measured twice, the statistical analyses treated each measurement as an independent observational unit. Consequently, the study was designed to characterise population-level associations rather than individual longitudinal changes, and future studies employing repeated-measures or mixed-effects models will be required to investigate within-animal morphometric trajectories across lactation.

Another limitation is that parity, reproductive status and pregnancy stage were not explicitly incorporated into the statistical models. These factors are likely to contribute to morphometric variability, particularly

for thoracic girth and the caudal cervical region, and should therefore be considered in future investigations.

Finally, cervical morphometry was assessed using external circumference measurements. Consequently, it was not possible to distinguish the relative contributions of muscle, adipose tissue and connective tissue to the observed regional differences. The proposed biological interpretations are therefore based on established anatomical and physiological knowledge (Klein, 2019; König and Liebich, 2020; Singh, 2017) and should be regarded as biologically plausible explanations.

4.4. Future perspectives

The present study provides a preliminary framework for the investigation of cervical morphometry in dairy cattle and identifies several directions for future research. Longitudinal studies including repeated measurements throughout complete lactation cycles would allow individual morphometric trajectories to be modelled and would facilitate the separation of physiological variation from long-term structural changes.

Future investigations should also incorporate parity, reproductive status and pregnancy stage into statistical models, together with direct

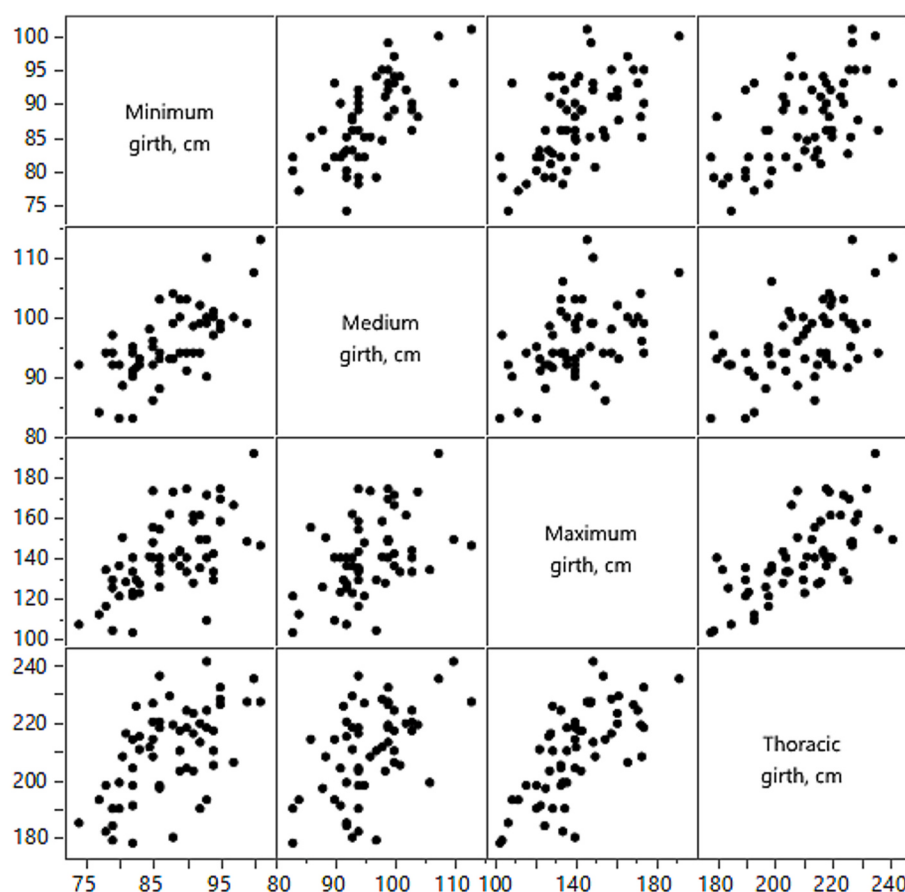


Fig. 13. Scatterplot matrix illustrating pairwise relationships among minimum, medium, and maximum neck girth and thoracic girth (cm). Each panel shows individual measurement events, highlighting generally positive and approximately linear associations across morphometric traits.

indicators of metabolic status and body composition. The integration of external morphometric measurements with complementary techniques such as ultrasonography, three-dimensional imaging or other body composition methods would help clarify the anatomical basis of regional cervical variation and the biological mechanisms underlying the morphometric patterns associated with physiological stage and age.

From an applied perspective, the observed regional differences in neck morphology may also be relevant for the design and positioning of neck-mounted PLF devices. Improved knowledge of cervical morphometry could contribute to the development of collars that better accommodate anatomical variability while enhancing sensor stability, animal comfort and data quality (Lamanna et al., 2026a, 2026b). Expanding these investigations across different breeds, production systems and environmental conditions will also be important for establishing reference values and supporting the wider application of cervical morphometry in dairy cattle research.

5. Conclusions

This pilot study provides a preliminary characterisation of regional cervical morphometry in Holstein dairy cows and explores its associations with lactation stage and chronological age. The results suggest that different cervical regions exhibit distinct patterns of variation, with minimum and medium neck girths showing stronger associations with lactation stage, whereas maximum neck girth and thoracic girth appear to be more closely related to age and structural development. These findings suggest that cervical morphometry may not represent a uniform

anatomical trait, but rather that different regions of the neck may provide complementary morphometric information.

Although the present study was exploratory and conducted in a single herd, it provides an initial descriptive framework for the assessment of cervical morphometry in dairy cows. The regional patterns observed are consistent with current anatomical and physiological knowledge, while the underlying biological mechanisms require further investigation through studies integrating morphometric, metabolic, and body composition measurements.

These findings represent a valuable starting point for future research on cervical morphometry in dairy cattle. Validation in larger and more diverse populations, together with longitudinal study designs, will be necessary to confirm the robustness of these preliminary observations and to explore their potential applications in bovine phenotyping, ergonomic assessment, and the development of PLF technologies.

CRedit authorship contribution statement

M. Lamanna: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **R. Colleluori:** Writing – review & editing. **M. Canova:** Writing – review & editing, Methodology, Conceptualization. **A. Grandis:** Writing – review & editing, Methodology, Conceptualization. **M. Bovo:** Writing – review & editing. **F. Petretto:** Writing – review & editing. **A. Formigoni:** Writing – review & editing. **D. Cavallini:** Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization.

Ethics approval

Not applicable. The study was conducted within routine herd management practices, without invasive procedures, experimental treatments, or modifications to standard handling, feeding, housing, or milking protocols; all activities complied with Directive 2010/63/EU and Directive 98/58/EC, and no additional ethical approval was required under national regulations.

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Declaration of competing interest

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

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Data availability

The datasets generated and analysed are available from the corresponding author on reasonable request.

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