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Effects of different feeding systems on growth, fat accumulation and semen quality of Merino-type sheep

A.M. du Preez[1], E.C. Webb[1#] & W.A. van Niekerk[1]
[1]Department of Animal Science, Faculty of Natural and Agricultural Sciences, University of Pretoria, P/Bag X20, Hatfield, Pretoria, 0028 South Africa

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Abstract

In South Africa, supplemental feeding is provided to improve the condition of breeding livestock before the animals are sold by the stud breeders to commercial farmers. This study aimed to evaluate the effects of different ram rearing systems on growth, fat accumulation and semen quality of Döhne Merino (DM), Merino (M) and South African Mutton Merino (SAMM) rams intended for breeding. The ram lambs were stratified according to weight and randomly allocated to one of three treatments, namely an extensive feeding treatment (ET), an extensive-intensive feeding treatment (EIT), and an intensive feeding treatment (IT). Rams in ET grazed for 200 days. Rams in EIT grazed for 73 days, followed by a concentrate diet for an additional 60 days. Rams in IT were fed the concentrate diet for 70 days. Scrotal neck fat was significantly less in IT rams compared with ET and EIT rams. Döhne Merino and M rams in EIT deposited significantly more scrotal fat than DM and M rams in ET. No adverse testicular pathology or differences in semen quality were observed. Regression analyses of pooled data showed extreme functional values for subcutaneous fat and total scrotal fat weight, which, when exceeded, may result in decreased semen volume, percentage normal spermatozoa and mass motility of spermatozoa. Sheep breeders should be cautious when feeding ram lambs in extensive-intensive systems for extended periods since this may affect semen quality adversely.

Keywords: percentage normal spermatozoa, over-conditioned, scrotal neck fat, semen volume
[#]Corresponding author: edward.webb@up.ac.za

Introduction

It is widespread practice for South African sheep farmers to feed supplements to rams before mating and before auction. This improves testicular growth, maximizes sperm production, and improves their condition, but producers should have their animals 'fit not fat', and thus not overfeed their rams (Combrink & Schoeman, 1993). Swanepoel *et al.* (2008) found that feeding high energy diets (11 MJ ME/kg DM) to eight-month-old beef bulls had detrimental effects on the percentage live sperm, percentage motile sperm, percentage dead sperm, percentage sperm defects and semen volume, although few published studies are available on young growing rams. The results of Swanepoel *et* al. (2008) confirmed that bulls fed a high energy diet (11 MJ ME/kg DM) had 36% more inactive seminiferous tubules, more tubuli demonstrating severe atrophy, and more scrotal fat deposition compared with bulls fed medium (7.5 MJ ME/kg DM) and low energy (7 MJ ME/kg DM) diets. The limited number of studies on rams evaluated only testes growth, fat deposition, and semen parameters, and not the histo-pathology of the testes. Further, these studies in mature rams reported conflicting results (Bester *et al.*, 2004; Fourie *et al.*, 2004; Kheradmand *et al.*, 2006).

Bester *et al.* (2004) tested the effects of various dietary energy concentrations (8.23 MJ ME/kg DM, 9.77 MJ ME/kg DM and 11.32 MJ ME/kg DM) on scrotal and semen traits in year-old Dorper rams and found that most of the fat deposited in the scrotum was set down in the neck of the scrotum, around the *Pampiniform venous plexus* (PVP), and that the amount of fat was related to the energy concentration of the diet. Although scrotal fat increased with increasing dietary energy intake, no differences were observed in semen volume, semen concentration, overall sperm motility, progressive motility or percentage normal and live sperm. The study by Kheradmand *et al.* (2006) produced similar results in 11- to 14-month-old rams.

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These contrast with the findings of Fourie *et al.* (2004), who observed adverse effects on progressive sperm motility, linear sperm progression, overall motility, and semen concentration in intensively fed (9.5 MJ ME/kg DM) year-old Dorper rams compared with extensively reared rams. This was attributed to poor thermoregulation of the testes owing to excessive fat deposition around the PVP. Bester *et al.* (2004), Fourie *et al.* (2004), and Kheradmand *et al.* (2006) observed improved testicular development (heavier testes weights and higher testes volumes) as the dietary energy concentrations of the feed increased.

Although the effects of nutrition on the fertility of older rams have been studied, the effects of different feeding systems to condition young growing ram lambs from weaning to auction have not been researched adequately on their semen quality in the subtropics. The study investigated the effects of various current feeding systems on the growth, gonadal development, scrotal fat accumulation, and semen quality of M, DM, and SAMM ram lambs during the growth phase (five to twelve months old).

Materials and methods

This research was approved by the Animal Ethics Committee of the University of Pretoria, reference number EC 027-08. The study was conducted at Vrede Veld Ram Club in Vrede district of the Free State province of South Africa, at latitude of 27.45° S and longitude of 29.15˚ E, altitude of 1675 m above sea level and average annual rainfall of 524 mm per year. The biome type in Vrede is classified as mixed Grassland (Acocks, 1975; Van Oudtshoorn, 1992) and has a flat and rolling topography. Both sweet and sour grasses are prevalent in this biome, but sour grasses predominate in the more acidic soils and higher rainfall areas (625 mm per year). Throughout the biome, C4 grasses dominate, but at higher altitudes C3 grasses prevail. *Themeda trianda* is the most prevalent grass type in this region (Mucina & Rutherford, 2006).

The study consisted of three feeding treatments, which represent three feeding practices that are commonly used for sheep production in South Africa. These treatments were extensive (ET), extensive-intensive (EIT), and intensive (IT). Ninety weaned five-month-old ram lambs, representative of DM, M, and SAMM, were stratified according to weight and randomly divided into one of these treatments. The ram lambs were all on rangeland before weaning and were all from farmers in Vrede district. Some ram lambs came from the same farmers, which made them genetically more similar. Before the onset of the trial, all the ram lambs were weighed, aged, and ear-tagged. The ram lambs were evaluated for structural soundness by a veterinarian, and sheared by a team from BKB Pty Ltd. Only animals with no anatomical deformities and with two symmetrical well-developed testes of normal consistency were selected. The three treatments differed in duration, and ET continued into another season, but the experimental design aimed to replicate what happens in the local sheep industry.

The extensive treatment is the most common feeding practice of South African sheep farmers. A total of 30 ram lambs (10 DM, 11 M, 9 SAMM) were reared on *Themeda trianda* grazing and a production lick was provided for the entire feeding period of 200 days.

The second treatment group was a combination of the extensive and intensive production systems, which is similar to the system used by Veld Ram Clubs in South Africa. This treatment had two phases, a 70-day rangeland feeding phase, followed by a 60-day intensive feeding phase, which comprised a treatment period of 133 days. A total of 10 DM, 10 M and 10 SAMM ram lambs were included in the EIT.

In the IT a total of 30 ram lambs (10 DM, 10 M, and 10 SAMM) were adapted for 21 days and then fed intensively for 70 days. The IT was short because the lambs in this study were still owned by the farmers, needed to sell them to recover costs. All rams were transported and slaughtered at Vrede Abattoir after the completion of each feeding treatment.

Rams in ET and the first phase of EIT grazed typical *Themeda trianda* on the mixed Sourveld of the Highveld. The ram lambs received a production lick supplement containing about 8 MJ ME/kg and 170 g/kg crude protein, on a dry matter basis, which is similar to the production lick used by Vrede Veld Ram Club. The raw materials used in this lick were salt (17%), Molatek Master 20 (Mhlati Farm, Malalane, Mpumalanga, South Africa) (21%), ground maize (31%), urea (2%), cottonseed oilcake (22%), fishmeal (2%), ammonium sulphate (2%), feed lime (2%), and BASF vitamins and minerals (1%). The lick was given ad libitum, and the average intake was estimated to be 100 g/ram/day.

The second phases of EIT and IT received the same commercial concentrate diet, which contained on a dry matter basis 11.4 MJ ME/kg, crude protein 13.5%, crude fibre 12%, 0.8% calcium, 0.35% phosphate, and 0.8% urea. The raw materials were maize meal, hominy chop, lucerne, cottonseed oilcake, molasses meal, feed lime, grade 1 salt, urea, vitamins, trace minerals, and lasalocid sodium. It was provided by Vrede Saamstaan Veevoere (Church Street, Industrial Area, Vrede) and was similar to the diet used by Vrede Veld Ram Club during their finishing off phase. The ram lambs were fed twice daily to stimulate intake, which was estimated to be 350 g/ram/day.

At the beginning of the study, after the ram lambs had been shorn, bodyweight was recorded with a scale, and body condition scores (BCS) of all the ram lambs were assessed. Body condition scoring was based on the principles described by Keinprecht *et al.* (2016) on a modified 9-point scale to simplify the 0.5 scoring on the 5-point scale. The numeral 1 represented an emaciated ram and 9 represented an extremely well-conditioned ram. Bodyweight, BCS, and average daily gain (ADG) were recorded monthly at the same time of day and at the end of every treatment after the rams had been shorn. Carcass weight, subcutaneous fat thickness, measured at the 13th rib, and carcass classification score were recorded at slaughter. This was used to compare the physiological age of the rams.

Scrotal circumference was measured with a flexible measuring tape (scrotum was wool free) while the ram lambs were in a standing position. The testes were palpated to the distal part of the scrotum and the measurement was taken at the widest part. The scrotum and testes were palpated to ensure symmetry and normal consistency. The epididymides were also palpated to ensure that there were no abnormalities. At the end of every treatment, scrotal circumference was measured again (any wool was shorn). Ultrasound scans of the neck of the scrotum of each ram were taken a week before the treatment's slaughter date. The ultrasound showed the fat accumulation in the neck of the scrota, around the *PVP*, which was used to determine the correlation between subcutaneous fat thickness and the fat accumulation in the scrotal neck, and the correlation between scrotal neck fat thickness and semen quality.

When the rams had been slaughtered, the total scrota were weighed. The scrota were then dissected into testes and scrotal fat. The testes were weighed, and the volume, width, length, and circumference were measured. Scrotal fat weight was recorded. All these measurements were correlated with the results from the semen and sperm analyses. After slaughter, the testes of all rams were collected and put into 10% formalin for histo-pathological analyses at the Faculty of Veterinary Science, University of Pretoria.

Semen samples were collected from the conditioned rams and analysed with an electro-ejaculator (Chella *et al.*, 2017). Semen samples were collected a week before the scheduled slaughter dates for each treatment. Standard gross evaluation procedures were conducted, which included semen volume, colour, total motility, forward progressive motility, percentage aberrantly motile spermatozoa, percentage immotile/dead spermatozoa, and percentage normal spermatozoa (Bester *et al.*, 2004; Fourie *et al.*, 2004; Elmaz *et al.*, 2007; Swanepoel *et al.*, 2008).

Gross analysis was done on farm by microscopic analysis. A calibrated test tube was used to measure the total volume of the ejaculate. The colour of the semen, which provides an indirect indication of sperm concentration, was determined on a scale of 0 to 5, where 0 represented a clear or cloudy appearance (low sperm concentration) and 5 represented a thick creamy appearance (high sperm concentration) (Bester *et al.*, 2004; Fourie *et al.*, 2004; Swanepoel *et al.*, 2008; Chella *et al.*, 2017).

To determine the percentage of normal spermatozoa and the morphology of the spermatozoa (normal vs abnormal) thin smears were made using 5 µl of dye (eosin-nigrosin and eosin B-fast green stains) and about 10 µL of semen (Tufarelli *et al.*, 2011). Two counts of 100 spermatozoa were evaluated, and the results were presented as percentages. Abnormal sperm cells were divided into major defects (knobbed acrosomes, abnormal loose heads, pyriform, dag defects, mid-piece reflexes, degenerative heads) and minor defects (distal droplets, normal loose heads, loose acrosomes, and curled end-piece) (Vilakazi & Webb, 2004; Palmer *et al*., 2005; Swanepoel *et al.*, 2008).

Samples were then taken to the Faculty of Veterinary Science, Department of Theriogenology, University of Pretoria, for microscopic analysis by computer-assisted sperm analyser (Kumar *et al.*, 2016). Semen samples were prepared as described by Kumar *et al.* (2016). Mass and progressive motility were evaluated. Mass motility was scored on a scale from 0 to 5 where 0 represented no swirl, with only sporadic oscillation, and 5 was a rapid and vigorous swirl. Each sperm cell's progression was determined by on a scale of 0 to 5, where 0 indicated no movement and 5 indicated rapid and vigorous forward movement (Tufarelli *et al.*, 2011).

Statistical analyses were done using the general linear model (GLM) procedure of IBM SPSS Statistics v. 23.0 software (IBM Corp., Armonk, New York, USA). The Bonferroni correction for multiple testing was used to determine significant differences among treatments, breeds, and their interaction. Differences were tested at the $P < 0.05$ level of confidence. To correct for ET entering a different season, season was included as a random factor. Non-linear regression analyses were conducted to assess the relationships between the variables while controlling for variation in initial weight. For these analyses, the data were pooled across feeding treatments and breeds.

Results and discussion

Final bodyweights and BCSs of all three breeds in IT were the lowest, whereas those in ET were the highest ($P < 0.05$) (Table 1). These results demonstrate the effects of the duration of the treatment and age of rams on growth, bodyweight, and condition. Although the ram lambs in IT received a concentrate diet, the

feeding period of this treatment was shorter than the other two treatments and these rams were younger than the rams of the other two treatments at the time of slaughter. Nevertheless, the purpose of this trial was to study the effects of these ram feeding systems that are currently used by South African sheep farmers, taking into consideration that feeding periods would differ.

Table 1 Effects of finishing treatment and breed on growth of Döhne Merino, Merino and South African Mutton Merino ram lambs from intensive, extensive-intensive, and extensive feeding treatments

Factors		Variables				
Finishing treatment	Breed	Initial weight, kg	Final weight, kg	BCS	ADG, kg/d	SCF, cm
IT	Döhne (n = 10)	28.8 ± 1.1	51.9^{A} ± 1.4	5^{A} ± 0.2	0.302^{A} ± 0.012	1.1^{A} ± 0.04
	Merino (n = 10)	30.1 ± 1.4	51.4^{A} ± 1.9	5^{A} ± 0.2	0.306^{A} ± 0.011	1.1^{A} ± 0.08
	SAMM (n = 10)	34.3 ± 1.3	57.7^{A} ± 1.8	4^{A} ± 0.2	0.304^{A} ± 0.011	1.0^{A} ± 0.07
EIT	Döhne (n = 10)	27.6_{a} ± 0.7	$66.0^{B}{}_{ab}$ ± 1.0	$5^{A}{}_{a}$ ± 0.4	0.289^{A} ± 0.008	$1.8^{B}{}_{ab}$ ± 0.03
	Merino (n = 10)	25.4_{a} ± 1.4	$59.9^{B}{}_{a}$ ± 2.3	$4^{A}{}_{b}$ ± 0.3	0.260^{AB} ± 0.015	$1.5^{AB}{}_{a}$ ± 0.06
	SAMM (n = 10)	37.4_{b} ± 0.9	$72.7^{B}{}_{b}$ ± 1.4	$7^{B}{}_{c}$ ± 0.3	0.270^{AB} ± 0.012	$2.2^{B}{}_{b}$ ± 0.15
ET	Döhne (n = 10)	28.9_{a} ± 0.8	72.5^{B} ± 1.3	7^{B} ± 0.2	0.218^{B} ± 0.005	$1.8^{B}{}_{a}$ ± 0.11
	Merino (n = 11)	25.7_{a} ± 1.0	75.3^{C} ± 1.6	7^{B} ± 0.3	0.247^{B} ± 0.005	$1.9^{B}{}_{ab}$ ± 0.11
	SAMM (n = 8)	34.9_{b} ± 1.8	80.6^{B} ± 1.9	8^{B} ± 0.2	0.228^{B} ± 0.008	$2.4^{B}{}_{b}$ ± 0.17

$^{A, B, C}$ Within a column, breed means with a common superscript were not different with probability P <0.05

$_{a, b}$ Within a column, finishing treatment means with a common subscript were not different with probability P <0.05 BCS: body condition score (on a scale from 1 to 9), ADG: average daily gain, SCF: subcutaneous fat depth the 13th rib, SAMM: South African Mutton Merino, IT: intensive feeding, EIT: extensive-intensive feeding, ET: extensive feeding

The final weight of the IT Döhne Merino rams was significantly lower compared to the final weights of the EIT and ET Döhne Merino rams. The smaller final weight difference between the EIT and ET Döhne Merino rams shows that the EIT rams responded well to an improved diet fed at an older age. The ET Döhne Merino group recorded lower ADG (P <0.05) compared with the IT and extensive-intensive DM groups. A similar trend was observed for SAMM rams. Similar to the DM rams, the SAMM rams in the EIT group responded well to an improved diet fed at an older age. The IT SAMM rams recorded higher (P <0.05) ADG compared with ET SAMM rams. Only M rams showed differences in final weight between all three treatments (P <0.05). The lowest weight was recorded in the IT group and the heaviest in the ET group. The ADG of the IT Merino rams was significantly higher than the ADG of the ET Merino rams.

Measurements of subcutaneous fat were recorded (Table 1). Growth curves show that adipose tissue is the last body component to develop and is considered 'primarily a tissue of maturity', which is used to store excess energy in the form of fat (Hammond, 1955; Batt, 1980; Hossner, 2005). The thickness of the subcutaneous fat layer of the rams increased with the lengths of the treatments. Thus, the lowest values were those of younger ram lambs in IT and the highest were those of the older ET rams. Intensively fed ram lambs had less (P <0.05) subcutaneous fat, indicating that energy utilization in this group was mainly for protein deposition. The ram lambs in IT had not yet reached their fattening phase. The concentrate diet was not fed for long enough to exceed the decreasing growth requirements of IT animals and to cause excess fat accretion. The fattening of ram lambs in EIT at a physiologically more mature age resulted in a faster shift from protein to fat accretion with more subcutaneous fat accretion. This could be observed in the similarity in subcutaneous fat thickness (Table 1) between the ET and the two-months-younger EIT rams. It illustrated that the fattening rate in the EIT rams was possibly higher than in the ET rams.

Nutrition influences testicular development (Setchell *et al.*, 1965; Cameron *et al.*, 1988; Hötzel *et al.*, 1998; Martin *et al.*, 2010) and a diet high in protein and energy stimulates testicular growth and development (Braden *et al.*, 1974; Hötzel *et al.*, 1998). However, treatment effects in the present study differed from the aforementioned studies, possibly because this study simulated typical South African farm conditioning practices, which involved differences in the duration of treatments with subsequent effects on the age, weight of rams at slaughter, and season of slaughter.

Table 2 Effects of finishing treatment and breed on scrotal traits of Döhne, Merino and South African Mutton Merino ram lambs from intensive, extensive-intensive, and extensive feeding treatments

Factors		Variables			
Finishing treatment	Breed	Initial scrotal circumference, cm	Final scrotal circumference, cm	Scrotal weight, g	Testes weight, g
IT	Döhne (n = 10)	22.5 ± 0.8	33.4 ± 1.0	635.5^{A} ± 38.1	217.6 ± 12.6
	Merino (n = 11)	20.7 ± 1.0	32.5 ± 0.8	555.1^{A} ± 30.6	191.4 ± 12.8
	SAMM (n = 8)	23.2 ± 1.1	32.3 ± 0.6	670.2^{A} ± 37.9	234.5 ± 16.3
EIT	Döhne (n = 10)	20.6_{a} ± 0.9	35.0 ± 0.7	880.8^{B} ± 45.2	258.0 ± 13.4
	Merino (n = 10)	18.9_{a} ± 0.9	34.1 ± 0.8	815.4^{B} ± 36.3	234.0 ± 9.6
	SAMM (n = 10)	25.9_{b} ± 0.5	33.4 ± 0.7	803.6^{AB} ± 29.6	227.4 ± 10.1
ET	Döhne (n = 10)	21.2_{ab} ± 0.7	35.4 ± 0.9	870.9^{B} ± 40.6	267.2 ± 16.4
	Merino (n = 10)	19.8_{a} ± 0.7	33.9 ± 0.9	801.5^{B} ± 45.8	235.8 ± 15.1
	SAMM (n = 10)	24.4_{b} ± 1.4	34.4 ± 0.9	905.6^{B} ± 38.2	277.0 ± 14.4

$^{A, B}$Within a column, breed means with a common superscript were not different with probability P <0.05
SAMM: South African Mutton Merino, IT: intensive feeding, EIT: extensive-intensive feeding, ET: extensive feeding

Initial scrotal circumference (SC) measurements (Table 2) did not differ between treatments within a breed (P >0.05). In EIT, the SAMM ram lambs recorded larger initial SC than the M (P <0.001) and DM ram lambs (P <0.01), and in the ET, initial SC of SAMM ram lambs was larger than that of M ram lambs (P <0.05). Because the SAMM breed is the largest of the three breeds, and SC is correlated with bodyweight, it was understandable that SAMM ram lambs had larger initial SC. Scrotal circumference taken at the end of the trial showed no differences (P >0.05) between breeds within the same treatments. This implied a limit to the size that SC could reach and that variations in SC between rams of different breeds that were approaching maturity could decrease.

Dissection of the scrotum and testes revealed differences (P <0.05) in scrotal weight (Table 2). Scrotal weight of IT rams was significantly lighter than the EIT and ET rams owing to the shorter duration of IT compared with ET and EIT, and the younger age of IT rams compared with the rams in the other treatments when scrotal weight was measured. Scrotal weight per se did not describe testicular development or the effects of nutrition on testicular development well, because scrotal weight included scrotal skin, testes, and scrotal fat. Therefore, testicular weights and volume are more useful to determine testicular development.

Testicular weights (Table 2) did not differ between breeds within a treatment or between treatments within breeds (P >0.05). The same was observed for testes volume (Table 3). Rams in IT, although the youngest and lightest, did not have significantly lighter testicular weights or lower volume than ET or EIT rams. The IT rams weighed between 51 and 57 kg at slaughter. Studies by Al-Haboby *et al.* (1994), Salhab *et al.* (2001) and Elmaz *et al.* (2007) reported that rapid increase in testicular dimensions occurred when rams reached weights of 28 - 34 kg, 37 - 43 kg, and 25 - 27 kg, respectively. This trial also demonstrated strong positive correlations between testicular measurements and bodyweight, as supported by the studies of Salhab *et al.* (2001), Bearden *et al.* (2004), and Elmaz *et al.* (2007). For the SAMM ram lambs in the IT correlations of final weight with testes volume and testes weight were 0.68 and 0.69, respectively (P <0.05). Correlations were also observed for DM rams in EIT between testes weight and final weight (r = 0.78; P <0.05). As all the IT rams were older than six months when slaughtered, and had reached puberty, although not maturity, they were expected to have well-developed testes (Bearden *et al.*, 2004). Further, the concentrate diet fed to IT ram lambs was high in energy and protein. Braden *et al.* (1974) found that diets with higher protein content increased testicular weight, and the addition of energy increased it even more. The rise in testes weight has been described as an increase in the volume of seminiferous epithelium and seminiferous tubule diameter (Braden *et al.*, 1974; Oldham *et al.*, 1978; Hötzel *et al.*, 1998; Fernandez *et al.,* 2004; Kheradmand *et al.*, 2006; Chella *et al.*, 2017). A factor that might have contributed to variations in testicular measurements was seasonal differences between the IT and the ET rams (Pelletier & Almeida, 1987; Schoeman & Combrink, 1987; Webb *et al.*, 2004; Sarlós *et al.*, 2013). To quantify this factor in the present study, season was included as a random factor in the analysis of variance procedures. Although the photoperiod effect is not so strong in southern hemisphere rams (Hafez, 1952; Martin *et al.*, 1999; Blache *et al.*, 2002), possibly owing to the small variations in day to night ratio between summer and winter, the rams

were slaughtered in different seasons. The rams in IT and EIT were slaughtered in summer, whereas the ET rams were slaughtered in autumn. Apart from the final SC, the testicular variables were influenced significantly by season. The movement into autumn would have stimulated further gonadal development in the ET rams. Similar findings were recorded in the studies of Pelletier and Almeida (1987), Schoeman and Combrink (1987), Webb *et al.* (2004), and Sarlós *et al.* (2013).

Table 3 Effects of finishing treatment and breed characterizing the testes and scrotum of Döhne Merino, Merino and South African Mutton Merino ram lambs from intensive, extensive-intensive, and extensive feeding treatments

Factors		Variables			
Finishing treatment	Breed	Testes volume, ml	PVP, cm	Scrotal fat weight, g	SSNF, cm
IT	Döhne (n = 10)	192.0 ± 10.5	52.3^{A} ± 1.4	27.5^{AB} ± 2.1	1.2^{A} ± 0.09
	Merino (n = 11)	166.0 ± 11.8	49.3^{A} ± 2.1	29.3^{A} ± 2.0	1.0^{A} ± 0.03
	SAMM (n = 8)	205.5 ± 14.1	57.6^{A} ± 1.7	32.2 ± 3.9	1.0^{A} ± 0.10
EIT	Döhne (n = 10)	231.5 ± 11.3	67.9^{B} ± 2.0	45.4^{A} ± 5.5	$1.7^{B}{}_{a}$ ± 0.06
	Merino (n = 10)	212.0 ± 7.1	63.8^{B} ± 2.9	52.5^{B} ± 5.9	$1.4^{B}{}_{b}$ ± 0.03
	SAMM (n = 10)	203.0 ± 9.3	65.2^{AB} ± 2.9	46.3 ± 3.6	$1.8^{B}{}_{a}$ ± 0.05
ET	Döhne (n = 10)	237.5 ± 15.6	69.5^{B} ± 1.9	24.1^{B} ± 2.8	1.6^{AB} ± 0.06
	Merino (n = 10)	208.2 ± 17.9	66.9^{B} ± 2.2	33.1^{A} ± 4.3	1.7^{B} ± 0.14
	SAMM (n = 10)	230.0 ± 21.8	68.8^{B} ± 2.4	34.6 ± 3.9	1.6^{B} ± 0.09

$^{A, B}$ Within a column, breed means with a common superscript were not different with probability P <0.05

$_{a, b}$ Within a column, finishing treatment means with a common superscript were not different with probability P <0.05

SAMM: South African Mutton Merino, IT: intensive feeding, EIT: extensive-intensive feeding, ET: extensive feeding, PVP: circumference of the *Pampiniform venous plexus*, SSNF: scanned depth of scrotal neck fat

An important additional measurement was the circumference of the *Pampiniform venous plexus (*PVP) (Table 3), which is where thermoregulation occurs, and which influences the functionality of sperm (Senger, 2003; Bearden *et al.*, 2004). The thickest (P <0.05), and therefore most developed PVP, was recorded in the ET in all three breeds. The least developed PVC (P <0.05) was recorded in the youngest rams, notably those in IT. Rams in ET were the oldest, and a better developed PVP was expected. Rams with a larger PVP such as those in ET should have had better testicular thermoregulation ability compared with those with a small PVP (e.g. those in the IT).

Treatment influenced scanned scrotal neck fat (SSNF) (Table 3) in all three breeds. Ram lambs in the IT had significantly less SSNF than the rams in EIT and ET, although they were fed the concentrate diet for the entirety of their treatment. These were young ram lambs, and the feeding period of IT was short, so the rams were not sufficiently physiologically mature (Hossner, 2005) to deposit excessive amounts of scrotal neck fat, which is typical of more mature rams or rams fed a concentrate diet for extended periods. Döhne Merino and SAMM rams in EIT, and M rams in ET had the thickest SSNF compared with their counterparts in other treatments. Döhne Merino ram lambs in IT differed (P <0.001) from the extensive-intensive DM rams. Similarly, SSNF differed (P <0.05) between IT and EIT for both the SAMM and M breeds. Ram lambs in the IT were young when they received the concentrate diet, so the nutrients were used predominantly for growth, leaving little for fat accretion. In the older rams in EIT, nutrient density was initially less and therefore tissue growth was less, followed by more nutrient-dense feeding in the final growth phase, which resulted in more fat deposition in the neck of the scrotum.

The EIT resulted in more scrotal fat deposition compared with the other two treatments (P <0.05) in all three breeds (Table 3). Rams fed the concentrate diet during the finishing off phase in EIT demonstrated improved scrotal and testicular development, but with more scrotal fat accumulation, because concentrate feeding occurred when rams were physiologically more mature (Hossner, 2005). Rams in ET, although older, were not fed a concentrate diet, which explains why they had less scrotal fat than those in the EIT. The rate of fattening was higher in EIT rams compared with ET rams.

Merino rams in the EIT recorded less (P <0.05) SSNF than the DM and SAMM rams in the same treatment group. The extensive-intensive M rams tended to deposit more scrotal fat than the DM and SAMM rams. In the IT Döhne Merino ram lambs had a thicker SSNF than M (P <0.05) and SAMM (P >0.05) ram lambs. Although numerically DM rams had the thickest SSNF, this breed recorded the lowest amount of scrotal fat of the three breeds in all three treatments (P >0.05). It appears that the location of fat deposition is breed specific. For example, DM and SAMM rams tended to deposit fat in the neck of the scrotum, whereas M rams deposited fat in the body of the scrotum. It follows that fat deposition in the neck of the scrotum rather than around the testes may be more detrimental in terms of thermoregulation (Coulter *et al.*, 1997; Lunstra & Coulter, 1997; Fourie *et al.*, 2004).

The volume of semen (Table 4) did not differ between treatments (P >0.05). This is in line with the findings of Elmaz *et al.* (2007), who found no increase in semen volume in rams between the ages of seven and 14 months. Further, semen volume is correlated with testicular weight, which, as stated, did not differ between treatments.

Treatment influenced the percentage normal sperm only in DM (Table 4). Döhne Merino rams in IT recorded a lower percentage of normal sperm than their counterparts in EIT (P <0.01) and ET (P <0.05). Since there were no differences in testes weight between the treatments, the differences in percentage normal sperm between treatments could be ascribed to the older age of the rams in the EIT and ET. This is in accordance with the accepted understanding that semen characteristics improve as animals age. For example, more abnormal sperm were found in the ejaculates of pubescent rams compared with adult rams (Courot, 1979; Alexopoulos *et al.*, 1991; Chella *et al.*, 2017).

Table 4 Effects of finishing treatment and breed on semen quality of Döhne Merino, Merino and South African Mutton Merino ram lambs from intensive, extensive-intensive, and extensive feeding treatments

Factors		Variables				
Finishing treatment	Breed	Semen volume, ml	Mass motility[1]	Progressive motility, %	Immotile/dead spermatozoa, %	Normal spermatozoa, %
IT	Döhne (n = 10)	1.9 ± 0.3	5 ± 0.2	70.0 ± 8.0	16.7 ± 4.1	57.9^{A} ± 7.8
	Merino (n = 10)	1.3 ± 0.2	4 ± 0.4	76.1 ± 6.9	11.7 ± 3.4	75.4 ± 4.3
	SAMM (n = 8)	2.1 ± 0.3	4 ± 0.6	68.5 ± 10.7	23.8 ± 10.6	73.3 ± 6.2
EIT	Döhne (n = 10)	2.7 ± 0.3	4 ± 0.1	77.5 ± 2.4	12.0 ± 1.3	82.2^{B} ± 3.9
	Merino (n = 10)	2.1 ± 0.3	3 ± 0.4	75.5 ± 6.8	14.0 ± 5.6	80.8 ± 3.4
	SAMM (n = 10)	2.1 ± 0.3	4 ± 0.3	78.0 ± 2.6	12.5 ± 1.5	82.2 ± 2.3
ET	Döhne (n = 9)	2.0 ± 0.2	4 ± 0.2	81.5 ± 2.6	11.5 ± 2.1	78.9^{B} ± 4.5
	Merino (n = 9)	1.5 ± 0.2	4 ± 0.3	79.5 ± 2.8	12.0 ± 2.0	83.9 ± 2.3
	SAMM (n = 10)	1.4 ± 0.3	4 ± 0.1	78.8 ± 2.2	11.3 ± 1.4	87.1 ± 2.3

$^{A, B}$ Within a column, breed means with a common superscript were not different with probability P <0.05

[1] on a scale of 0 to 5

SAMM: South African Mutton Merino, IT: intensive feeding, EIT: extensive-intensive feeding, ET: extensive feeding

The ET recorded slightly better progressive spermatozoa motility and fewer immotile/dead spermatozoa than the other two treatments, although not significantly. The findings of the present study agree with those of Alexopoulos *et al.* (1991) and Elmaz *et al.* (2007), who reported no major fluctuations in semen motility in rams between the ages of seven and 14 months. However, Alexopoulos *et al.* (1991) reported that abnormal spermatozoa percentage decreased rapidly after five months old.

Bester *et al.* (2004) recorded no significant improvement or decline in semen parameters when rams were fed high-energy diets. This was in contrast with Braden *et al.* (1974), who found that increasing the energy intake improved the daily sperm production of M rams. In the present study, rams in the IT were in their pubescent stage, which explains why they did not show an improvement in semen characteristics. It may be possible that the concentrate diet improved their semen characteristics via improved testicular growth. This was supported by the positive correlation seen in the IT Döhne Merino rams between testes weight and percentage normal spermatozoa (r = 0.76, P <0.05) and the negative correlation between testes weight and aberrant motile spermatozoa in SAMM rams (r = -0.75, P <0.05).

Although rams in the EIT recorded thicker SSNF than those in the IT (P <0.05), no detrimental effect was observed on the percentage normal spermatozoa. This could be ascribed to the larger PVP of the EIT rams and an increase in age of rams. Larger PVP provides a larger surface area for better thermoregulation. Precautions should be taken if DM ram lambs are fed intensively for longer, since more SSNF would accumulate, which would be detrimental to semen quality traits, owing to the observed correlations between SSNF and progressive motility (r = -0.76, P <0.05), percentage normal spermatozoa (r = -0.80, P <0.05), aberrant motile spermatozoa (r = 0.78, P <0.05), and immotile/dead spermatozoa (r = 0.73, P <0.05). It is advisable to ensure that SSNF does not become too thick in DM ram lambs.

Regression analyses were carried out on data that were pooled across feeding treatments and breeds. The relationship between weight gained and SSNF was curvilinear (Figure 1). Scanned scrotal neck fat increased at a decreasing rate with increasing weight gain (R^2 = 0.43, P <0.05). Weight gain also had a positive correlation (r = 0.60; P <0.05) with SSNF. Similar analyses revealed nonlinear relationships between subcutaneous fat and SSNF, and between subcutaneous fat and scrotal fat weight. Increased subcutaneous fat depth was associated with concomitant increases in SSNF (R2 = 0.40; P <0.001) and scrotal fat weight (R2 = 0.06, P <0.05). Subcutaneous fat and SSNF were positively correlated (r = 0.56, P <0.05).

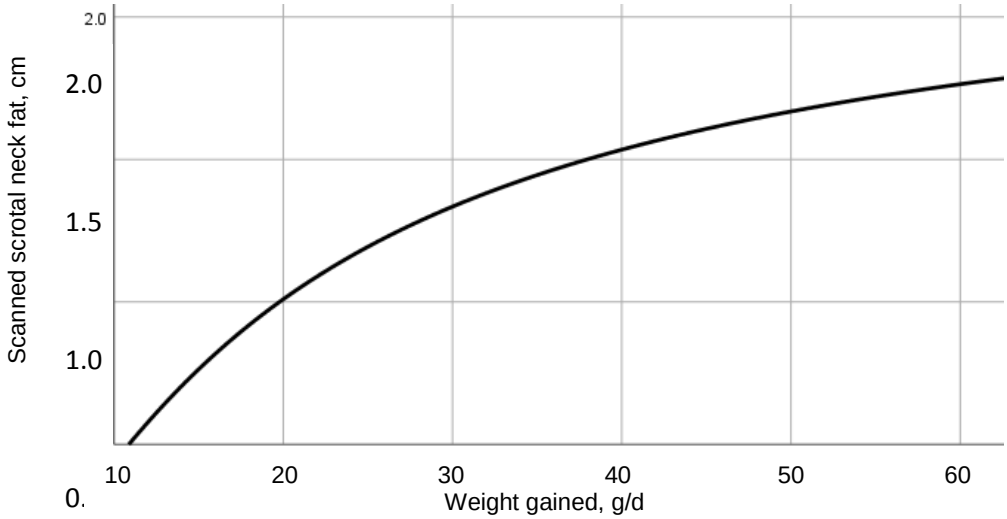

Figure 1 Regression of scanned scrotal neck fat on weight gained pooled over breeds and treatments

There was a detrimental relationship between the accumulation of subcutaneous fat and semen volume as demonstrated by the quadratic function shown in Figure 2 (R^2 = 0.07, P <0.05). Semen volume initially increased with fattening, but when subcutaneous fat thickness exceeded the extreme functional value of 1.6 cm, semen volume decreased. The adverse effects of fattening on semen volume were also associated with increase deposition of fat in the scrotal neck and in the scrotum (R^2 = 0.40; P <0.001 and R^2 = 0.06, P <0.05, respectively).

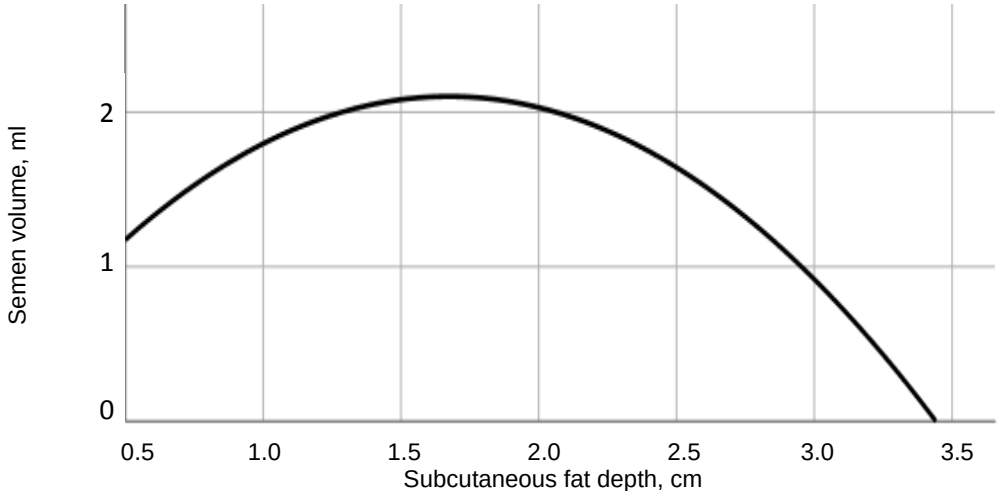

Figure 2 Regression of semen volume on subcutaneous fat depth pooled over breeds and treatments

Figure 3 illustrates a quadratic relationship of the percentage of normal spermatozoa with subcutaneous fat (R^2 = 0.19, *P* <0.001). The functional value of the regression is maximized at a fat depth of approximately 2.2 cm. Higher levels of fatness may result in a decreased percentage of normal spermatozoa.

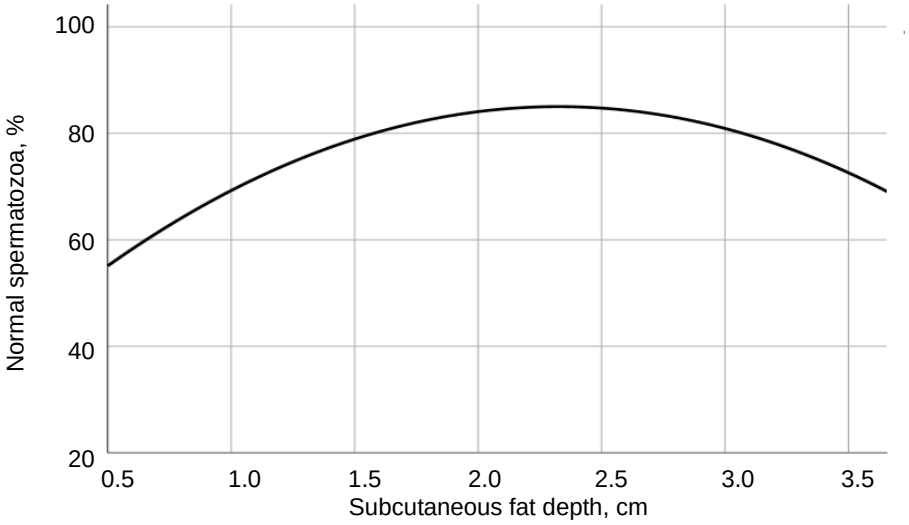

Figure 3 Regression of percentage normal spermatozoa on subcutaneous fat depth pooled over breeds and treatments

The regression of spermatozoa mass motility on scrotal fat weight was quadratic (Figure 4), indicating maximum mass motility at approximately 39 g of scrotal fat (R^2 = 0.07, *P* <0.05). These adverse effects of fattening on the three semen parameters (semen volume, normal spermatozoa percentage, and mass motility) can be attributed to an increase in SSNF and scrotal fat deposition (R^2 = 0.40; *P* <0.001 and R^2 = 0.06, *P* <0.05 respectively). The coefficients of determination were small, but the significant relationships indicated clear extreme functional values.

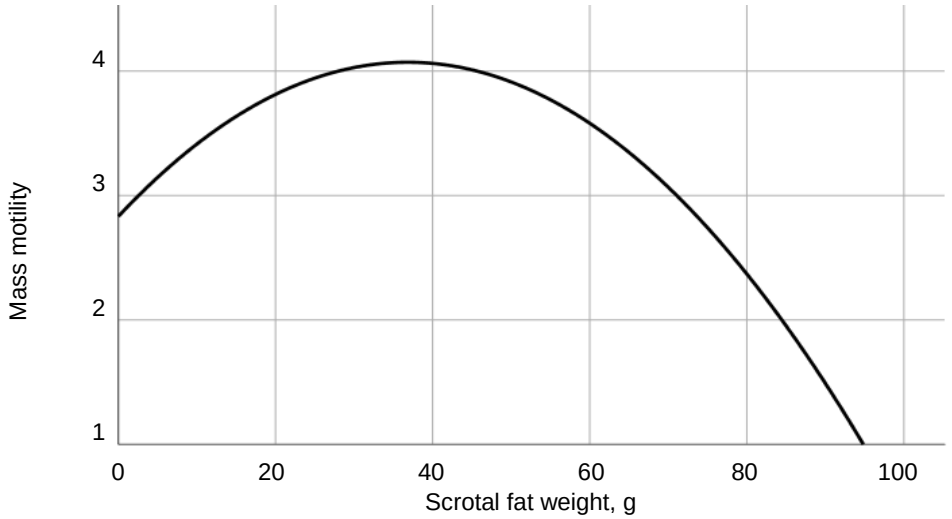

Figure 4 Regression of mass motility on scrotal fat weight pooled over breeds and treatments

Conclusions

For breeds that tend to accumulate more fat in the neck of the scrotum, the ram fattening system should be managed with caution so that testicular thermoregulation is not compromised. Differences in semen quality between feeding systems and breeds were small, but regression analyses revealed increasingly adverse effects of fat accumulation in the scrotum and scrotum neck on semen volume, mass motility, and percentage normal sperm over extended feeding periods. Efficient feeding programmes of ram lambs should make provision for differences among breeds in physiological maturation. More research is required to study the effects of various ram feeding systems over extended periods in different breed types on the accumulation of fat in the scrotum and its effects on semen quality.

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Authors' contributions

This research was conceptualised by ECW and WAVN. AMDP formulated and refined the research article as part of her MSc (Agric.) degree in Animal Science under the supervision of ECW and WAVN. AMDP, ECW and WAVN revised and edited the article.

Conflict of Interest Declaration

None of the authors has a conflict of interest to declare.

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