

Andrological characteristics of tropical milking criollo Bulls

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ABSTRACT

Objective: To assess the testicular and semen characteristics of Tropical Milking Criollo (CLT) bulls in the subhumid Mexican tropics.

Design/methodology/approach: Eight bulls were evaluated and distributed in two groups: G1 (n=5): young bulls and G2 (n=3): adult bulls. All bulls were managed under grazing and evaluated throughout a year. From each bull, the following measurements were taken once a month: live weight, body condition score, scrotal circumference, and testicular width, length and volume. Semen was obtained every 3 months via an artificial vagina. The evaluated semen variables were: aspect, volume, mass and individual motility, and sperm concentration and morphology.

Results: Live weight and testicular measurements linearly increased during the study in bulls from both groups. All bulls had a scrotal circumference larger than the minimum threshold value for cattle and high-quality semen.

Study limitations/implications: The low availability of CLT bulls prevented the inclusion of a larger number of animals in the research. This low animal availability makes it necessary to establish standard values for testicular measurements and semen characteristics in the CLT breed in order to select the best individuals as sires and contribute to its conservation.

Findings/conclusions: The CLT bulls had good scrotal circumference and semen quality from a young age and into adulthood. These traits make CLT bulls an important alternative for livestock breeding in the tropics.

Keywords: Semen quality, scrotal circumference, semen.

INTRODUCTION

The testicular measurements and semen quality evaluation are essential in the assessment of the fertility of bulls. Out of the testicular measurements, the scrotal circumference (SC) is the most used, given its easy measurement, high repeatability, and high correlation with sperm concentration, semen quality, testicular size, and body weight (Martínez-Velazquez *et al.*, 2003). Bulls with higher-than-average SC produce more semen, of better quality and reach puberty earlier (Silva *et al.*, 2012). The live weight of the animal is positively correlated to the testicular weight, which in turn, has a high correlation with the amount of spermatic tissue and with the SC, so the SC is the basis for estimating the testicular size and selecting sires (Silva *et al.*, 2012).

The populations of Tropical Milking Criollo cattle (CLT; *Bos taurus taurus*) are scarce and distributed only in certain tropical regions of Mexico (De Alba, 2011; Parra-Cortés and Magaña-Magaña, 2019). This breed is characterized by its precocity, high fertility, ease of calving, survival, resistance to diseases and ectoparasites, and longevity (De Alba, 2011; Rosendo-Ponce and Becerril-Pérez, 2015). However, little has been documented on the andrological characteristics of males in warm climate conditions;

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this is necessary to select specimens to increase their population or for crossbreeding schemes with European or Zebu breeds in the tropics, which would improve their profitability and the sustainability of the cattle herds in these regions (Parra-Cortés and Magaña-Magaña, 2019).

Although reproductive traits have been characterized in males from *B. taurus taurus* breeds in the Latin American tropics (Palmieri *et al.*, 2004; Madrid-Bury *et al.*, 2011), there is no information on the CLT breed, despite its importance as a dairy genotype adapted to the tropics. Therefore, the objective of this research was to assess the testicular and seminal characteristics of CLT males in the subhumid Mexican tropics.

MATERIALS AND METHODS

Geographical location and characteristics of the study

The research was conducted in a CLT cattle herd owned by the Colegio de Postgraduados, Campus Veracruz, located in the state of Veracruz, Mexico, on the central coast of the Gulf of Mexico, at latitude 19° 16' N and longitude 96° 16' W, at 20 m altitude, with a warm sub-humid climate, mean annual temperature of 26.5 °C and annual precipitation of 1 230 mm. The study was conducted over one year and included four periods: 1=September 22 to December 20, 2=December 21 to March 19, 3=March 20 to June 20, and 4=June 21 to September 21.

Experimental animals and management

Eight bulls were selected from a group of 14 CLT males, which had no sight, limbs, or reproductive problems that could affect the results of the study. The bulls were distributed in two groups according to their age: G1 (n=5): young males that at the beginning of the study were 19.3±0.4 months old, weighed 260±9 kg (live weight, LW) and had body condition (BC) from 2.5 to 3.0 on a scale of 1 to 5 (1=emaciated and 5=obese; Wildman *et al.*, 1982); and G2 (n=3): adult males at 30.5±3.1 months old, weighing 371±29 kg LW and with BC similar to G1. The animals grazed in paddocks of *Megathyrsus maximum*, *Brachiaria mutica*, *Cynodon nlemfluensis*, and *Paspalum* spp., at a stocking rate of 2 AU ha⁻¹, and did not receive feed or mineral supplementation. All males were kept together, and none had been used as sire.

Live weight and body condition

The LW of each animal was recorded at the beginning of the study and subsequently every 30 d for one year. The weighing was done on a commercial cattle scale, and the BC was visually evaluated by the same technician, using the scale from 1 to 5 described above.

Testicular characteristics

The scrotal circumference (SC), testicular width (TW), testicular length (TL), testicular volume (TV) and testicular consistency (TC) were measured in all animals at the beginning of the study and every 30 d thereafter. Scrotal circumference was

measured with a scrotal tape measure; TW and TL were measured in each testicle using a vernier; and TV was calculated with the TW and TL measurements using the equation $TV=0.5236 (TW) (TL)^2$ (Bailey *et al.*, 1996). The TC was assessed by palpation of each testis, which were graded as firm or soft, and testicular tone was graded as elastic, flaccid or rigid.

Seminal characteristics

Every three months, a semen sample was collected from each animal with an artificial vagina. For semen collection, the bull was placed with a cow previously synchronized with PGF₂α (20 mg dose of Lutalyse®, Lab. Zoetis, USA) during the same days of the evaluation of testicular characteristics. Immediately after collection, semen volume was determined by direct observation in the collection tube, and mass (MM) and individual (IM) sperm motility were determined. To establish MM, a 10 μL semen drop was placed on a slide pre-warmed to 37 °C, observed under a phase-contrast microscope (10x and 40x) and assigned a value from 0 to 100%. To determine the IM, a 10 μL drop of semen was placed between a prewarmed slide and coverslip at 37 °C, observed for progressive rectilinear motility with phase-contrast microscopy (10x and 40x) and assigned a value from 0 to 100%. The spermatozoa concentration (SC) in the ejaculate was determined with a hemocytometer, for which 10 μL of semen were diluted in 2 mL of formol saline solution; then, two chambers of the hemocytometer were filled and the number of spermatozoa present in five large squares in each chamber was counted using a phase-contrast microscope (40x); the total number of cells counted was multiplied by 10⁶ to obtain the number of spermatozoa per milliliter of semen. To determine sperm morphology, 20 μL of semen were diluted in 1 mL of formalin-saline solution and analyzed following the criteria by Blom (1973), using a phase-contrast microscope (1000x); 400 cells were evaluated, and the percentage of abnormal cells recorded.

Statistical analysis

Descriptive statistics were used to characterize the evaluated variables. For testicular (SC and VT) and seminal characteristics (volume, MM, IM, SC and abnormal spermatozoa) descriptive statistics were performed and mean comparisons were made using Tukey's tests when significant F tests were detected (P<0.05).

RESULTS AND DISCUSSION

Changes in body weight and body condition

Weight increased in both groups of bulls and all maintained body condition from 2.0 to 2.5 (Table 1). At the end of the study, G1 bulls weighed 362.8±13.4 kg and G2 bulls weighed 451.3±22.2 kg; during the study, G1 bulls gained 103.1 kg and G2 bulls 80.2 kg.

The weight changes observed in the G2 bulls suggest a stabilization of the growth and weight of the CLT bulls between 36- and 42-months age, as suggested for other

breeds, depending on their management (Vásquez and Arango, 2002). This will be necessary to verify in future studies with a larger number of animals.

Table 1. Age, live weight, and body condition of young (G1) and mature (G2) Tropical Milking Criollo bulls throughout the seasons.

Group	Time periods			
	1	2	3	4
Age (months)*				
G1	19.3±0.4	21.6±0.2	24.8±0.4	27.8±0.4
G2	30.5±3.1	33.3±3.1	36.5±3.1	39.2±3.1
Live weight (kg)*				
G1	260±9	266±9	285±16	363±13
G2	371±29	365±45	377±67	450±42
Body condition				
G1	2.5	2.5	2.0	2.5
G2	2.5	2.5	2.5	2.5

*Mean ± standard deviation. 1=September 22 to December 20, 2=December 21 to March 19, 3=March 20 to June 20, 4=July 21 to September 21.

Testicular characteristics

Upon palpation, all bulls had testes of firm consistency and elastic tone in all evaluations, indicating good testicular consistency. The SC linearly increased during the study in both groups, which can be attributed to weight gain and age (Table 2). An increase in SC has been reported as age advances in Zebu (Torres-Junior and Henry, 2005) and European breeds (Jiménez-Severiano, 2002).

Table 2. Testicle measures of young (G1) and mature (G2) Tropical Milking Criollo bulls, throughout the seasons.

Group	Time periods			
	1	2	3	4
Scrotal circumference (mm)*				
G1	29.1±0.7 ^d	30.5±0.2 ^c	31.5±0.5 ^b	32.9±0.2 ^a
G2	32.0±1.0 ^d	32.8±0.7 ^c	33.2±0.7 ^b	34.9±0.5 ^a
Scrotal width (cm)*				
G1	6.1±0.3	6.7±0.2	7.1±0.2	7.5±0.2
G2	7.2±0.2	7.6±0.6	7.8±0.4	8.0±0.4
Scrotal length (cm)*				
G1	9.7±0.4	9.9±0.4	10.3±0.3	10.2±0.4
G2	10.5±0.7	11.1±0.5	11.3±0.5	11.5±0.4
Testicle volume (cm ³)*				
G1	373±22 ^b	426±17 ^b	529±26 ^a	567±34 ^a
G2	585±44 ^b	685±43 ^a	708±37 ^a	724±31 ^a

a,b,c,d Means having different superscript letter within a row differed (p<0.05).

*Mean ± Standard deviation. 1=September 22 to December 20, 2=December 21 to March 19, 3=March 20 to June 20, 4=July 21 to September 21.

It was also observed that at 24.8 ± 0.4 months of age, the G1 bulls had a SC of 31.5 ± 0.5 cm, slightly lower than the 32 cm suggested as a minimum for bulls of criollo breeds at the age of 24 m (Irons *et al.*, 2007). In this regard, it is worth mentioning that the bulls used in this study are genetically small in body size, and their diet was based on low nutritional quality grass without supplement, which probably affected the SC.

The SC of the evaluated bulls at different ages was comparable to that of the horned Costeño, Romosinuano (Palmieri *et al.*, 2004) and Guzerat (Torres-Junior and Henry, 2005) bulls, the latter with greater weight than the CLT and the Criollo Limonero (Ocanto *et al.*, 1991; Madrid *et al.*, 1995). However, the SC in CLT bulls was lower than that of *Bos taurus indicus* \times *Bos taurus taurus* bulls (Prieto *et al.*, 2007), with higher body weight than the CLT.

Racial differences have been noted that cause some *Bos taurus taurus* breeds to reach puberty at a younger age, lower weight and smaller SC than others. It has been proposed that males should be evaluated according to a minimum SC standard for the breed and not according to the one established by the Society for Theriogenology (Coulter *et al.*, 1987). This could be applied to this study, that is, to establish a SC standard for the CLT breed in particular, because they are smaller in size and weight less than most *B. taurus taurus* breeds.

Like the SC, TW and TL also increased linearly throughout the evaluation in all animals (Table 2). The TW was higher in comparison to Guzerat bulls (Torres-Junior and Henry, 2005); TL was higher than that of Guzerat bulls (Torres-Junior and Henry, 2005) and lower than that of *B. taurus indicus* \times *B. taurus taurus* bulls (Prieto *et al.*, 2007). The TV was lower in comparison to Guzerat bulls (Torres-Junior and Henry, 2005).

Seminal characteristics

Ejaculate volume and seminal concentration significantly increased until the last evaluation (Table 3), which was when the bulls were one year older. The greatest ejaculate volume (EV) observed in both groups of bulls was similar to that of Holstein bulls in temperate climates (Brockett *et al.*, 1994) and *B. taurus indicus* \times *B. taurus taurus* bulls (Prieto *et al.*, 2007), and was higher than that of Criollo Limonero (Madrid *et al.*, 1995), horned Costeño, and Romosinuano (Palmieri *et al.*, 2004) bulls.

Spermatozoa concentration in CLT bulls was similar to that of Holstein bulls in a temperate climate (Brockett *et al.*, 1994) and Criollo Limonero bulls (Madrid *et al.*, 1995) in a tropical climate, although it was lower than that of horned Costeño and Romosinuano (Palmieri *et al.*, 2004) bulls, also in a tropical climate. Although the semen appearance coincided with that of this last study, the highest concentration of spermatozoa obtained in CLT bulls was around 1 billion mL^{-1} , while in horned Costeño and Romosinuano (Palmieri *et al.*, 2004) it was 1600 million mL^{-1} .

Table 3. Semen characteristics of young (G1) and mature (G2) Tropical Milking Criollo bulls during the study.

Group	Time periods			
	1	2	3	4
Semen volume (mL)*				
G1	3.4±0.2 ^b	3.8±0.4 ^b	3.3±0.4 ^b	4.7±0.6 ^a
G2	3.8±0.6 ^b	4.0±0.1 ^b	5.5±0.8 ^a	6.5±1.4 ^a
Total sperm count (x10 ⁶ mL ⁻¹)*				
G1	560±112 ^b	586±103 ^b	714±56 ^b	974±110 ^a
G2	667±16 ^b	667±167 ^b	747±167 ^b	1 000±312 ^a
Mass motility (%)*				
G1	88±2	88±2	88±2	84±4
G2	80±2	80±2	80±2	70±10
Individual motility (%)*				
G1	88±2	88±2	88±2	84±4
G2	80±2	80±2	80±2	70±10
Abnormal sperm (%)*				
G1	-	12.0±1.2 ^a	13.4±0.9 ^a	6.6±0.7 ^b
G2	-	13.3±1.7 ^a	16.3±0.9 ^a	3.7±0.3 ^b

a,b Means having different superscript letter within a row differed (p<0.05). * Mean ± Standard deviation. 1=September 22 to December 20, 2=December 21 to March 19, 3=March 20 to June 20, 4=July 21 to September 21.

The MM and IM were high in CLT bulls from 19.3±0.4 months of age, contrary to reports that indicate that progressive motility gradually increases with age (Torres-Júnior and Henry, 2005). The results indicate that CLT bulls show high MM and IM since a young age, which are maintained at least until 39.2±3.1 months of age. In CLT bulls IM was higher than that reported in Criollo Limonero (Madrid *et al.*, 1995), horned Costeño, Romosinuano (Palmieri *et al.*, 2004), Guzerat (Torres-Júnior and Henry, 2005), and *B. taurus indicus* × *B. taurus taurus* (Prieto *et al.*, 2007) bulls.

The AE percentage in both groups of CLT bulls was less than 30 %, a value established as a minimum by the Society of Theriogenology. The AE percentage decreases as age increases (Torres-Júnior and Henry, 2005). The percentage of AE in young CLT bulls at 19.3±0.4 months of age was similar to that obtained at similar ages in Criollo Limonero (Madrid *et al.*, 1995), and lower than that of horned Costeño, Romosinuano (Palmieri *et al.*, 2004), and *B. taurus indicus* × *B. taurus taurus* (Prieto *et al.*, 2007) bulls.

It is important to know the reproductive characteristics of the criollo breeds, in particular the CLT, in order to select the best specimens to be used to improve and increase the number of individuals of their breed, or in crossbreeding schemes with European or Zebu breeds in the tropics, to increase the profitability of the cattle herds in these regions and preserve this breed (Parra-Cortés and Magaña-Magaña, 2019).

CONCLUSIONS

The CLT bulls had good scrotal circumference and seminal quality from a young age through adulthood. The smaller scrotal circumference of the CLT breed, with respect

to other breeds, does not affect its seminal quality and is in proportion to the smaller body size of these animals. The CLT bulls have similarities and differences with other breeds, for this reason, it is important to have specific information about this breed.

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