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Evaluation of the breeding soundness test for Bonga sheep in the context of a community-based breeding programme in Ethiopia

Zelalem Abate [a], Ebadu Areb [b,c,*], Nahom Belay [a], Adisu Gebremikael [a], Tegebaru Gebresilase [a]

[a]*Southwest Agricultural Research Institute at Bonga Agricultural Research Centre, Bonga, Ethiopia*
[b]*South Agricultural Research Institute at Werabe Agricultural Research Centre, Werabe, Ethiopia*
[c]*College of Animal Science and Technology, Northwest A&F University, Yangling, China*

Abstract

The aims of this study were to evaluate sire selection traits and semen characteristics for Bonga sheep. A total of 101 sires with different birth year from the Bonga sheep community based breeding programme (CBBP) were used to collect data. The data was analysed by using the GLM procedure of SAS and least square means was separated by using adjusted Tukey-Kramer method. The breeding soundness tests consisted of physical examinations, sire body condition score (BCS), scrotum circumference, estimated breeding value (EBV), six months body weight (SMWT), and semen characteristics (volume, motility, and concentration) collected using an artificial vagina. The overall least square means ± SE for scrotum circumference, EBV, and six-month body weight of Bonga sheep were 28.20 ± 0.381 cm, 2.13 ± 0.326, and 24.69 ± 0.547 kg, respectively. Similarly, semen volume, mass motility, sperm concentration, and total spermatozoa of Bonga sheep was 1.14 ± 0.076 ml, 4.28 ± 0.138 million/ml, 4.25 ± 0.195 billion/ml, and 4.86 ± 0.393 billion/ml, respectively. A sire with thin BCS resulted in low scrotum circumference ($P < 0.001$) and questionable semen characteristics ($P < 0.05$). The body weight and the scrotum circumference of the Bonga sheep increased with age, but the breeding value fluctuated. Both a large scrotum and a good BCS sire produce a high volume of semen and has simultaneously better motility and concentration. This will enable to increase the fertility of a sire under CBBP. The scrotum circumference of Bonga sheep in relation to their body weight can be categorised as either satisfactory or excellent. Body weight of Bonga sheep was increased across sire birth year but fluctuating trend for breeding value. Scrotum circumference increased with increasing age from grower to mature stage of a sire. Both large scrotum circumference and good BCS sire produces high semen volume and has simultaneously better motility and concentration. This will enable to increase fertility of a sire under CBBP. We can categorize scrotum circumference of Bonga sheep either satisfactory or excellent with the proportion of their body weight.

Keywords: breed improvement, optimisation, selection, semen, sire

1 Introduction

Sire contributes up to 75 % of the genetic variation in a flock. Therefore, it is important to select them carefully as the evaluation of the reproductive traits of an animal is key to improving its productivity (Elmaz *et al.*, 2007). The improved reproductive success could lead to greater genetic progress for the selected traits (Carvajal-Serna *et al.*, 2018). The reproductive capacities of a sire are directly involved in the reproductive process either through natural mating or artificial insemination (AI) (Allaoui *et al.*, 2014). Maintaining high fertility through genetically superior rams producing large numbers of high-quality spermatozoa leads to an improvement in the fertility of the herd (Rege *et al.*, 2000).

In community based breeding programmes (CBBP), community is both breeder and producer; being suggested as viable options for the genetic improvement programmes of small ruminants in low-input smallholder production systems (Gizaw & Getachew, 2009). It is a designed suitable breeding schemes that enable communities to imple-

* Corresponding author – ebaduareb1@gmail.com

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ment breed improvement activities under uncontrolled village breeding practice. This designing and implementation of CBBP require a good understanding of the production system and importance of the different constraints, breeding objectives of the farmers and identifying the superior genotypes (Baker and Gray, 2003). It contributes to efficient utilization and conservation of animal genetic resources. This includes procedures for the selection and use of superior breeding stock and prediction of genetic progress under village conditions (Adisu *et al*., 2015). A community-based breeding programme is focused on identified traits of farmers before starting the program. In case of Bonga sheep the identified traits were growth rate, tail type, polled, twining rate and coat colour of the breed (Tadele *et al*., 2010). Selection was carried out separately within for each CBBP cooperative. Selection of male lambs is being carried at two stages; screening of heavy at weaning (3-month) followed by selection at six months of age (post weaning). This method had own advantage to avoid negative selection. Breeding soundness examination (BSE) is the process of identifying a male capable of settling a female. Therefore, the selected breeding sire should be certified in its BSE. Certification of a breeding sire should incorporate origin and breeding value, screening for sexually transmitted diseases, physical and reproductive organs examination, and sperm production and viability serving capacity.

Scrotal circumference reflects the weight of the gonad and the ability of sperm production (Salhabe *et al*., 2003) and a bigger scope for genetic improvement of fertility in ram lambs than semen characteristics (Rege *et al*., 2000). It is a highly heritable trait that is considered an excellent indicator of sperm production (Moghaddam *et al*., 2012). Males with larger testis have higher daily sperm production and tend to have their daughters reach puberty earlier and ovulate more ova during each oestrus period (Söderquist & Hultén, 2006).

Sperm collected from younger rams is characterised by incomplete spermatogenic activity and maturation. The number of doses produced for each ejaculate per sire depends on components of semen quality such as volume, concentration, and motility, and is affected by both environmental and genetic factors (David *et al*., 2007). These components of semen quality evaluation allow the calculation of the total number of sperm in the ejaculate, the gross motility and the individual sperm motility of a diluted sample, as well as the morphologically normal sperm. Semen with a high concentration of spermatozoa could enable more ewes to be serviced and the conception rate would be increased (Elmaz *et al*., 2007).

The selection and dissemination of sires for mating under a community-based breeding programme (CBBP) were based on simple physical evaluation, estimated breeding value (EBV), scrotum circumference, and pedigree information. Only these methods do not guarantee the productivity and fertility of sires. There is no information on the standard testicle size, body condition score (BCS), and appropriate age of the sire for semen characteristics under CBBP. Therefore, this study aimed to compare semen characteristics of different testicle sizes and set standards to determine better semen production age of the sire and body condition score, and to generate optimisation information for selection of breeding sires kept under CBBP for Bonga sheep.

2 Materials and methods

2.1 Study area

The study was conducted in Kaffa zone of the southwest regional state of Ethiopia. This zone is situated at 7º 34´ N latitude and 37º 6´ E longitude and is 467 km from the capital city Addis Ababa, Ethiopia. The area has one major rainy season that extends from May to October, and the dry season lasts from October to April. The altitude range of the study area is between 1600 to 3348 m a.s.l. and the minimum and maximum temperatures were 14 and 32 °C, respectively with an average of 24 °C. The major production systems are mixed crop-livestock systems (Mirkena *et al*., 2012).

2.2 Experimental animals

A total of 101 experimental sires were selected from Bonga CBBP. In this breeding program, the pedigree, growth, reproductive and morphological characteristics data were recorded after identification by ear tag of the animal. Selection of breeding sire was done at six months of age. Estimated breeding value, physical examination, and farmer preferences were the criteria for the selection of breeding sires. The physical examination included lameness, blindness, conformational defect, teeth, abnormal testicular shape, abdominal hernia, cryptorchidism, abnormal spermatic cord, injury, and penile abnormalities. The farmer preferences included coat colour of the animal and absence of horn.

2.3 Data collection

Sires' pedigree information, six months' body weight, sire age, birth type, and birth season data were taken from the CBBP dataset of Bonga sheep. However, scrotum circumference, sire location, BCS, and semen characteristics data were collected during the experimental period. A scrotum circumference was measured by using measuring tape on the wider part of the scrotum in centimetres. Semen was collected from each sampled sire by using an artificial vagina (AV) with a temperature of 42–43 °C. Before collection, the prepuce was cleaned to prevent contamination of the semen. Semen volume was recorded using a graduated collecting glass with 0.1 ml accuracy. Measurement of the sperm concentration was done by using a portable spectrophotometer pre-calibrated for ram semen (ovine-caprine accuread photometer; IMV®, France). Sperm cell concentration was estimated after taken of 10 μ litre of fresh semen by using a micropipette and mixing it gently with 4 ml of normal saline (0.9 %) on the UV Macrocell (UV Macro Cell 2.5 to 4.5 ml, Great Britain) and measuring the concentration using AccuRead IMV Technologies SA, 232 Spectrophotometer.

Sperm mass motility was estimated subjectively by using a light microscope. Semen was taken with a pipette, dropped on the slide, and observed with the 10× magnification on the objective lens. The mass motility was graded according to Evans & Maxwell (1987) from zero to five (0 = all spermatozoa are motionless, 5 = 90 % or more of the spermatozoa are very rapidly moving waves) score based on the passion of the wave motion. Total spermatozoa/ejaculation was calculated as multiplication of volume of ejaculation by its concentration in billion/ml.

2.4 Data analysis

The data was analysed using the General Linear Model (GLM) procedures of the Statistical Analysis System (SAS, 2012). The least square means ± SE was separated by using Adjusted Tukey-Kramer method in SAS. The statis-

Table 1: *Least square means (± SE) for sire selection criteria of Bonga sheep by different fixed effects.*

Fixed effects	*N*	*SC (cm)*	*EBV at SMWT*	*SMWT (kg)*
Overall	*101*	*28.20 ± 0.381*	*2.13 ± 0.326*	*24.69 ± 0.547*
CV		*6.43*	*75.27*	*10.23*
Sire age	*P-value*	*0.0041*	*0.4341*	*0.2949*
4 years	9	29.88 ± 0.703[a]	1.62 ± 0.601	23.24 ± 1.010
3 years	33	29.22 ± 0.455[ab]	2.49 ± 0.389	24.80 ± 0.653
2 years	26	28.09 ± 0.546[bc]	2.58 ± 0.467	24.19 ± 0.785
1 year	7	27.20 ± 0.899[cd]	1.63 ± 0.761	26.09 ± 1.277
< 1 year	26	26.64 ± 0.617[d]	2.32 ± 0.528	25.14 ± 0.886
Location	*P-value*	*0.5583*	*0.0956*	*0.0003*
Buta-Shuta	45	28.09 ± 0.441	1.84 ± 0.377	25.79 ± 0.634[a]
Boka-Guta	56	28.32 ± 0.421	2.42 ± 0.360	23.60 ± 0.605[b]
Birth season	*P-value*	*0.5246*	*0.3997*	*0.4109*
Dry	23	28.38 ± 0.495	2.33 ± 0.423	25.02 ± 0.711
Wet	78	28.03 ± 0.442	1.93 ± 0.378	24.37 ± 0.635
Birth type	*P-value*	*0.0209*	*0.2108*	*0.0097*
Single	74	27.65 ± 0.379[b]	1.87 ± 0.328	25.58 ± 0.545[a]
Twin	27	28.76 ± 0.505[a]	2.38 ± 0.432	23.80 ± 0.725[b]
BCS	*P-value*	< .0001	< .0001	< .0001
2.5	15	26.12 ± 0.648[c]	1.56 ± 0.554[b]	23.70 ± 0.930[b]
3	27	27.59 ± 0.498[b]	1.30 ± 0.426[b]	23.71 ± 0.716[b]
3.5	26	27.85 ± 0.501[b]	1.63 ± 0.429[b]	23.80 ± 0.720[b]
4	28	30.07 ± 0.474[a]	3.96 ± 0.406[a]	27.98 ± 0.681[a]
Dam parity	*P-value*	*0.4919*	*0.4811*	*0.4776*
1	6	27.98 ± 0.853	1.61 ± 0.730	23.04 ± 1.225
2	16	27.89 ± 0.633	2.33 ± 0.542	24.65 ± 0.910
3	20	29.00 ± 0.505	2.47 ± 0.432	24.65 ± 0.726
4	13	28.16 ± 0.602	1.49 ± 0.515	24.50 ± 0.865
5	17	28.22 ± 0.516	2.34 ± 0.442	24.97 ± 0.742
6	18	27.72 ± 0.570	2.57 ± 0.487	25.79 ± 0.818
≥ 7	11	28.45 ± 0.631	2.07 ± 0.540	25.2 ± 0.906

N = sample size; SC = scrotum circumference; BCS = body condition score; EBV = estimated breeding value; SMWT = six months body weight. Means with different letters in column within fixed effects are significantly different ($p < 0.05$)

tical model fitted for the data was:

$$y_{ijklmn} = \mu + S_i + B_j + D_k + T_l + L_m + C_n + e_{ijklmn}$$

Where: y_{ijklmn} = Semen characteristics traits of each ram; μ = overall mean; S_i = effect sire age; B_j = effect of sire birth season; D_k = effect of dam parity; T_l = effect of sire birth type; L_m = effect of location of sire within breed; C_n = effect of sire BSC; and $e_{ijklmno}$ = residual effect. Sire selection traits such as six months body weight, estimated breeding value, and scrotum circumference and semen characteristics namely semen volume, motility, concentration, and total spermatozoa were dependent factors. Breeding value was estimated by restricted maximum likelihood method with an animal model using WOMBAT software (Meyer, 2012).

3 Results

3.1 Sire selection traits

The overall least square means ± SE for scrotum circumference, estimated breeding value, and six-month body weight of Bonga sheep were 28.20 ± 0.381 cm, 2.13 ± 0.326, and 24.69 ± 0.547 kg, respectively (Table 1). The effect of sire age, birth type, and BCS of sire was significant ($P < 0.05$) for scrotum circumference (Table 1). Similarly, sires BCS had a significant effect on its estimated breeding value. Also, sire location, birth type, and BCS were significant effects on the six months body weight (SMWT) of a sire ($P < 0.05$) (Table 1).

3.2 Semen characteristics traits

The overall least square means ± SE of semen volume, mass motility, sperm concentration, and total spermatozoa of Bonga sheep was 1.14 ± 0.076ml, 4.28 ± 0.138, 4.25 ± 0.195 billion sperm/ml, and 4.86 ± 0.393 billion sperm/ml, respectively (Table 2). Both semen volume and total spermatozoa were significantly ($P < 0.05$) affected by sire age and BCS. However, none of the studied fixed effects significantly affect mass motility and sperm concentration for Bonga sheep (Table 2). To get quality semen, the scrotum circumference of a breeding sire should be above 26 cm.

4 Discussion

Estimated breeding value and body weight comparisons were done by using sires SMWT. The older sires have better SMWT than the youngers but statistically non-significant. The possible reason may be the selection of a large body-weight sire as a parent might have a positive effect on the body weight of offspring. The scrotum circumference was increasing with aging of the sire. This positive correlation has also been described by Pezzanite *et al.* (2019); Shaaledin *et al.* (2018); Van Metre *et al.* (2012). Similarly, different studies explained that sire body weight and scrotum circumference have a positive association (Al-kawmani, 2019; Elmaz *et al.*, 2007; Gemeda and Workalemahu, 2017; Shaaeldin *et al.*, 2018). In agreement to the current finding on an Ethiopian sheep breeds such as Horro, Bonga, and Menz, there is an observed trend of the average scrotal circumference rising from 25 cm at one year old to almost 30 cm by the age of four. In the case of larger breeds like Awassi, the desired scrotal circumference range is set at 36-38 cm (Rekik, 2016). The yearling body weight of Bonga sheep was 34.0 ± 0.84 kg (Areb *et al.*, 2021). Therefore, based on the positive association of body weight with scrotum circumference; the breed can be grouped under either excellent or satisfactory scrotum circumference. The size of the scrotum circumference of mature Bonga sheep was related to Turkish Kivircik sire (29.68 ± 0.69cm), Arsi (28 ± 0.45cm), and Menz sheep (28.8 ± 0.13cm) (Elmaz *et al.*, 2007; Endale *et al.*, 2009; Goshme *et al.*, 2020). Bonga sheep scrotum circumference was larger than Afar (20.5 ± 2.10cm), Long-eared Somali (21.4 ± 1.67cm), and Woyto-Guji (20.6 ± 1.93cm) goats (Gemeda & Workalemahu, 2017) but smaller than Merino (32.1cm), Dorper (32.3 ± 0.15cm), and cross of Awassi with Menz (30.4 ± 0.13cm) sheep (Duguma *et al.*, 2002; Goshme *et al.*, 2020). The implication of value of scrotum circumference might indicate the size of a sire.

The semen characteristics of Bonga sheep was not statistically affected by SC (Table 2). In terms of numbers, however, SC and semen characteristics are directly related. According to Duguma *et al*, (2002), several authors explained that a sire with a large testis has either high sperm production or higher daily sperm output. This will enable to increase in the fertility of a sire under CBBP. Rapid sperm motility will result in more sperm being able to cross the female reproductive tract and penetrate the outer membranes of the oocyte (David *et al.*, 2015), increase the chance of fertilisation. Similarly, a sire with a high BCS will produce a higher volume of semen with a better motility and higher concentration. Also, BCS had a positive effect on scrotum circumference, EBV, and body weight. As a result, improvement of sire management is important to increase the number of doses for artificial insemination and fertility. At an age of 8 months, a Bonga sire fully expressed male characteristics such as libido, testicle size, semen volume, concentration, and motility. This phenomenon might be the reason for the non-significant for semen characteristics across the studied

Table 2: *Least square means (± SE) for semen characteristics traits Bonga sheep by different fixed effects.*

		Ejaculation	*Mass mobility score*	*Sperm conc.*	*Total sperm.*
Fixed effect	*N*	*(ml)*	*(0 to 5)*	*(10^{12} sperm ml^{-1})*	*(10^{12} sperm ml^{-1})*
Overall	*101*	*28.20 ± 0.381*	*2.13 ± 0.326*	*24.69 ± 0.547*	*4.86 ± 0.393*
CV		*37.39*	*15.41*	*22.90*	*47.74*
Sire age	*P-value*	*0.0638*	*0.5651*	*0.4353*	*0.2516*
4 years	9	0.97 ± 0.140	3.98 ± 0.257	3.99 ± 0.363	3.79 ± 0.728
3 years	33	0.98 ± 0.094	4.43 ± 0.172	4.47 ± 0.242	4.40 ± 0.487
2 years	26	1.06 ± 0.108	4.31 ± 0.197	4.31 ± 0.278	4.74 ± 0.557
1 year	7	1.45 ± 0.174	4.32 ± 0.318	3.94 ± 0.449	5.66 ± 0.900
< 1 year	26	1.27 ± 0.123	4.39 ± 0.224	4.57 ± 0.317	5.75 ± 0.635
Location	*P-value*	*0.6045*	*0.7788*	*0.2394*	*0.9673*
Buta-Shuta	45	1.12 ± 0.087	4.31 ± 0.159	4.37 ± 0.225	4.87 ± 0.450
Boka-Guta	56	1.16 ± 0.083	4.26 ± 0.153	4.13 ± 0.216	4.86 ± 0.433
Birth season	*P-value*	*0.0240*	*0.7196*	*0.6102*	*0.0650*
Dry	23	1.27 ± 0.097a	4.32 ± 0.178	4.33 ± 0.251	5.38 ± 0.505
Wet	78	1.02 ± 0.088b	4.25 ± 0.160	4.19 ± 0.227	4.35 ± 0.455
Birth type	*P-value*	*0.0303*	*0.1779*	*0.4473*	*0.0390*
Single	74	1.04 ± 0.074b	4.17 ± 0.136	4.16 ± 0.192	4.50 ± 0.385b
Twin	27	1.25 ± 0.102a	4.40 ± 0.186	4.35 ± 0.262	5.50 ± 0.527a
SC (cm)	*P-value*	*0.0818*	*0.2744*	*0.2726*	*0.1087*
≤ 25	24	0.95 ± 0.122	4.10 ± 0.223	4.24 ± 0.314	4.01 ± 0.631
26 to 29	48	1.18 ± 0.095	4.21 ± 0.174	4.04 ± 0.245	4.82 ± 0.492
≥ 30	29	1.30 ± 0.102	4.53 ± 0.186	4.48 ± 0.263	5.77 ± 0.528
BCS	*P-value*	*0.0380*	*0.2339*	*0.3514*	*0.0276*
2.5	15	0.93 ± 0.129b	3.99 ± 0.240	4.19 ± 0.338	4.03 ± 0.679bc
3	27	1.03 ± 0.098b	4.05 ± 0.179	3.92 ± 0.253	4.09 ± 0.508bc
3.5	26	1.15 ± 0.098ab	4.28 ± 0.180	4.04 ± 0.254	4.61 ± 0.511bc
4	28	1.32 ± 0.108a	4.32 ± 0.197	4.21 ± 0.278	5.68 ± 0.558ab
4.5	5	1.44 ± 0.180a	4.78 ± 0.329	4.91 ± 0.465	6.74 ± 0.933a
Dam parity	*P-value*	*0.4640*	*0.4371*	*0.8686*	*0.6187*
1	6	1.32 ± 0.167	4.57 ± 0.304	4.65 ± 0.430	5.97 ± 0.863
2	16	1.15 ± 0.124	4.35 ± 0.227	4.21 ± 0.320	4.86 ± 0.642
3	20	0.97 ± 0.104	4.14 ± 0.190	4.34 ± 0.268	4.17 ± 0.538
4	13	1.17 ± 0.119	4.37 ± 0.218	4.19 ± 0.309	4.81 ± 0.619
5	17	1.15 ± 0.102	4.42 ± 0.186	4.08 ± 0.263	4.73 ± 0.528
6	18	1.09 ± 0.113	4.04 ± 0.206	4.09 ± 0.291	4.58 ± 0.585
≥ 7	11	1.17 ± 0.125	4.09 ± 0.228	4.22 ± 0.322	4.95 ± 0.647

Sperm conc. = Sperm concentration; Total sperm. = total spermatozoa; SC = scrotum circumference; BCS = body condition score. Means with different letters in column within fixed effects are significantly different ($p < 0.05$)

age category. Therefore, early selection of sire is possible. Elmaz *et al.* (2007) showed for Kivircik sire that with increased age semen concentration improved but volume and motility did not improved. Semen volume and concentration of Bonga sheep were found to be higher than for Lacaune and Manech tête rousse sires, Turkish Kivircik sire, Spanish dairy sheep, Colombia wool sheep, and Creole sire (Carvajal-Serna *et al.*, 2018; David *et al.*, 2007; Elmaz *et al.*, 2007; Pelayo *et al.*, 2019). However, Bonga sheep semen volume, concentration, and mass motility were lower than found for the Assaf sheep breed (Pelayo *et al.*, 2019). A high score for sperm wave movement of mass motility can guarantee an improvement in fertility in Bonga, sheep but its disadvantage was its subjective assessment. However, sperm mass motility is an indicator of sheep fertility in French AI centres (David *et al.*, 2015).

In conclusion, a sire with a large scrotum circumference and a good BCS produced a high semen volume and the sperm showed at the same time better motility and higher concentration. As a result, both scrotum circumference at the wider part and BCS traits should be given special attention during sire selection in addition to its EBV. In the CBBP, genetic progress over generations was expected through selection and a successful reproductive process. Therefore, not only body weight but also scrotum circumference at the wider part and semen traits namely semen volume, concentration and motility should be taken into account when selecting a sire. To improve farmer's participation, their trait preference should be considered such as non-black colour and hornless. A limitation of the current study was that its inability to incorporate health parameters.

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Conflict of interest

The authors declare that they have no conflict of interest.

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