RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v22i3.10

Madziga *et al.*/Sokoto *Journal of Veterinary Sciences*, **22**(3): 214 - 222.

Evaluating the marbling potential of four indigenous Nigerian cattle breeds

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Publication History:
Received: 21-06-2024
Revised: 14-08-2024
Accepted: 17-08-2024

Abstract

This study investigated the peculiarities of cattle marbling. Four breeds of cattle were raised in an experimental setting before being slaughtered. Azawak, Bunaji, Rahaji, and Sokoto Gudali bulls were used; with an age range of 1½ to 2 years old, and their live weights were 200–235 kg. Through computerized image analysis, marbling features in the *longissimus* muscle (LM) and semitendinosus muscle were identified and categorized. There were variations in the amount, composition, and arrangement of the marbling specks in both muscles among the breeds. Compared to other breeds, Azawak bulls have significantly less fat deposited on them. Marbling fleck regions were bigger ($P < 0.05$) in Rahaji bulls. Rahaji still had the largest ($P < 0.05$) number and the most consistent ($P < 0.05$) distribution of the marbling specks. Additionally, when compared to the other breeds under investigation, the marbling features of Rahaji bulls displayed a higher quantity and a slightly finer structure, followed by Sokoto Gudali. There was an increase ($P < 0.05$) in the proportion of marbling flecks as the structure of the flecks grew larger ($P < 0.05$) and coarser. As the proportion and quantity of marbling flecks increased, the distribution of these particles was regular ($P < 0.05$). The findings imply that adipocyte hyperplasia contributes significantly to marbling in cattle during muscle growth.

Keywords: Adipocytes, Breed difference, Cattle, Growth, Hyperplasia

Introduction

Nigeria is by far the biggest livestock producer in Central and West Africa in terms of both population and animal production capacity, holding 25% of the subregion's herds. Nearly twenty-two million (22,378,374) native cattle make up its national herd; these are mostly multipurpose breeds (Abubakr, 2022). Adamawa Gudali, Azawak, Bunaji, Keteku, Kuri, Muturu, N'dama, Rahaji, Shuwa Arab, and Sokoto Gudali are some of these breeds which are mostly Zebu or *Bos indicus* varieties, are believed to be in the country's northern regions FAO (2021).

Marbling refers to the white flecks and streaks of intramuscular fat visible within the lean parts of meat, resembling a marble pattern. This fat enhances flavour, tenderness, and juiciness, playing a key role in determining the quality and grading of meat cuts, particularly beef (Tan & Jiang, 2024). Marbling affects meat's juiciness, tenderness, texture, and flavour—attributes that determine the "eating experience." In this case, more of the above is better. Intramuscular fat should not be confused with intermuscular fat, which is the fat between the muscles. That fat, which is typically trimmed off, does not enhance a piece of meat. Marbling has a very positive effect on the eating quality of some cuts but it is only one of the many factors affecting eating quality. High-quality cuts from young cattle that have low marbling can have good eating quality, however, cuts from high-marbling carcasses can fail to grade if other factors are poorly managed. All factors that interact to determine eating quality need to be managed together. However, where all else is equal, enhanced marbling will improve eating quality (MLA, 2018).

Adipocytes implanted in a connective tissue matrix near a blood capillary network constitute the cellular component of marbling (Park *et al*., 2018). Vopri–Lagreca *et al*. (2021) discovered a quadratic pattern for the development of marbling scores in *longissimus* muscle (LM). On the other hand, Sperber *et al*. (2024) found that both the intramuscular fat content and the marbling score steadily rise during growth. As a result, fat in LM does not always build slowly.

The specific characteristics of marbling are provided by computerized image analysis (CIA), a technique for quantitatively quantifying visible fat in muscle (Aredo *et al.*, 2017; Mendizabal *et al*., 2021; Erena *et al.*, 2024). A technique developed (Cai *et al*., 2024) uses pigmented muscle slices to increase contrast and allow the distinction between muscle, connective tissue, and fat. Using this technique allows for the inclusion of even the smallest marbled particles in the measurement.

Prior studies by Lamidi *et al.* (2007) and Madziga *et al*. (2013) on institutional beef cattle fattening trials in Nigeria focused exclusively on the performance of the Zebu breeds, which tend to fatten at low weights of roughly 250–330 kg, the weight at which few months old temperate breeds do. There is currently a dearth of research in Nigeria on the effects of breed on marbling features, such as the distribution, size, and quantity of marbling flecks which can provide new insights into the accumulation of intracellular fat. However, according to personal correspondence with a former Executive Director of the National Animal Production Research Institute (NAPRI) at Ahmadu Bello University Zaria, Nigerian cattle breeds are capable of performing well in terms of marbling. During one of the Institute's Seminar series, when he was still the Executive Director, he stated that the Azawak breed of cattle has the potential to become as fat and marbled as the Bunaji (White Fulani) breed. The acceptance of beef by its consumers is directly related to sensory attributes including tenderness, juiciness and flavour and marbling is one of the major factors perceived for these. To the best of our knowledge, there is a dearth of information on the marbling ability of the indigenous cattle breeds in Nigeria hence the purpose of the current study was to evaluate the marbling capacity of four breeds of Nigerian cattle under the same management, based on the notion that Azawak marbles like Bunaji.

Materials and Methods

Study site

The investigation was conducted at the NAPRI in Shika, Zaria. Shika is situated in the Northern Guinea Savanna Zone of Nigeria, between latitudes 11°N and 12°N and longitudes 7°E and 8°E, at an elevation of 640 meters above sea level (Encarta, 2009). The rainy season, which starts in April or May, settles in June and lasts until October is well-known for this zone. Seasonally, the maximum temperature ranges from 27 to 35℃. The average yearly rainfall is 1100 millimetres, and the relative humidity is about 72%. A period of cold, dry weather known as harmattan, which occurs from October to January heralds the beginning of the dry season. From February to April, dry, hot weather follows the harmattan. During this time, the relative humidity is 37%. The research was conducted between June and September.

Animals and design of experiment

Ten bulls from each of the Bunaji, Rahaji, Sokoto Gudali, and Azawak breeds were used. The bulls were 1½ to 2 years old, with a live weight of between 200 and 235 kg. After the bulls were bought from the open market in the states of Kaduna, Yobe, and Sokoto, they were quarantined for eight weeks. Albebdazole® and Amitix® solution were used to deworm the bulls and were dipped against endo- and ectoparasites, one week before the trial started. *Digitaria smutsii* hay served as the experiment's basal diet, and a concentrate consisting of 20% cotton seedcake, 60% maize offal, and 20% poultry litter was used (Table 1).

The bulls were kept in individual pens in a completely randomised design (CRD) arrangement, with each breed serving as a treatment. At 9.00 hours, the animals were fed their diets following the collection

Table 1: Chemical composition (%) of *Digitaria smutsii* hay, maize offal, poultry litter, cotton seed cake and the concentrate diet

Ingredients					
Nutrients%	*D. Smutsii*	Maize offal	Cottonseed cake	Poultry Litter	Concentrate diet
Dry Matter	92.72	91.92	92.99	92.71	92.37
Organic matter	83.90	81.87	88.01	65.05	80.82
Crude protein	5.86	14.31	30.88	26.13	19.63
Ether Extract	4.05	8.03	11.05	4.40	10.01
Crude Fibre	41.09	31.21	37.23	21.05	27.40
Neutral Detergent Fibre	68.89	53.92	50.21	60.26	35.95
Acid Detergent Fibre	42.35	34.99	42.35	35.95	51.27
Ash	8.82	10.05	4.98	27.66	11.55
ME (MJ/kg DM)	11.03	10.88	11.51	11.09	10.52

The ME values of the experimental feeds' ingredients were calculated as per Alderman & Contril (1985) as follows:
ME = (MJ/kg DM) 11.78+0.00654CP+ $(0.000665EE)^2$ – CF (0.00414EE) -0.0118A
Where DM = Dry Matter, CP = Crude Protein, EE = Ether extract, CF = Crude Fibre, A= Ash

and weighing of the refusals from the previous day's feeding. Prior to hay *ad libitum*, each animal was fed a concentrate ration equivalent to 2% of its body weight at each meal. Each animal's concentration feeding trough received a cube of mineral salt. The animals were given 30L of fresh water each morning. To determine the voluntary feed intake, daily records of the amount of feed provided and refused were taken. Every two weeks, the bulls' weights were recorded, and the amount of feed provided was modified correspondingly. At the start and finish of the experiment, their starting and final weights were recorded. The study lasted for ninety days.

Tissue collection

After the animals were slaughtered, LM and semitendinosus muscles were harvested from the left side of the carcasses and were refrigerated at 4℃ for 24 hours. After removing any extra fat, the carcass was reweighed. Two 2 cm thick muscle slices were extracted from LM's 12^{th} ribs and the thickest part (muscular belly – middle portion) of the semitendinosus muscle. A single muscle slice was used to determine the fat content. Before being immersed in the oil red O stain solution for six to eight hours, muscle slices were fixed in 5% formaldehyde, cut into smaller pieces (2 mm), and rinsed in distilled water for at least twelve hours. The parent solution of oil red O was made by dissolving 0.5 grams of stain in 100 milliliters of pure isopropanol. This was diluted 3:2 (3 parts oil red 0 solution to 2 parts water) using distilled water and filtered to achieve a usable solution. Staining slices were rinsed for a minimum of 12 hours, differentiated with 70% isopropanol for two to four hours with continuous, and then rinsed for an extra night, as stated by Cai *et al.* (2024).

Marbling

Using photographic standards, marbling was graded on a 6-point scale, with 1 representing extremely low marbling and 6 representing extremely high marbling. Only one skilled operator completed the scoring to exclude subjective variations between operators.

Computerized image analysis (CIA)

According to Cai *et al.* (2024), a CIA system (Quantimet 570, Cambridge Instruments, Leica, Bensheim, Germany) was used to quantify the marbling features in the images taken with a color video camera (DXC-930P, Sony, Japan). To put it briefly, the measurement began with the cross-sectional area of the muscle. Later, a preprocessing method improved the transitions between the muscular backdrop and the marbling particles. The number of marbling flecks, the proportion of marbling fleck area (calculated by dividing the total marbling fleck area by the area of muscle), and the size of marbling flecks (calculated by calculating the average marbling fleck area) were the three quantitative marbling characteristics that were ascertained as a result.

There were three measurements made of the structural characteristics: 1) the maximum skeleton line length of marbling flecks; 2) the proportion of the three largest areas (found by dividing the total marbling fleck area by the sum of the three largest areas); and 3) the proportion of long marbling flecks (found by dividing the total marbling fleck area by the total number of long-shaped areas (identified by a shape factor).

The two distribution traits that were examined were: 1) the number of marbling flecks and 2) the regions of marbling flecks. Lower values indicate a more even distribution for the latter two features, which were computed as coefficients of variation of the number or total marbled fleck area in 8 (LM) or 4 (semitendinosus muscle) regions of the muscle cross-section.

Statistical analysis

The General Linear Model technique of the Statistical Analysis System (SAS, 2002) was used to examine all study data to assess the performance of the four cattle breeds. Significant differences between treatment means were compared using the SAS package's Duncan Multiple Range Test.

Y_{ij}=μ+ B_i + e_{ij} was the feed intake model that was em ployed.

Where e_{ij} stands for random error, Bi for the breed's effects on 1, 2, 3, and 4, μ for overall mean, and Y_{ij} for breed observations. Every statistical test was conducted with a 5% probability threshold.

Results

Table 2 displays the fat content and marbling. According to the findings, Rahaji had the greatest (P<0.05) fat level at 1.98%, while Azawak had the lowest fat percentage at 1.12%. In the same vein, the marbling reveals that the Bunaji breed had the lowest value (1.21) while Rahaji had the highest value (1.8).

As shown in Table 3, the proportion of the marbling fleck area and the number and size of the marbling flecks increased (*P*<0.05) in the muscle cross-sections of both muscles. This development is different among breeds. In the longissimus muscle (LM), Rahaji had the greatest (P<0.05) value (8.7%) and Azawak and Bunaji had the lower marbled fleck numbers, according to Table 3`s distribution. In the same vein, Rahaji had The largest marbling fleck area in LM (11.5%), whereas Azawak and Bunaji had lower values. The distribution of marbled flecks in the semitendinosus muscle was highest (P<0.05) in Rajaji and lowest (P<0.05) in Azawask and Bunaji (9.78%). Rahaji had the highest (P<0.05) marbled fleck area distribution (27.2%). Additionally, the results indicate that Rahaji had the largest proportion of marbled fleck area in LM (P<0.05) at 75%, while Azawak had the lowest value, recorded at

Table 2: Fat content and marbling scores of samples in LM of different cattle breeds

	Breed			
Trait	Azawak	Bunaji	Rahaji	S/Gudali
Fat content (%)	1.12±0.012[b]	1.34±0.014[b]	1.98±0.022[a]	1.69±0.018[a]
Marbling	1.5±0.016[b]	1.2±0.013[b]	1.8±0.02[a]	1.6±0.017[a]
No of samples	10.0±0.111	10.0±0.111	10.0±0.111	10.0±0.111

[a, b] Means bearing different superscripts within the same row differ significantly (P<0.05)

Table 3: Distribution and proportion of marbling fleck area located ventral in LM and cranial in semitendinosus muscle of different cattle breeds

Trait	Breed			
	Azawak	Bunaji	Rahaji	S/Gudali
Distribution				
LM				
Distribution of marbling fleck number (%)	3.5±0.039[b]	3.6±0.04[b]	8.7±0.089[a]	7.8±0.087[a]
Distribution of marbling fleck areas (%)	7.8±0.087[c]	8.7±0.089[c]	11.5±0.127[a]	10.5±0.117[b]
Semitendinosus				
Distribution of marbling fleck number (%)	10.8±0.12[b]	9.7±0.107[c]	18.70.207[a]	11.1±0.123[b]
Distribution of marbling fleck areas (%)	19.1±0.212[c]	19.8±0.22[c]	27.2±0.302[a]	20.3±0.226[b]
Proportion of marbling fleck area				
LM				
Proportion of marbling fleck area (%)	62.5±0.694[c]	63.1±0.701[c]	75.0±0.833[a]	67.9±0.744[b]

[a, b] Means bearing different superscripts within the same row differ significantly (P<0.05)

Table 4: Quality and structure traits of marbling in LM and semitendinosus muscle of different cattle breeds

Trait	Breed			
	Azawak	Bunaji	Rahaji	S/Gudali
Quantity				
LM				
Proportion of marbling fleck area (%)	3.21±0.035^{c}	3.22±0.035^{c}	3.92±0.044^{a}	3.51±0.039^{b}
Number of marbling flecks	166±1.844^{c}	167±1.855^{c}	287±3.188^{a}	254±2.822^{b}
Size of marbling flecks (mm^2)	0.97±0.011^{c}	0.99±0.011^{c}	1.32±0.015^{a}	1.12±0.012^{b}
Semitendinosus				
Proportion of marbling fleck area (%)	2.14±0.024^{b}	2.17±0.024^{b}	2.60±0.029^{a}	2.56±0.028^{a}
Number of marbling flecks	71±0.789^{c}	73±0.811^{c}	114±1.267^{a}	85±0.944^{b}
Size of marbling flecks (mm^2)	1.05±0.011^{d}	1.08±0.012^{c}	2.05±0.023^{a}	1.96±0.022^{b}
Structure				
LM				
Proportion of 3 largest marbling fleck areas (%)	26.6±0.296^{c}	28.7±0.318^{b}	36.7±0.408^{a}	29.2±0.324^{b}
Length of maximum skeleton line (mm)	3.38±0.038^{c}	3.40±0.038^{c}	6.01±0.067^{a}	4.78±0.053^{b}
Proportion of long marbling flecks (%)	50.5±0.561^{c}	57.2±0.636^{b}	68.3±0.758^{a}	66.6±0.74^{a}
Semitendinosus				
Proportion of 3 largest marbling fleck areas (%)	38.8±0.426^{bc}	39.8±0.442^{b}	47.8^{a}±0.531	40.8±0.453^{b}
Length of maximum skeleton line (mm)	3.30±0.0367^{c}	3.34±0.0379^{c}	5.39±0.599^{a}	5.09±0.066^{b}
Proportion of long marbling flecks (%)	55.6±0.618^{c}	56.9±0.632^{b}	76.2±0.847^{a}	75.9±0.843^{a}

$^{a, b}$ Means bearing different superscripts within the same row differ significantly (P<0.05)

62.5%. The fraction of marbling fleck area in the semitendinosus muscle reveals that Bunaji recorded the lowest value at 68.7%, while Rahaji still maintains the highest value at 83.6% (P<0.05). According to the quality and structural characteristics of marbling in the LM and semitendinosus muscle of the cattle breeds shown in Table 4, Rahaji had the largest (P<0.05) number of marbling flecks in both muscles, with 2.6 % in the semitendinosus muscle and 3.92 % in the LM muscle. The next highest (P<0.05) was obtained by Sokoto Gudali, followed by Azawak and Bunaji. Rahaji exhibited the greatest (P<0.05) count of marbling flecks in both muscles (287 in the LM and 114 in the semitendinosus muscle). Sokoto Gudali, with 254 in LM and 85 in semitendinosus muscle, came next. With 166 in the LM and 71 in the semitendinosus muscle, Azawak had the lowest count in both muscles. The proportion of the 3 largest marbling fleck areas Table 4) is great if the marbling is dominated by large marbling flecks. The proportion of the 3 largest marbling fleck areas in the LM decreased (*P* < 0.05) during growth. The proportion of the area of long marbling flecks in LM increased during growth in all the breeds. However, Azawak and Bunaji bulls showed no changes (*P* > 0.05) in this trait in LM. This trend of marbling flecks becoming longer as the fattening days increased was clearer in the semitendinosus muscle. In all 4 breeds, the proportion of the area of long marbling flecks increased (*P* < 0.05) during growth.

Rahaji had the largest marbling flecks (P<0.05) in both muscles (1.32 mm^2 in the LM and 2.05 mm^2 in the semitendinosus muscle), according to the evaluation of fleck size. In both muscles, Azawak had the smallest specks (0.97 mm^2 in the LM and 1.05 mm^2 in the semitendinosus muscle). Rahaji possessed the greatest (P<0.05) percentage of the three largest marbling fleck areas in both muscles (36.7% in the LM muscle and 17.8% in the semitendinosus muscle) in terms of structure. Rahaji also had the longest maximum skeleton line (P<0.05) (6.01 mm^2 in LM and 5.39 mm^2 in semitendinosus muscle). In Rahaji and Sokoto Gudali, the long marbling fleck proportion was highest (P<0.05) in both muscles (68.3 and 66.6% in LM and 76.2 and 75.9% in semitendinosus muscle).

Discussion

Differences existed in most of the parameters considered in the current study. These variations are in line with the findings of many researchers. The current study's results on fat content and marbling align with those of Martinez *et al*. (2023), who found a favourable correlation between beef cow fat content and marbling scores. Similarly, Park *et al*. (2018) discovered a correlation between increased marbling ratings and increased longissimus muscle fat content. The results of this study support those of Lee & Choi (2019), who found a positive correlation between marbling scores and the marbling fleck distribution for quantity attributes of marbling. In a similar line, Kim *et al.* (2020) study discovered a correlation between increased marbling fleck area and increased fat content in the LM.

The results of this study's evaluation of the marbling structural attributes in LM and Semitendinosus muscle are consistent with those of Lee & Choi (2019), who found that more developed marbling structure in beef cattle was associated with higher levels of marbling quality and fat content. The current study's findings also support a report by Park *et al.* (2018) that found a correlation between greater marbling scores and larger marbling flecks. Since the weight of the animal at slaughter impacts the carcass yield and, consequently, the fat content and the marbling ability, the variations in the results obtained in the parameters evaluated across all the breeds in this study may be related to breed-specific weight variances (Nguyen *et al*., 2021). The outcome of this study validates the findings published by Pečiulaitienė *et al*. (2015) about the impact of age and pre-slaughter weight of various dairy breeds and their hybrids with beef breeds on carcass muscularity class and carcass yield.

Research on the correlation between marbling and meat quality frequently refers to eating quality, which is measured by tenderness, juiciness, and flavour, as well as subjective marbling scores (Thompson, 2004; Shahrai *et al.*, 2021). Marbling has more of an impact on flavour and juiciness, with a relatively little and varied effect on tenderness (Warner *et al.*, 2021). As a result, meat graders in abattoirs evaluate marbling as a reliable indicator of meat quality. An earlier quantitative and morphological study of i.m. fat accumulation in cattle LM was presented by Jaborek *et al*. (2023). A great deal of work has gone into creating techniques to improve the impartiality and repeatability of marbling evaluation.

The prediction of beef's internal fat content or marbling score is already being done using computerized image analysis (Ozkaya., 2012; Stinga *et al.*, 2020; Aredo *et al.*, 2017). The most recommended technique for obtaining these data is the CIA.

In comparison to subjectively rated marbling in beef (Cai *et al.*, 2024) and pork (Teixeira *et al.*, 2021), marbling features measured by the CIA present a far more diverse image (Figure 1). By using the CIA to estimate the size of marbling specks, Erena *et al*. (2024) divided them into three size classes. There were more little marbling particles. Nonetheless, there was a larger summed fat area from the big marbling specks. Furthermore, they concluded that the big marbling specks exaggerate the marbling scoring. In the current study, we also found that the appearance of greater fat streaks overestimates marbling. On the other hand, marbling is undervalued if there are only a few tiny marbling flecks. However, the presence of membrane phospholipids and muscle fiber lipids in the retrieved fat further limits the precision of the chemical extraction techniques. Stain-treated muscle slices can be objectively measured without any restrictions of any kind. A single, tiny, marbled fleck made up of a few adipocytes can provide a different outcome than zero. As a result, the CIA statistics are clearer and more helpful for comparing breeds and changes across time.

Most research on marbling development focuses on LM and the final stage. Regressing marbling scores versus days on feed, Vopri–Lagreca *et al.* (2021) demonstrated that marbling developed quadratically before reaching a plateau, which occurred when animals reached their genetic potential to deposit intramuscular fat. Peña *et al*. (2013) demonstrated in their tests that, animals with high marbling at slaughter had higher beginning values and increased at a faster rate of marbling over a 180-day feeding period. Sperber *et al*. (2024) came to a similar conclusion that fat in LM is not always a late-developing trait. Their findings demonstrated that there is a continuous rise in i.m. fat during growth when regressed as a growth component over HCW. Park *et al*. (2018) proposed in their study that producers should concentrate on initiating more preadipocytes in the muscles of animals that are genetically predisposed to marble. These preadipocytes differentiate and fill with lipids to create evident marbling during finishing. Additionally, Tan & Jiang (2024) reported that there is a phase of preadipocyte hyperplasia in addition to a significant hypertrophy of adipocytes during the postnatal expansion of intraperitoneal fat. Our findings on the quantity of marbling flecks imply that hyperplasia is a significant factor in marbling. Only with the recruitment and filling of preadipocytes do new marbling flecks form.

According to the results of this study, there is more fat in the semitendinosus muscle compared to previously published data on this topic (Picard & Gagaoua, 2020). This means that semitendinosus has a larger percentage of marbling fleck area. Drey *et al.* (2019) reported that the semitendinosus muscle experiences a smaller rise in fat content throughout growth. A lower mean size results from LM because more little marbling flecks—that is, smaller marbling flecks—are formed and marbling fat has a finer structure and a more uniform distribution of marbling flecks throughout the muscle cross-section. The reason that could be advanced for the differences in the results of the present study and previous studies might be due to breed and management as well as the environment as all these are factors that affect marbling. More important is the method employed as it does not give the exact results but an estimate.

The development of fat depots is similar in both muscles, beginning in the deeper layers of the muscle, near large blood vessels, where nutrients are better provided. Li *et al.* (2020) described a connection between the size of arterioles and the size of fat depots. In the semitendinosus muscle of the investigated breeds in the current study, often large blood vessels were surrounded by fat and located near the branching points of the large connective tissue streak. These little marbling particles combined into a single, thick streak in bigger animals. Further flecks of marbling were visible at a considerable distance from the central major blood vessels in the tertiary muscle fibre bundles.

In conclusion, the CIA data not only accurately depict the subjectively determined marbling score in an objective setting, but also bridged the gap between the macroscopically observed marbling and the microscopically observable adipocytes. The given results demonstrate marbling in several cattle breeds concerning the number, structure, and distribution of marbling flecks for the first time using CIA hence the hypothesis that Azawak marbles like Bunaji is proven although both breeds marble less than Rahaji and Sokoto Gudali.

Acknowledgement

The authors are grateful to the Executive Director and Management of the National Animal Production Institute, Ahmadu Bello University, Zaria for funding this research. All staff of the Beef Research Programme, NAPRI are being appreciated for managing the bulls.

Funding

This research was funded by the National Animal Production Research Institute, Ahamdu Bello University, Shika–Zaria, Nigeria.

Conflict of Interest

The authors declare that there is no conflict of interest.

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