

# **Reproductive traits in Ethiopian male goats**

**With special reference to breed and nutrition**

**Yoseph Mekasha Gebre**

*Division of Reproduction, Department of Clinical Sciences, Faculty of  
Veterinary Medicine and Animal Science, Swedish University of  
Agricultural Sciences*

*Uppsala*

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## Abstract

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This thesis characterizes reproductive traits of Ethiopian male goats raised under extensive husbandry and subjected to differential nutritional management. A total of 177 extensively-managed indigenous bucks of 5 breeds (i.e., *Arsi-Bale* [AB], *Central Highlands* [CH], *Afar*, *Boran* and *Woito-Guji* [WG]) were selected following stratified random sampling. The bucks were compared according to three age classes (<14 mo [young], 14–19.5 mo [intermediate] and 19.6–24 mo [old]) to determine whether breed and age influenced body size, testicular traits and epididymal sperm morphology. Bucks of a single goat breed (Ogaden;  $n=35$ ), selected by simple random sampling, were used to determine responses to differential nutritional treatments. Dietary treatments consisted of native hay fed *ad libitum* (control, C), native hay supplemented with 1% of body weight (BW) of agro-industrial by-products (Treatment 1, T1), native hay supplemented with 1% of BW of khat (*Catha edulis*) leftovers (Treatment 2, T2) and khat leftovers fed *ad libitum* (Treatment 3, T3). Breed, age and their interaction affected ( $P<0.05$  to  $P<0.001$ ) BW, body condition score (BCS), scrotal circumference (SC) and testicular weight (TW). Comparing all age classes, Boran displayed the highest ( $P<0.05$ ) BW, greatest SC and heaviest TW, while Afar displayed the lowest values for these characteristics. Expressed as percentage of BW, Afar bucks had the highest TW. Regarding epididymal sperm morphology, most acrosome defects were displayed by CH ( $P<0.05$ ) while AB mainly showed loose sperm heads ( $P<0.05$ ). Bucks from a lowland agro-climate (i.e., Afar, Boran and WG) displayed more total sperm-head abnormalities ( $P<0.05$ ) than did bucks of highland breeds (i.e., AB and CH). Younger bucks showed more ( $P<0.05$ ) loose sperm heads, while older bucks had more ( $P<0.05$ ) acrosome defects. Bucks fed according to the T1–T3 treatments improved ( $P<0.05$ ) their BW, BCS, testicular size and testicular weight compared to controls. Goats in treatment groups T1–T3 showed higher ( $P<0.05$ ) sperm motility, sperm concentration per mL and total number of spermatozoa per ejaculate compared to controls. Of groups T1–T3, bucks in T3 had the highest BW and testicular size. Feeding goats according to the T1–T3 regimes improved ( $P<0.001$ ) feed dry matter (DM) and nutrient intake, and the occurrence of morphologically normal spermatozoa, compared to controls. Of the T1–T3 treatments, feeding according to T3 and T2 resulted in higher ( $P<0.05$ ) DM, organic matter and gross energy intakes, while T1 followed by T3 resulted in the highest ( $P<0.05$ ) crude protein intake, and T3 the highest occurrence of morphologically normal spermatozoa. In conclusion, body size, testicular traits and sperm morphology of Ethiopian bucks raised under extensive management were influenced by breed and age. Nutritional supplementation with khat leftovers and an agro-industrial by-product mix improved feed intake, growth and semen characteristics; these feedstuffs could be considered alternative feed resources to enhance goat production under smallholder farming systems in Ethiopia.

**Key words:** body weight, body condition score, scrotal circumference, sperm morphology, sperm motility, sperm numbers, age, khat leftovers, agro-industrial by-products.

**Author's address:** Yoseph Mekasha Gebre, Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Box 7054, SE-750 07 Uppsala, Sweden; Department of Animal Sciences, Haramaya (formerly Alemaya) University, P.O. Box 138, Dire Dawa, Ethiopia.  
E-mail: y\_mekasha@yahoo.com; yoseph.gebre@kv.slu.se

**To**

***My parents***

***My wife, Addisalem Lemma***

***My children, Rahel, Tinsaye & Tsiyon***

# Contents

## Appendix

## Abbreviations

## Introduction, 9

## General background, 11

Goat production systems in Africa, 11

Socio-economic role of goats, 11

Goat genetic resources in Ethiopia, 12

Puberty and sexual maturity in male tropical goats, 13

Goat breeding system and reproductive management, 13

Breeding soundness evaluation in goats, 14

Characteristics of tropical feed resources, 15

Testicular traits, 17

Sperm production and sperm morphology, 18

Factors influencing body size, testicular traits and sperm features, 19

*Effect of nutrition, 19*

*Effect of breed, 20*

*Effect of age, 21*

## Aims of the study, 23

## Methodological considerations, 24

Overview of the study, 24

Study location, 24

Animals, 25

*Goat population and their distribution in Ethiopia, 25*

*Animal sampling and management, 25*

Dietary treatments and feed sampling, 26

Data collection procedures and measurements, 26

*Chemical analysis of feedstuff, 26*

*Feed intake, 26*

*Scrotal measurements, 27*

*Body size and testicular measurements, 27*

*Semen and epididymal sperm collection, and evaluation, 27*

Statistical analyses, 28

## **Results, 29**

Feedstuff composition and feed intake, 29

Assessment of scrotal traits, 29

Body mass and testicular traits, 29

Seminal traits, 31

*Morphology of epididymal spermatozoa in relation to breed and age class, 31*

*Semen characteristics in relation to nutrition, 31*

Correlation among reproductive traits, 32

## **General discussion, 33**

Effect of breed and age class on reproductive traits, 33

Effect of nutrition on reproductive traits, 38

**General conclusions, 44**

**Recommendations, 45**

**Scope of future research, 46**

**References, 47**

**Acknowledgements, 54**

# Appendix

## Papers I–IV

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Mekasha, Y., Tegegne, A., Abera, A. & Rodriguez-Martinez, H. 2007. Body size and testicular traits of tropically adapted bucks raised under extensive husbandry in Ethiopia. *Reproduction in Domestic Animals*, Published Online: 26-Jul-2007 (doi: 10.1111/j.1439-0531.2007.00877.x.)

II. Mekasha, Y., Tegegne, A. & Rodriguez-Martinez, H. 2007. Sperm morphological attributes in tropically adapted bucks raised under extensive husbandry in Ethiopia. *Animal Reproduction*, In Press.

III. Mekasha, Y., Tegegne, A. & Rodríguez-Martínez, H. 2007. Effect of supplementation with agro–industrial by-products and khat (*Catha edulis*) leftovers on testicular growth and sperm production in Ogaden bucks. *Journal of Veterinary Medicine Series A* 54, 147–155.

IV. Mekasha, Y., Tegegne, A. & Rodriguez-Martinez, H. 2007. Feed intake and sperm morphology in Ogaden bucks supplemented with either agro–industrial by-products or khat (*Catha edulis*) leftovers. *Reproduction in Domestic Animals*, In Press (doi: 10.1111/j.1439-0531.2007.00931.x).

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## Abbreviations

AB	Arsi–Bale
ADF	acid detergent fibre
ADG	average daily gain
AI	artificial insemination
BCS	body condition score
BD	body depth
BL	body length
BSE	breeding soundness evaluation
BW	body weight
C	control
Ca	calcium
CH	Central Highlands
CP	crude protein
CSA	Central Statistics Authority
DAGRIS	Domestic Animal Genetic Resources Information System
DM	dry matter
FAO	Food and Agricultural Organization of the United Nations
FARM-Africa	Food and Agricultural Research Management – Africa
GDP	gross domestic product
GE	gross energy
GENMOD	generalized linear model
GLM	general linear model
HAW	height at wither
HG	heart girth
HW	hip width
ILCA	International Livestock Centre for Africa
IVDMD	<i>in vitro</i> dry matter digestibility
IVOMD	<i>in vitro</i> organic matter digestibility
K	potassium
MEDaC	Ministry of Economic Development and Cooperation
Mg	magnesium
Mn	manganese
NBE	National Bank of Ethiopia
NDF	neutral detergent fibre
OM	organic matter
SAS	Statistical Analysis System
SC	scrotal circumference
SD	standard deviation
SLU	Swedish University of Agricultural Sciences
TC	testicular consistency
TW	testicular weight
TWPBW	testicular weight expressed as percentage of body weight
WG	Woito Guji

## Introduction

Goat (*Capra hircus*) husbandry is integral to livestock production in the tropics and subtropics, where ~80% of the world's 710 million goats are found (Knight & Garcia, 1997). This is primarily related to their better adaptation to harsh tropical environments. The abilities to reduce their metabolism, efficiently use water, minimize nitrogen requirements and efficiently digest high-fibre forage are among the adaptive features of goats (Gilboa *et al.*, 2000; Silanikove, 2000; Morand-Fehr *et al.*, 2004; Mengistu, 2007). It is generally accepted that goats require low inputs and are easy to manage, contributing to a sustainable economic stock for most smallholders in the tropics and subtropics (Acharya & Battacha, 1992).

Goats account for approximately one-third of the total livestock genetic resources in Africa (Galal, 2005). In Ethiopia, domestic goats play an important role in the national economy. It has been estimated that there are approximately one hundred million domestic animals in Ethiopia, 23% of which are goats (CSA, 2004). The Ethiopian livestock sub-sector accounts for 13–16% of the gross domestic product (GDP) and 30–35% of the agricultural GDP (MEDaC, 2000). Livestock-related exports account for ~15% of the total export revenue of the country, third in importance after coffee and khat (*Catha edulis*) (MEDaC, 2000). However, livestock provides other non-food products, not included in this estimate, indicating that its real economic contribution is likely underestimated. It has been demonstrated that goat meat accounts for ~26% of Ethiopia's total red meat consumption *per capita* (ILCA, 1991). Besides, Ethiopia generates hard currency through exporting live goats, goat meat and goat skins. In the 1996–1998 period alone, Ethiopia earned USD 1.3 million from exports of live goats and goat meat (FAO, 1999). Moreover, because of the currently favourable market in the Middle East, exports of live goats and goat meat have significantly increased from those of earlier periods and in contrast to exports of other livestock and meat. This has substantially contributed to smallholder earnings in particular, as well as to the overall national economy.

However, as in other tropical areas, productivity of the livestock population in Ethiopia has been hampered by the low fertility of the breeding herd (Payne & Wilson, 1999). Although female sub- or infertility is often linked to this productivity problem, the fertility of individual males has a greater influence on herd performance than does the fertility of individual females (McGowan, 2004); influencing reproductive efficiency in the herds irrespective of whether males are used for natural breeding or artificial insemination (AI) (Chacon *et al.*, 1999). Several factors influence male fertility, including the ability to produce normal spermatozoa and to mate. Therefore, evaluating the fertility of a breeding male animal, other than by directly evaluating its ability to produce offspring, can be done indirectly by evaluating its semen characteristics (Hafez, 1993), in particular, sperm viability (e.g., motility), sperm morphology and sperm production rates (Lagerlöf, 1934; Söderquist *et al.*, 1991). Screening males intended for breeding is thus relevant to improving Ethiopian goat production.

Male reproductive traits are influenced by several factors, nutrition in particular, but also by genotype, season, management, disease and parasite infestation levels (Barth & Oko, 1989; Dowsett & Knott, 1996; Bielli *et al.*, 2000; Karagiannidis, Varsakeli & Karatzas, 2000). Unlike temperate goat breeds, indigenous Ethiopian goats are adapted to the tropics, where direct seasonal effects on male reproduction are minimal but where nutrition – indirectly regulated by fluctuations in seasonal effects on vegetation – plays the major role in modulating their sexual activity (Delgadillo, Malpoux & Chemineau, 1997). Under tropical conditions in Africa, feed resources are limited in quantity and quality; their availability fluctuates with the season, seriously challenging optimum reproduction. Among the strategies attempted to boost the potential of low-quality fodder use is grain supplementation. However, the application of conventional grain supplements is obviously far beyond the reach of African smallholders; moreover, the priority use of grain is for human consumption. There is thus a need to seek alternative supplementation strategies that best fit the possibilities of the region and its smallholders.

Ethiopia has diverse goat genetic resources, which have not, however, been characterized in terms of functional traits. Moreover, selection for reproductive traits has not been performed, hampering breed improvement. The prevalence of diverse agro-ecologies each with distinct physico-geographic and climatic characteristics poses another challenge to efficient reproduction. The major production system is extensive husbandry and the dominant animal breeding system is uncontrolled natural mating, both of which can conspire against efficient reproduction and result in low fertility if -for instance- sub-fertile males are allowed to breed. The breeding soundness examination (BSE) of male goats under such a system is almost non-existent. Inadequate reproductive efficiency definitely leads to economic loss, as reproductive merit is more important than growth performance and carcass quality (Trenkle & William, 1977).

Despite the importance of the above considerations, information is scarce regarding the reproductive traits of Ethiopian male goats under both extensive husbandry and controlled management. Thus, a comprehensive characterization of the reproductive traits of male goats under diverse husbandry conditions is vital. Since nutrition has an overriding effect on reproduction, and since conventional supplements are expensive for most low-input goat producers, it is essential to seek alternative non-conventional by-products/leftovers that could improve goat performance. Such research would increase our understanding of goat biology under diverse production systems and help us design mechanisms for improving goat production, thereby fostering the sustainable use and conservation of Ethiopia's genetic biodiversity.

## **General background**

### **Goat production systems in Africa**

Goat husbandry practices in Africa follow the diverse agro-ecologies (classified depending on altitude and rainfall) prevalent across the continent, and are broadly classified as pastoral, agro-pastoral, mixed and commercial systems (Lebbie, 2004; Peacock, 2005). Goats are kept in all agro-ecological zones (Payne & Wilson, 1999), but owing to their evident ability to survive in harsh arid and semi-arid environments, their presence is comparatively higher than other livestock in such areas (Silanikove, 2000). In pastoral and agro-pastoral production systems, found in arid and semi-arid agro-ecological zones, goats are kept by nearly all pastoralists, often in mixed flocks with sheep, freely grazing or browsing in the rangelands. Arid and semi-arid zones comprise 55% of the area of sub-Saharan Africa, supporting 50–60% of the livestock and 40% of the people in that area (Silanikove, 2000). However, as arid and semi-arid agro-ecology zones receive low moisture most of the year and feed is scarce in the dry season, pastoralists move their animals from place to place in search of feed and water. Such a management strategy helps them survive the dry season with minimum losses. In a mixed crop-livestock production system, prevalent in humid, sub-humid and highland agro-ecological zones, goats are kept by smallholders and graze together with sheep and/or other livestock such as cattle. In these mixed-species grazing systems, goats complement cattle and sheep rather than compete with them for feed, because of their inherent ability to eat a wider variety of plant species (Lebbie, 2004). These mixed herds usually freely graze on communal pastures and seasonally on fallow cropland. However, due to the increasing population pressure in areas with this production system, free grazing is becoming limited and goats are now being tether fed, reflecting the challenge of procuring sufficient feed in this system (FARM-AFRICA, 1996). Furthermore, in highland agro-ecology, as in central Ethiopia, increased human population has led to decreased farm size and a gradual shift from keeping large to small ruminants, mainly goat and sheep (Peacock, 2005). The production system generally ranges from pastoral to mixed farming, but it is basically characterized by low inputs. Commercially, goats are seldom intensively managed; instead, they are raised in small flocks (small-scale intensive farming), particularly for fattening purposes. Although increasing in incidence, the management of large flocks in ranches is still rare (Peacock, 2005).

### **Socio-economic role of goats**

Goats are socio-economically important in developing countries, ensuring food and fibre supply and providing income to small households (Lebbie, 2004; Sahlu *et al.*, 2004; Sahlu & Goetsch, 2005). Often the only animal protein source, goat meat and milk help ensure infant development and sustain human health. Owing to increased demand for goat products, more livestock producers are raising goats in developing countries, including Ethiopia (Sahlu & Goetsch, 2005). Human population growth, increased urban income in several African countries, and new opportunities for export, have encouraged the marketing of goats from rural

households and pastoral communities. Goat production thus helps meet local meat demands, keeps hard currency from being spent on importing meat, and increases hard currency reserves through exports of goat meat and skins. Goat production provides employment for poor rural families, especially for women and children (Lebbie, 2004). Goats can also serve as a store of value and a security system. They can be sold to attain immediate cash assets for poor goat holders, helping them improve livestock and crop farming and financing social events (Morand-Fehr *et al.*, 2004). Especially during droughts when crops fail, goats, due to their adaptation capabilities, goats can survive on woody browses and infrequent watering; coupled with their high reproductive rate and short generation interval, goats enable their owners to recover quickly and economically (Lebbie, 2004; Peacock, 2005). The value of goats for the use of the vast areas of natural grasslands, regions where crop production is yet impracticable, should not be overlooked (Lebbie, 2004). Goats are also important in various cultural activities, especially in pastoral and agro–pastoral production systems.

### **Goat genetic resources in Ethiopia**

Unlike breeds from/in temperate regions, which are often highly selected for functional traits, most of the goat genetic resources of the tropics have been naturally selected for adaptive traits that help them fit into the prevailing environment. Thus, most tropical goats are commonly described as dual/multi-purpose animals. Because of not having been selected for functional traits, the reproduction performance and production of most tropical goat breeds are both low. This situation should be improved, and goats should be selected for their abilities to produce the desired products, reproduce efficiently and survive in the environments in which they are kept (Bretzlaff, 1995). Breed characterization should thus be prioritized, if we are to select superior animals. Descriptions of tropical goats are based on certain physical, morphological and functional characteristics (Banerjee, Animut & Ermias, 2000). Based on these variables and other goat breed descriptors listed by the FAO, Ethiopian goats have been phenotypically classified into 13 breeds, these diverse genetic resources distributed according to distinct agro–ecologies and climatic conditions (FARM-AFRICA, 1996; DAGRIS, 2006). With the most breeds of any African country, Ethiopia is considered a centre of animal genetic resource diversity (Galal, 1983).

Though such phenotypic characterization is an essential, initial step in breed identification, little further effort has been made towards an in-depth genetic characterization of indigenous goat breeds. A lack of information on genetic resource characteristics may lead to the underutilization, replacement and dilution through crossbreeding of local goat breeds, despite their local adaptation to environmental constraints.

The diversity of goat genetic resources might be revealed by variation in phenotypic appearance, which should be compared, across breeds, at a similar physiological stage (i.e., age) for evaluation purposes. This would assist in the selection of superior animals for productive or reproductive functions. However,

such a comparison is lacking for tropical goat breeds raised under extensive husbandry in general and for Ethiopian goats in particular. As well, information on the reproductive traits of indigenous goat breeds is scarce; this is especially true for male reproductive traits, which were basically ignored in the breed characterization venture cited above (FARM-AFRICA, 1996). Certain easily recordable reproductive traits, such as scrotal circumference (SC, linked to testicular growth), have high heritability, suggesting that selection for higher SC would result in higher sperm production at puberty (Bailey *et al.*, 1996).

### **Puberty and sexual maturity in male tropical goats**

Like other tropical goats, indigenous Ethiopian goats have evolved over a long period of natural selection for survival traits. Consequently, survivability has generally attained prominence at the expense of reproductive features in low-input production systems in which fulfilling nutrient requirements is the major challenge. The time taken for male goats to reach puberty varies based on genotype, nutrition, season and other environmental factors (Abi-Saab *et al.*, 1997). Not only is information scarce on age at puberty for tropical goat breeds, but whatever information is available in the literature is not consistent. Depending on nutritional status and breed, most tropical male goats attain puberty at approximately eight months of age (Delgadillo, Malpaux & Chemineau, 1997; Madani & Rahal, 1988); Payne and Wilson (1999) reported that tropical male goats reach puberty at approximately 97 days and sexual maturity at 132 days of age. These results contrast with reports of male Nubian goats reaching puberty at 32 weeks of age, based on the first collection of an ejaculate containing motile spermatozoa (Chakraborty, Stuart & Brown, 1989). Furthermore, it was demonstrated that Libyan male goats reached puberty and sexual maturity at approximately 158.7–192.1 and 239.4–254.4 days, respectively (Madani & Rahal, 1988). These obvious variations in findings could be attributed to differences in breed, nutritional management, frequency of semen collection and other environmental factors. The onset of puberty in males refers to the appearance of the first motile spermatozoa in the ejaculate and it results from complex interactions between the hypothalamus, anterior pituitary gland and gonads (Chakraborty, Stuart & Brown, 1989). However, animals may not be fully sexually competent at puberty, since puberty may often be reached before animals have grown enough to physically support reproduction. Thus, periodically evaluating the spermogram of an individual at different ages is worthwhile in monitoring its sperm profile, which in turn indirectly indicates the degree of testicular and epididymal maturity. Such studies are extremely relevant in the case of Ethiopian indigenous goats, for which information is scarce regarding the relationship of age and body weight to onset of puberty and attainment of sexual maturity – prerequisites for genetic improvement through selection.

### **Goat breeding system and reproductive management**

Although goats are seasonal breeders in higher latitude, they breed the year round in tropical environments (Delgadillo, Malpaux & Chemineau, 1997), most often

by uncontrolled natural mating, since there is no tradition of using improved breeding techniques in extensive production systems (Gatenby, 1986). In such systems, both fertile and infertile male and female goats are kept together in the same grazing/browsing field. This negatively influences flock fertility, be it in a single or multi-herd system. Besides, in a country like Ethiopia, which has several goat breeds, such management systems might increase the risk of crossbreeding, and losses of pure-bred genotypes. In places where limited controlled natural mating is practiced, selection of the bucks is usually phenotypically-based, without paying attention to basic andrological examinations.

Artificial insemination (AI) is neither well developed nor organized –at national level- in Ethiopia. There is only one national AI centre, mainly providing service for cattle. Some scattered AI activities have also been undertaken by some governmental and non-governmental organizations, but these are primarily intended for research and teaching purposes. The national AI centre has mostly been providing services for crossbred and purebred dairy cows located in urban and peri-urban areas, because of increased urban demand for dairy products. Very recently, however, an attempt was made to extend AI services to some rural towns. Despite this, the centre has focused on crossbreeding exotic dairy cattle genotypes with indigenous breeds, to produce F<sub>1</sub> descendants with higher milk yields; little attention has been paid to breeding pure-bred, well-adapted indigenous stock. Besides the incentive for increasing milk production in urban areas, a lack of rural infrastructure – including the necessary facilities and equipment for AI in the countryside – has jeopardized the development of AI in non-urban regions of Ethiopia. As can be inferred from the above, the use of AI for developing national livestock resources other than dairy cattle has not received due attention in Ethiopia, mostly owing to the prevalence of traditional extensive breeding and the resultant lack of identification and selection of the best breeding males. Recording and evaluating the testicular and seminal traits of goat bucks at various physiological stages is a prerequisite for any herd improvement efforts.

### **Breeding soundness evaluation in goats**

Independently of whether natural mating or AI is used for breeding, the reproductive competence of a herd is strongly influenced by the male used (Chacon *et al.*, 1999). Breeding soundness examination (BSE) is thus valuable, and probably represents the most practical tool with which to select the potentially best breeding male animal in a flock, as demonstrated in beef bulls (Kennedy *et al.*, 2002). The BSE also helps disclose prevalent breeding problems in a flock, being useful for a farm or breeding centre at time of animal purchase, as such screening will help prevent the introduction of infertility problems into a flock. The consistent use of BSE improves reproductive efficiency by identifying sub-fertile males and assessing the current status of males of previously good fertility (Coe, 1999). BSE is based on clinical examination of male genitalia and behaviour, and the evaluation of semen production and quality (Ott & Memon, 1980; Kennedy *et al.*, 2002). Evaluation of the spermogram, including semen volume, sperm motility, sperm concentration and sperm morphology, provides an

important indicator of the breeding potential of males and helps predict the animal's fertilizing ability. In male goats, where ejaculate volume is usually under 2 mL, selecting bucks that produce larger semen volumes and, it is hoped, more spermatozoa with intact sperm attributes could allow the insemination of many more does through AI breeding programmes. However, unlike bull BSE in which standard criteria are continuously evolving, such examinations are still uncommon for small ruminants in general and goat bucks in particular (Ball, Ott & Mortimer, 1983; Chacon *et al.*, 1999; Kennedy *et al.*, 2002). Given the increasing socio-economic importance of goats and the increased requirements for proper goat husbandry, which demands the best breeding bucks for profitable production, a functional BSE system is needed for goats in Ethiopia.

### **Characteristics of tropical feed resources**

Most feed resources for livestock in the tropics are derived from natural grasslands and crop residues. However, these resources are marginal in nutritive value and of insufficient abundance. According to Bediye and Sileshi (1995), most dry forages in tropical environments contain less than 8% of crude protein (CP) and a large fibre fraction. Feed resources containing less than 7% CP cannot support optimum rumen fermentation (Whiteman, 1980), while feed resources with large fibrous fractions inhibit feed intake due to their slow digestion (Van Soest, 1994; Mekasha *et al.*, 2004). Consequently, such feedstuffs are unable to meet the year-round nutrient requirements of livestock beyond maintenance levels (Nsahlai, Zinash & Bediye, 1996). Apart from this, steadily increasing human populations have converted much grazing/browsing land into cropping land, exacerbating feed scarcity in the tropics (Berhanu, Yami & Hailemariam, 1999). Besides, the availability of tropical feed resources is seasonal, being governed by seasonal rainfall dynamics. Animal productivity is thus determined by the fluctuation of feed resources, and it is negatively affected in the dry season when feed is scarce and of low quality. Reproduction is among the first parameters to be jeopardized in critical dry periods, as nutrient partitioning is primarily meant for maintenance.

Among the various suggested strategies to ameliorate this situation is supplementing the fibrous low-quality basal diet with more nutritious and digestible feedstuffs; this improves voluntary feed intake and animal performance by increasing rate of forage digestion and passage (Ellis, 1978; Mekasha *et al.*, 2002; Mekasha *et al.*, 2004). However, as concentrates, especially cereals and oilseeds, are too expensive for smallholders in the tropics (and moreover are valued as human food), the focus is on finding alternative, unconventional feed resources (Mekasha *et al.*, 2002). Agro-industrial by-products (e.g., cereals and oilseed by-products) and farm leftovers (e.g., khat leftovers) are such potential feed resources. Grasser *et al.* (1995) have emphasized that by-product feeds are becoming increasingly important in the food and fibre system, because they are available for use as livestock feeds at competitive prices relative to other feed commodities. As small ruminants, goats in particular, are mostly kept by resource-limited smallholders in mixed crop-livestock production systems, such a strategy would help them produce at the lowest cost.

Khat (*Catha edulis*) leftovers comprise the unused part of the khat crop and are among the non-conventional feed resources abundant in Ethiopia. Khat is a perennial plant and, along with coffee and oil-seeds, it is an important export crop in Ethiopia (MEDaC, 2000). The innermost part of this cash crop has been used since ancient times as a stimulant for social, cultural and recreational purposes by several million people in East Africa and southern Arabia; more recently, its use has become widespread worldwide (Carvalho, 2003; Adeoya-Osiguwa & Fraser, 2004; Mwenda *et al.*, 2006). Khat consumption, primarily by chewing, is documented to lead to increased levels of energy, alertness and self-esteem, to produce a sensation of delight and to enhance imaginative ability and the capacity to associate ideas (Carvalho, 2003). Khat can also be consumed as a tea after boiling the dried leaves, hence its common name, “Abyssinian tea”.

Meanwhile, most of the unused parts of the khat plant, the hard leaves, branches and twigs, are thrown away; these either decompose or, more often, are picked up by livestock, especially goats. However, despite their wide availability and distribution in crop farming areas, there has been little investigation of the nutritional value of these leftovers and the effect of their consumption on livestock reproduction.

The human consumption of khat is controversial, since the plant contains naturally-occurring alkaloids (Al-Mottareb, Baker & Broadley, 2002; Mwenda *et al.*, 2006). Chewing the leaves of khat releases cathinone, which is fairly unstable and metabolized relatively quickly to the more stable and less potent molecules cathine and norephedrine (Adeoya-Osiguwa & Fraser, 2004). Cathinone, cathine and norephedrine are related to ephedrine, a stimulant present in prescription pharmaceuticals and over-the-counter medications and products, such as herbal dietary supplements for weight loss and asthma treatment (Greenway, 2001). The fact that cathinone is, structurally, closely similar to amphetamine, sharing pharmacodynamic features with it, has led to the conclusion that cathinone has major pharmacological effects (Hollister, 1995). The common, albeit individual adverse effects of khat consumption are reported to include insomnia, anorexia, hyperthermia, endocrinological disturbances, delayed gastric emptying and toxicity (Brenneisen *et al.*, 1990; Al-Habori *et al.*, 2002), causing khat to be classified as an addictive drug in many countries but yet considered as a traditional herbal tonic in many others.

Most studies of khat have focused on its pharmacology and chemistry, little attention being paid to its biological implications, including toxicity (Al-Habori *et al.*, 2002; Al-Mottareb, Baker & Broadley, 2002). According to available toxicological studies, including khat in the diet of New Zealand white rabbits caused nephrotoxic and hepatotoxic effects (Al-Habori *et al.*, 2002). In humans, a depressive stage follows the end of a khat session of consumption, and cognitive impairment and neurological illness are among other reported side effects of khat consumption (Morrish *et al.*, 1999; Al-Habori *et al.*, 2002; Carvalho, 2003).

Reports regarding effects of khat on male reproduction are controversial. On one hand, it has been demonstrated that injecting pure cathinone decreased sperm count and motility, as consequence of degenerative changes in testicular morphology and reduced plasma levels of testosterone in male rats (Islam *et al.*, 1990). Comparing two groups of human males, khat consumers vs. non-consumers, has also demonstrated that sperm concentration, morphology and motility were poorer among the former (El-Shhoura *et al.*, 1995).

On the other hand, it has been demonstrated that the khat metabolites, cathine and norephedrine, accelerated sperm capacitation in uncapacitated mouse- and human spermatozoa and inhibited spontaneous acrosome reaction in capacitated mouse spermatozoa, suggesting that, at an appropriate dose, these drugs might even have specific effects on sperm function (Adeoya-Osiguwa & Fraser, 2004). Supplementing rabbits with graded levels of dried khat leaves has been demonstrated to stimulate spermatogenesis, without affecting either the Leydig cells or the epithelium of the epididymal duct (Al-Habori *et al.*, 2002). Crude khat extract up-regulated plasma testosterone levels and reduced prolactin and cortisol levels in baboons, without affecting the histology of the testis, epididymis, liver or pituitary gland (Mwenda *et al.*, 2006). These positive findings run contrary to earlier reports by Islam *et al.* (1990).

The above description of findings from the literature indicates that studies of the effects of khat on reproduction have achieved divergent results. Most of the experiments were done entirely on monogastric animals and the methods of administration have been diverse, and not only *per os*. Since the pre-stomachs of ruminants, where various physico-chemical and biological reactions take place, could well affect some of the compounds contained in khat, studies should be undertaken to determine the potential of these leftovers. This is especially true in the case of goats, which cannot only efficiently digest browses including tannin-rich feeds, but also have the potential to detoxify them (Silanikove *et al.*, 1996). Given that khat leftovers are ubiquitous and cheap, albeit controversial, and that inadequate nutrition appears as the major bottleneck threatening livestock production, investigating the nutritional value of this non-conventional feed resource and its effect on male goat reproduction is worthwhile.

## **Testicular traits**

Testicular traits are important variables closely associated with sperm features and animal fertility. The shape and content of the scrotum are associated with fertility parameters (Coulter & Foote, 1977). Scrotal circumference (SC) and testicular consistency (TC) have been widely used in predicting the reproductive capacity of male domestic animals. This is mainly because SC is an indirect measurement of testicular mass and a reliable indicator of testicular growth and, indirectly, of the spermatogenic capacity of the testis – normally also related to TC (Daudu, 1984). Similarly, testicular weight (TW) is a reliable variable for estimating the sperm production capacity of males; together with the other variables, it can be used to select males for testicular size at puberty (Coulter, Larson & Foote, 1975). These

traits, SC in particular, are most heritable components of fertility and should thus be included in the BSE (Bailey *et al.*, 1996). Even though several studies have characterized the testicular traits of bulls (Coulter, Larson & Foote, 1975; Coulter & Foote, 1976), comparable information is scarce regarding goat bucks in general and Ethiopian goats in particular. The fact that Ethiopia has a diverse agro-ecology with diverse climatic conditions suggests that indigenous goat breeds might differ in testicular traits as an adaptive response, for example, to promote better thermoregulation of spermatogenesis. Studying such basic matters is obviously of utmost importance.

### **Sperm production and sperm morphology**

Spermatogenesis is a long process of cellular generation and differentiation in which a spermatogonial stem cell proceeds through several mitotic divisions, meiosis and cytological transformations leading to the generation of elongated spermatids (Barth & Oko, 1989) that ultimately differentiate into testicular spermatozoa. Following maturation in the epididymis, these terminal and highly specialized cells, are available for delivery to the female (Rodriguez-Martinez, 2003). The function of the spermatozoon is, primarily, to deliver a message from the paternal side, contained in the highly condensed nuclear genomic material, within a certain period after deposition in the female (Rodriguez-Martinez *et al.*, 1997). To achieve this goal, spermatozoa depend on the intactness of the processes of spermatogenesis and sperm maturation.

Under normal conditions, spermatozoa traverse the seminiferous tubules after synthesis, mature along the epididymal duct and are then stored in the cauda epididymides until ejaculation. Since sperm production and maturation are continuous processes, delicate in nature and proceeding under constraints (i.e., thermic, nutritional and endocrinological), the sperm population in the epididymal reserves and in the ejaculate are, per definition, heterogeneous in nature. Such heterogeneity can be reflected in differences in morphology (even within the limits of accepted normality), viability and, ultimately, in fertilizing capacity (Rodriguez-Martinez & Barth, 2007). Thus, evaluating the sperm profile *in vitro* is a valuable way to determine the normality of the spermatozoa and, to some extent at least, predict their, and by extension, the male's, fertilizing potential. Semen evaluation is of high diagnostic value in assessing testicular and epididymal function and/or the genital tract of the male, allowing the elimination of clear-cut cases of infertility or even of potential sub-fertility (Rodriguez-Martinez, 2003). Furthermore, by evaluating semen one can determine its degree of normality before it is processed for AI (Koonjaenak, 2006). To this end, assessment of sperm motility, number and morphological characteristics has commonly been included in such evaluation, although more elegant assessment methods are available (Rodriguez-Martinez, 2006). Sperm evaluation should thus be considered a prerequisite for selecting breeding males, including goat bucks.

## **Factors influencing body size, testicular traits and sperm features**

Goats are important meat animals in the tropics. Growth performance and body weight (BW) changes are thus crucial to attaining the required market BW with good carcass composition. Similarly, growth in testicular size is an important indicator of the breeding potential of male animals. Animal size, as BW, is closely associated with testicular mass (Nsoso *et al.*, 2004). However, the patterns of the growth and sperm production capacity of bucks, as in other domestic male animals, are influenced by factors such as nutrition, breed, age, season and health status (Roca *et al.*, 1992; Bielli, 1999; Karagiannidis, Varsakeli & Karatzas, 2000).

### *Effect of nutrition*

Nutrition is a major environmental cue that influences growth and sperm production in domestic animals. In a tropical environment, where the influence of photoperiod is minimal, nutrition appears to be the major modulator of sexual activity in small ruminants. This is unlike the situation in higher latitudes, where seasonal factors have an overriding impact on reproduction, since improving the nutritional status cannot reverse a seasonal reduction in testicular size once it has started, as demonstrated in Corriedale rams (Bielli, 1999). However, such testicular regression is less prominent in rams grazing on improved pasture than in rams grazing only on natural pastures, higher feeding levels hastening testis size recovery after winter size reduction (Bielli, 1999). Ruminant domestic animals in general require nutrients primarily for maintenance of their body metabolism, followed by growth, production and reproduction. Supplying nutrients as required is thus indispensable for optimum livestock performance.

Improving feed quality by improving its protein content results in improved BW, testicular size, semen characteristics and fertility (Abi-Saab *et al.*, 1997; Fernandez *et al.*, 2004). Increasing the energy intake has also been reported as favourably influencing testicular growth and sperm production (Braden *et al.*, 1974; Murray *et al.*, 1990). Nutritional regimes that improve average daily growth (ADG) also enhance testicular development and sexual maturity in ram lambs (Mukasa-Mugerwa & Ezaz, 1992; Bielli, 1999). Increased feeding levels lead to heavier TW (Bielli *et al.*, 1997; Bielli *et al.*, 1999), indicating a link between better nutrition and higher sperm-producing parenchyma in the testis of the animals. Higher-quality semen is usually collected in the rainy rather than in the dry season in the tropics, ultimately indicating the positive influence of nutrition, as rainy season feeds have more nutrients than dry season feeds do (Carmenate & Gamcik, 1982). Supplementing highland Ethiopian rams on a low-quality basal diet with *Leucaena leucocephala* leaves increased the percentage of individual and mass sperm motility, sperm concentration/mL and sperm number (Dana, Tegegne & Shenkoru, 2000). Furthermore, digestible energy intake is closely related to live weight change, both of which are excellent indicators of testicular growth (Murray *et al.*, 1990). Fernandez *et al.* (2004) have demonstrated that improved protein supply in the form of rumen un-degradable protein increased ADG, SC, testicular volume and semen production, suggesting that spermatogenesis is positively

affected by increasing feeding levels. It has also been demonstrated that feeding with sown pasture and grain increases the number of elongated spermatids per Sertoli cell compared to those of controls fed on native pasture (Bielli *et al.*, 2001). Similarly, improved nutrition maintains and increases the secretion of gonadotrophins and thereby enhances the proportions of morphologically normal spermatozoa (Walkden-Brown, Restall & Taylor, 1994).

On the other hand, inadequate nutrient supply depresses growth and reproduction in domestic animals. Supplying low-quality feeds to rams decreases the proportion of morphologically normal spermatozoa and increases the proportion of immature spermatozoa (Dana, Tegegne & Shenkoru, 2000). Nsahlai *et al.* (2000) have also demonstrated that protein undernutrition severely affects sperm motility and morphology. Oldham *et al.* (1978) have associated depressed testicular growth and spermatogenesis with protein deficiency in rams. It has been reported that undernutrition reduces serum luteinizing hormone and follicle stimulating hormone secretion, impairing testicular growth in ram lambs (Martin & White, 1992). Poor maternal nutrition during pregnancy reduces the number of Sertoli cells per testis, and hence, the future capacity for sperm production and fertility in the newborn lambs (Bielli *et al.*, 2002). It is thus essential to supply the required nutrients for the optimum functioning of the testicular parenchyma, which in turn ensures optimum spermatogenesis and, indirectly, normal sperm maturation in the epididymis. However, information is scarce on the response of Ethiopian goats to differential nutritional supplementation of a low-quality basal diet.

#### *Effect of breed*

Breed is one factor that influences body size, testicular and seminal traits in domestic animals. This has been demonstrated in Alpine, Saanen and Damascus goat breeds, in which differences between breeds affected most seminal characteristics (Karagiannidis, Varsakeli & Karatzas, 2000). Similarly, genotype differences in testicular size, testicular and epididymal weight and gonadal and extragonadal sperm reserves have been reported in bulls (Tegegne, Dembarga & Kassa, 1992). Consequently, large-sized breeds grow faster but mature sexually later than small-sized breeds do (Taylor, 1971). Likewise, large-sized breeds are heavier and have larger testicular measurements than small-sized breeds, and continue to grow until late in their maturation (Al-Ghalban, Tabbaa & Kridli, 2004).

Thus, by selecting males with larger testes, more females could be bred per sire, resulting in greater production efficiency, especially if AI is applied (Coulter, Larson & Foote, 1975). Larger SC is associated with greater ejaculate volume, higher sperm concentration, better seminal quality, and higher daily sperm production (Mukasa-Mugerwa & Ezaz, 1992; Coulter, Cook & Kastelic, 1997). The higher sperm production due to larger testicular mass is advantageous for semen banking from superior bucks of preferred breeds and for the conservation of genetic material.

Differences in breed have also been reported to influence sperm morphology in domestic animals. Söderquist *et al.* (1991) found variation in proximal cytoplasmic droplets and abnormal sperm head shapes in ejaculated spermatozoa between two bull breeds studied; Dowsett and Knott (1996) also reported differences in similar variables between stallion breeds. The prevalence of between-breed variation in abnormal spermatozoa has also been reported in goats (Karagiannidis, Varsakeli & Karatzas, 2000). In Africa, Ethiopia has the largest number of goat breeds, which are widely distributed within the country due to its agro-climatic diversity. Thus, it is logical to infer that such diversity in goat breeds and agro-climate is an important factor governing body size and testicular and seminal traits. Even though tropical breeds have evolved through natural selection for adaptive traits, they exhibit non-uniformity in production and reproduction variables, as demonstrated earlier (Tegegne, Dembarga & Kassa, 1992; Tibbo, 2006). Meanwhile, differences in productivity and seminal attributes have been reported in different breeds of small ruminants (Karagiannidis, Varsakeli & Karatzas, 2000; Tibbo, 2006), indicating that one can improve the performance of tropical goats by applying pertinent breeding systems. However, information on how and to what extent breed influences the body size and testicular and seminal attributes of tropical goats in general and Ethiopian goats in particular, is scanty.

#### *Effect of age*

Differences in physiological stage due to age influence body size and testicular growth in domestic animals (Karagiannidis, Varsakeli & Karatzas, 2000). Under normal conditions, animals grow fast when younger, but grow slowly when they reach maturity. Testicular development is also rapid at an early age, which is followed by a period of slow growth in goat kids (Nsoso *et al.*, 2004). Brito *et al.* (2002) have also demonstrated that SC increases rapidly in young bulls, but only gradually in mature bulls, and even decreases in old bulls. In goat bucks too, the increase in SC is curvilinearly related to age (Bongso, Jainudeen & Siti Zahrah, 1982). However, comparing the growth of male animals in terms of BW and testicular mass, growth of the latter is sluggish especially at higher ages. Similarly, Nishimura *et al.* (2000) reported that growth in TW as a percentage of BW was higher before 12 months of age, but that it decreased by 24 months, in the Tokara (Japanese) goat. Age also influences sperm morphology in domestic male animals. Earlier studies have indicated that abnormal sperm head shape was more prevalent at a younger age, gradually decreasing after sexual maturity, but becoming higher again at an older age (Söderquist *et al.*, 1991; Dowsett & Knott, 1996; Amann, Seidel & Mortimer, 2000). This is apparently due to immature spermatogenesis at a younger age, a slight propensity to testicular (seminiferous epithelium) degeneration with age and slight disturbances in epididymal function at older ages (Jackson & Dowsett, 1994; Dowsett & Knott, 1996). All this suggests that domestic male animals should be used for breeding or semen collection in an age range within which growth performance and spermiogram attributes are optimal. Furthermore, it suggests that different breeds of domestic animals should be compared at similar ages when evaluating reproductive traits, which are important indicators for the selection of superior breeding animals (Harder, Lunstra &

Johnson, 1995). The lack of systematic research in this regard, into bucks of tropical breeds, makes it difficult to determine the optimum age range for better breeding and semen collection. In Ethiopian male goats, information is almost non-existent with regard to the body size, testicular traits and semen features of different age classes.

## Aims of the study

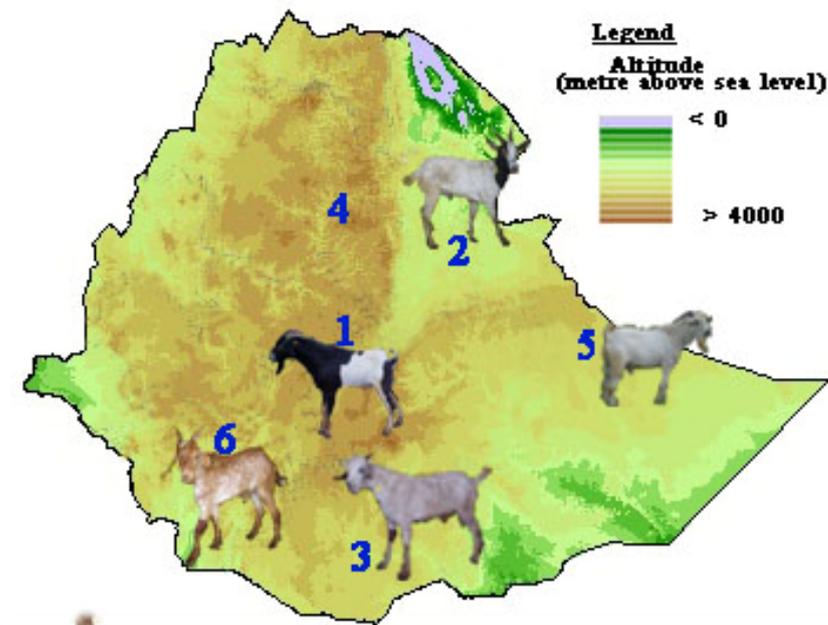
The overall aim of this thesis was to characterize the corporal, testicular and semen traits of male goats, indigenous to Ethiopia, in relation to breed, age and nutritional status. More specifically, it aimed to determine:

- the influence of breed and age class on the body size and testicular traits of tropical bucks raised under extensive husbandry;
- whether the morphological attributes of the spermatozoa of tropical bucks raised under extensive husbandry were affected by breed and age;
- the effect of feed supplementation with agro–industrial by-products and khat (*Catha edulis*) leftovers on testicular growth and sperm production; and
- whether feed supplementation with agro–industrial by-products and khat (*Catha edulis*) leftovers impacted feed intake and sperm morphology.

## Methodological considerations

### Overview of the study

This study investigated the reproductive traits of tropically adapted indigenous male goat breeds under both extensive (**Papers I and II**) and controlled management with differential nutritional regimes (**Papers III and IV**). Six major indigenous male goat breeds distributed over more than 50% of Ethiopia's landmass, and representing diverse agro-ecologies, were included (Figure 1). Several breeds were compared across constant age classes, while a selected breed was evaluated in depth under differential nutritional treatments.



**Figure 1.** Approximate distribution of indigenous goat types (breeds) included in this study: 1: Arsi-Bale; 2: Afar; 3: Boran [Long-eared Somali]; 4: Central Highlands [not depicted]; 5: Ogaden [Short-eared Somali]; 6: Woito-Guji.

### Study location

The field research (data sampling) was carried out in Ethiopia, and examined both at extensive (**Papers I and II**) and controlled (**Papers III and IV**) management systems. The material and data for **Papers I and II** was sampled at two export abattoirs, namely, HELMEX (Debre-Zeit; 10° 34' N & 35° 47' E) and MODERN (Mojo; 8° 35' N & 39° 7' E). The abattoirs are Ethiopia's major exporters of small ruminant meat, and their importance in providing relevant genetic information is described in **Paper I**. The studies presented in **Papers III and IV** were undertaken

at Haramaya University's goat research centre (9.0°N and 42.0°E). In all cases, sperm morphology was evaluated at the sperm laboratory, Division of Reproduction, Department of Clinical Science, Swedish University of Agricultural Sciences (SLU), Sweden.

## **Animals**

### *Goat population and their distribution in Ethiopia*

In this study, bucks of five major tropically adapted breeds raised under extensive husbandry in Ethiopia were evaluated across three constant age classes (**Papers I and II**). The buck breeds included (see Figure 1) were *Arsi-Bale* (AB), *Central Highlands* (CH), *Afar*, *Boran* (also known as long-eared Somali), and *Woito-Guji* (WG). Furthermore, the *Ogaden* breed (also known as short-eared Somali), dominating most of the eastern part of the country, was employed in investigating the response of indigenous bucks to varied nutritional regimes under controlled husbandry conditions (**Papers III and IV**). In general, due to diverse agro-climates and production systems, the distribution of goat breeds differs in the country, as reported earlier (FARM-AFRICA, 1996) and summarized in **Paper I**.

### *Animal sampling and management*

To characterize the body size, testicular traits and epididymal sperm attributes of bucks raised under extensive husbandry, apparently healthy bucks were selected from each of the five breeds supplied to the abattoir and considered as a slaughter flock representing different agro-climates of origin (**Papers I and II**), employing stratified randomly sampling. The total number of bucks included in the study was 177 (AB= 35, Afar= 35, CH= 33, Boran= 36 and WG= 38) (**Paper I**). However, sperm morphology based on epididymal spermatozoa was studied in only 74 bucks (AB= 15, Afar= 15, CH= 10, Boran= 16 and WG= 18) (**Paper II**). The age of the bucks was approximately determined from dentition, based on the number of permanent incisors, as previously reported for indigenous African goats: all primary teeth= <14 mo (younger), one erupted pair of definitive incisors= 14–19.5 mo (intermediate age) and two erupted pairs of definitive incisors= 19.6–24 mo (older) (Wilson, 1989). The effects of breed and age on sperm morphology were studied based on caudal epididymal spermatozoa, sperm maturation was studied using spermatozoa aspirated from three anatomical segments of the epididymis (caput, corpus and cauda).

The on-station study, concerned with the influence of nutrition, involved one-year-old Ogaden bucks purchased from a local open market (**Papers III and IV**). The bucks were clinically examined for any obvious abnormalities at time of purchase, and the apparently healthy animals were further screened. Upon arrival at the university, the animals were quarantined for three weeks and vaccinated against ovine pasteurellosis and treated for endo- and ectoparasites. After the quarantine period, initial measurements were made of body weight (BW) and scrotal circumference (SC) before feeding the animals. Forty apparently healthy bucks of uniform ( $P>0.05$ ) initial BW (mean  $\pm$  SD;  $15.5 \pm 1.5$  kg) and SC ( $20.0 \pm$

1.4 cm) were selected and randomly assigned to one of the four dietary treatments ( $n= 10$  each). The animals were kept indoors in individual pens, and adapted to the experimental feeding and facilities for an additional two weeks. The animals had free access to clean, fresh water. However, five bucks assigned to Treatments 1 ( $n= 3$ ) and 2 ( $n= 2$ ) died during the experimental period. At the end of the experiment, 16 bucks, four from each treatment, were euthanized for evaluation of their genital organs. The actual experiment lasted 12 weeks.

### **Dietary treatments and feed sampling**

Nutritional treatments were as follows: feeding native hay (dominated by *Hypernia rufa*) *ad libitum* (control, C), native hay supplemented with agro-industrial by-products at 1% of BW on a dry matter (DM) basis (Treatment 1, T1), native hay supplemented with khat (*C. edulis*) leftovers at 1% of BW on a DM basis (Treatment 2, T2), and feeding khat (*C. edulis*) leftovers *ad libitum* (Treatment 3, T3). The agro-industrial by-products, mixed as needed, comprised 40% wheat bran, 25% wheat middlings and 35% groundnut cake. The daily allowance of native hay and khat leftovers was provided in two portions, at 9:00 and 16:00 hrs, while the agro-industrial by-products mix was fed only once a day. Feed refusals were collected the next morning before refreshing the feed. Samples were taken of each feed type, from the offer and refusal, once a week for chemical composition analysis. Animals had free access to mineral blocks irrespective of treatment (**Papers III and IV**).

### **Data collection procedures and measurements**

#### *Chemical analysis of feedstuff*

The dry matter, organic matter and ash content of the feedstuff were assayed according to AOAC (1990). The nitrogen content of the feed was determined using the Kjeldahl procedure, while the energy content was determined using bomb calorimetry. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) compositions of the feed were assessed according to Van Soest *et al.* (1991). *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963). The mineral composition of the feed was analyzed using atomic absorption spectrophotometry (**Papers III and IV**).

#### *Feed intake*

The quantities of fresh feed offered and feed leftovers were measured daily for individual animals, during the entire experimental period. Feed dry matter and nutrient intakes were estimated based on the chemical composition of the feed and the amounts of feed offered and refused (**Paper IV**).

### *Scrotal measurements*

Scrotum shape (normal ovoid or long ovoid), scrotum anatomy (undivided or split) and degree of testicular symmetry (symmetrical or not) were visually assessed and categorized. The scrotal content was palpated for freely moving testicles, testicular tone (soft, normal or hard) and presence of ducti epididymides (**Paper I**).

### *Body size and testicular measurements*

The BW of the animals was measured using a scale, BCS was scored subjectively (ranging from 1= emaciated to 5= obese) and SC was measured using a measuring tape at the broadest part of the scrotum (**Papers I and III**). Testicular length and width, however, were measured using a digital caliper (**Papers I and III**). Heart girth (HG), body length (BL) and hip width (HW) were measured using a plastic measuring tape, while height at wither (HAW) and body depth (BD) were measured using a metal measuring tape (**Paper I**).

For **Paper III**, 16 animals (4 bucks from each treatment) were sacrificed at the end of the experiment for post-mortem testicular measurements, while 74 were sacrificed for **Paper II** and 177 for **Paper I**, as indicated above. In all cases, the right and left testes of each buck were removed separately; each was placed in a different pre-labelled plastic bag and transported to the laboratory in an icebox. At the laboratory, epididymal segments were carefully dissected from the testes and separated into three anatomical segments: Caput (head), corpus (body) and cauda (tail). The weights and dimensions of the testes and epididymides were measured using a scale and a digital calliper, respectively, while testicular volume was estimated by means of water displacement (**Papers I, II and III**).

### *Semen and epididymal sperm collection, and evaluation*

Semen was collected from the goat bucks with the aid of a portable, battery-operated electro ejaculator (**Papers III and IV**), while epididymal spermatozoa were collected post-mortem from the epididymides (**Paper II**). In the latter case, each segment of the right epididymis (caput, corpus and caudal) was incised using a scalpel blade and the spermatozoa were aspirated using a pipette into a labelled vial containing buffered formalin solution (Hancock, 1957) for fixation.

Immediately after semen collection, semen volume and sperm motility were measured. Sperm mass activity was subjectively assessed by placing a drop of semen on a pre-warmed slide and using a light microscope at 100× magnification (scored from 1= no activity to 5= maximal activity), while the percentage of individually progressively motile spermatozoa was subjectively assessed on an extended drop of semen under a cover slide at 400× magnification (scored at 10% unit intervals). Sperm concentration was measured in a Bürker chamber, after diluting the semen with distilled water (1:1000). The spermatozoa were counted under a phase contrast microscope (100× magnification) by the same operator (**Paper III**).

Drops of semen samples were fixed with buffered formaldehyde solution for sperm morphological examination (**Paper IV**). From the semen and epididymal samples, two hundred spermatozoa each in wet smears were counted using a phase contrast microscope with an immersion objective lens at 1000× magnification. Spermatozoa displaying morphological abnormalities, including proximal and distal cytoplasmic droplets, loose heads, acrosome defects and abnormalities, nuclear pouches, abnormal mid-pieces and tail defects, were identified. Spermatozoa were also evaluated in air-dried smears stained with Williams solution (carbol–fuchsin–eosin), as described by Williams (1920) and modified by Lagerlöf (1934). Five hundred spermatozoa were examined in a light microscope at 1000× magnification in these slides, to count sperm head shape abnormalities (pear shape, narrowness at the base, abnormal contour, undeveloped, narrowness, abaxial tail implantation and abnormal loose heads). The relative percentage of morphologically normal spermatozoa was estimated from both the wet smears and William-stained slides (the proportion of spermatozoa without any defects according to either method) and presented as a range following our reference laboratory protocol. Smears with dense ridges were also prepared and stained with haematoxylin–eosin, according to a method described by Papanicolaou (1942), to detect the presence of cells other than spermatozoa (such as cells of the seminiferous epithelium, epididymides and genital tract as well as inflammatory cells). Their relative abundance (scored as 0= absent, 1= scarce, 2= moderate, and 3= rich-to-very rich) was determined at magnifications of 100×, 250× or 400× using light microscopy (**Papers II and IV**).

### **Statistical analyses**

Data regarding body mass and testicular traits (**Paper I**), and regarding sperm morphology based on caudal epididymal spermatozoa (**Paper II**), were stratified into breed and age classes and analyzed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS) software, version 8 (SAS Institute, Cary, NC, USA, 2004). Segments of the epididymides were also considered as class variables when evaluating sperm maturation (**Paper II**). As significant interaction between breed and age was apparent for body size and testicular traits, they were included in the model (**Paper I**). For the feed supplementation experiment (**Papers III and IV**), data were stratified into treatment groups and observation period and analyzed according to an entirely randomized design with repeated statements using the “mixed” procedure of SAS. The model also accounted for the effect of interaction between the class variables. Sperm concentration was log transformed (**Paper III**), while sperm morphology and the relative quantity of foreign cells were square-root transformed (**Papers II and IV**) before analysis. Differences due to class variables and their interactions were examined using Tukey’s adjustment (**Papers I–IV**). The Spearman coefficient of correlation was employed to assess the existence of associations between growth, testicular and sperm production variables (**Paper III**) and between body size and testicular traits (**Paper I**). The generalized linear model (GENMOD) procedure of SAS was employed to analyze data regarding subjective clinical measurements of testicular traits (**Paper I**). Differences due to the class variables and their interactions were declared significant at  $P < 0.05$ .

## Results

### Feedstuff composition and feed intake

The crude protein (CP) content of khat (*Catha edulis*) leftovers was moderate, with a low fibre content expressed as neutral detergent fibre and acid detergent fibre. While hay had marginal CP and high fibre contents, wheat bran, wheat middlings and groundnut cake had high CP but lower fibre contents than khat leftovers did. Gross energy (GE) was high for khat leftovers and wheat middlings, moderate for wheat bran and groundnut cake and low for hay. Groundnut cake and wheat middlings had high true IVOMD, but it was moderate for wheat bran and khat leftovers and low for hay. Furthermore, khat leftovers contained higher Ca and Mg but lower K and Mn levels than did other feeds (**Papers III and IV**). Khat leftovers contained high lignin (7.8%) and tannins (soluble= 26.2%; condensed= 16.75 abs/g).

Consequently, goats assigned to the three treatment groups (T1–T3) consumed higher ( $P<0.001$ ) mean total feed dry matter (DM) than did their counterparts assigned to the control (C) group. Similarly, mean total feed nutrient intakes (CP, OM and GE) were higher for the T1–T3 than for the C groups. Of the treatment groups, the T2 and T3 feeding regimes resulted in higher ( $P<0.05$ ) DM, GE and OM intakes than did T1 feeding; however, CP intake was higher ( $P<0.01$ ) in T1 followed by T3 and then T2. Consumption of feed DM, CP, OM and GE was higher for the bucks in the last feeding period (period IV) than the first (period I), though such a trend was not observed for the control bucks. The mean overall NDF intake was higher ( $P<0.05$ ) in T1, T2 and C than in T3. NDF intake was higher in period IV than period I for T1 and T3 goats, but not appreciably so for T2 and C goats (**Paper IV**).

### Assessment of scrotal traits

The present study revealed the existence of non-uniformity ( $P<0.05$ ) in scrotum shape among indigenous goat breeds. A normal ovoid scrotal shape was more often seen in AB (71.4%) than in WG (40.5%) or Afar (34.2), while CH (59.3%) and Boran (52.7%) had intermediate proportions of this scrotum shape. The percentage of bucks with anatomically split scrotums was highest ( $P<0.05$ ) in Afar and Boran breeds. Regarding testicular symmetry, most bucks of the Boran (94.2%) and WG (92.1%) breeds had symmetrical testicles, unlike CH (71.4%) and AB (67.7%) bucks. Basically, there was no difference in testicular consistency among the five goat breeds studied (**Paper I**).

### Body mass and testicular traits

Breed differences were reflected ( $P<0.001$ ) in physical linear traits (heart girth, body length, height at wither and hip width), while HG as well was significantly influenced by age. Thus, Afar breeds maintained low HG while the other breeds

had higher HG, BL and HAW across all age classes compared. Afar bucks had a greater HG in the intermediate age class, while Boran, WG and AB bucks had a greater HG in the older age class. However, HG, BL and HAW measurements were slightly higher for Boran and CH than for AB and WG in both the younger and intermediate age classes. The hip width measurement was the largest ( $P<0.05$ ) for Boran, medium for WG followed by CH and the least for Afar in both the young and intermediate age classes. In the oldest age class, Boran and WG had the widest hips and Afar the narrowest (**Paper I**).

Body weight and SC were influenced differently ( $P<0.01$ ) by breed and age class. Boran bucks were heaviest and had the greatest SC of all the studied breeds, across all age classes ( $P<0.05$ ). Afar bucks were smallest and attained their highest weight in the intermediate age class, while AB, Boran, and WG attained their highest weights in the oldest age class. AB and Afar bucks had the greatest SC in the intermediate age class, and Boran and WG bucks in the old age class. BCS was affected by breed ( $P<0.001$ ) and its interaction with age ( $P<0.05$ ); Boran bucks had better BCS than the other breeds did in the young age class, and together with WG bucks had the highest BCS in both the intermediate and old age classes. While CH bucks had the lowest BCS, Afar and AB bucks achieved intermediate values (**Paper I**).

The effects of breed and age were apparent ( $P<0.001$ ) for testicular weight (TW). Accordingly, TW was higher for Boran, WG and CH bucks than for AB and Afar bucks in the young age class. Boran bucks retained the highest ( $P<0.05$ ) TW in the intermediate and old age classes. Testicular weight expressed as a percentage of live weight (TWPBW) was affected by both breed ( $P<0.001$ ) and age ( $P<0.05$ ), Afar bucks displaying the highest values in both the young and intermediate age classes; in the older age class, however, TWPBW was similar among breeds. The influence of breed was also evident ( $P<0.05$ ) in total epididymis weight; however, differences in epididymis weight attributable to age class and its interaction with breed did not reach significance, owing to similar values among the youngest bucks. Boran had the highest ( $P<0.05$ ) total epididymis weight in the intermediate and old age classes. The weight of the epididymides for the rest of the bucks was similar (**Paper I**).

Nutritional supplementation with khat leftovers (T2 and T3) and an agro-industrial by-products mix (T1) improved ( $P<0.001$ ) BW and BCS in indigenous male goats compared to those of unsupplemented goats fed grass hay only (C). As well, these dietary treatments (T1–T3) ameliorated testicular traits, as indicated by increased SC and paired testicular and epididymis weight and dimensions. Of treatments T1–T3, feeding bucks on a diet exclusively comprising khat leftovers (T3) increased ( $P<0.05$ ) mean BW, average daily gain, BCS, SC, testicular length and width compared to feeding them either the T1 or T2 diets. There was no difference ( $P>0.05$ ) in BW, SC and testicular measurements between T1 and T2. Feeding bucks the T1–T3 diets increased body and testicular size across the observation periods compared to feeding them the control diet. Apart from sustaining a lower body and testicular size across the experimental period, C bucks experienced negative ADG (**Paper III**).

## Seminal traits

### *Morphology of epididymal spermatozoa in relation to breed and age class*

The proportions of head shape abnormalities in spermatozoa collected from the caudae epididymides were similar among the bucks of different breeds ( $P>0.05$ ), though numerically highest in Boran and lowest in AB bucks. The CH bucks accounted for the highest proportion of acrosome defects ( $P<0.05$ ), while the AB bucks accounted for the highest proportion of loose heads. There was no difference in occurrence of proximal and distal cytoplasmic droplets among breeds. Afar and CH bucks accounted for the highest ( $P<0.05$ ) proportion of tail defects, while WG and Boran bucks had the lowest. Overall, WG and AB bucks had the highest proportion of morphologically normal spermatozoa, while CH had the lowest (**Paper II**).

There were no significant differences in the occurrence of sperm head shape abnormalities, proximal cytoplasmic droplets and abnormal mid-pieces in the cauda epididymides, among the different buck age classes. However, young bucks had the highest proportion ( $P<0.05$ ) of loose sperm heads while old bucks had the highest ( $P<0.05$ ) proportion of acrosome defects. In addition, old bucks had a higher proportion ( $P<0.05$ ) of distal cytoplasmic droplets and tail defects than did those in the young or intermediate age classes. Bucks of intermediate age had a numerically higher percentage of morphologically normal spermatozoa than did either the young- or old age classes (**Paper II**).

Sperm morphology differed ( $P<0.05$ ) along the different segments of the epididymis, the caudae having a higher proportion of morphologically normal and mature spermatozoa than the other segments did. Spermatozoa collected from the caput and corpus had more ( $P<0.05$ ) loose heads than did those from the cauda epididymides. The occurrence of both acrosome defects ( $P<0.05$ ) and acrosome abnormalities was higher in the corpus than in the cauda epididymides. The highest ( $P<0.05$ ) proportion of proximal cytoplasmic droplets was seen in the caput, while those collected from the cauda had the highest ( $P<0.05$ ) proportion of distal cytoplasmic droplets. The occurrence of tail defects was highest ( $P<0.05$ ) in spermatozoa retrieved from the caput. Spermatozoa from the cauda had a lower ( $P<0.05$ ) incidence of abnormal mid-pieces and tail defects than did spermatozoa from the other segments (**Paper II**).

### *Semen characteristics in relation to nutrition*

The ejaculate characteristics of indigenous goat bucks were influenced by nutritional management. Feeding goats the T1–T3 diets improved ( $P<0.05$ ) sperm concentration per mL, total sperm number per ejaculate, sperm mass activity and individual sperm progressive motility compared to feeding them the control diet (C). Among bucks in treatment groups T1–T3, those fed the T3-diet produced ejaculates with the highest ( $P<0.05$ ) volume and sperm mass activity. While supplemented bucks maintained the production of sperm with normal attributes

throughout the collection weeks, such a trend was not observed in the control bucks. Ejaculate volume and other sperm variables, however, were similar ( $P>0.05$ ) for bucks in T1 and T2 (**Paper III**).

Sperm morphological features were also influenced by nutritional management. Bucks fed agro-industrial by-products at 1% of body weight on top of a basal diet of hay (T1) and khat leftovers *ad libitum* (T3) had a higher proportion of morphologically normal spermatozoa than did those fed hay only (C) or hay *ad libitum* and khat leftovers at 1% of body weight (T2). The proportion of total abnormal sperm head shapes was under 10% for the T1–T3 group bucks but higher (11.1%) for the controls. The proportion of pathological sperm head shapes was significantly lower for T1 and T3 bucks than for T2 or control bucks. Furthermore, T1 and T3 bucks had the lowest proportion of pear-shaped sperm heads ( $P<0.05$ ), under-developed spermatozoa ( $P<0.01$ ) or acrosome abnormalities ( $P<0.001$ ), compared to T2 or C bucks. Proportions of loose sperm heads, however, were lower ( $P<0.05$ ) for goats in all experimental groups (T1–T3) than for C-goats. Feeding bucks T1–T3 diets also reduced ( $P<0.001$ ) the proportions of immature spermatozoa (those with proximal cytoplasmic droplets). There was no difference ( $P>0.05$ ) in the occurrence of sperm tail defects among nutritional treatments. Samples from the control bucks contained higher ( $P<0.05$ ) relative abundances of spermatogenic cells than did samples from the bucks in the treatment groups (T1–T3) (**Paper IV**).

### **Correlation among reproductive traits**

There were positive linear associations between body size, testicular traits and sperm production variables. Accordingly, BW was favourably associated with SC, TW and TS (**Papers I and III**), but only slightly associated with sperm variables (**Paper III**). Testicular growth as measured by SC and TS was strongly correlated with TW and moderately associated with BCS (**Papers I and III**) or sperm attributes (**Paper III**). Body weight, SC and TW were also associated with physical linear measurements (**Paper I**).

## General discussion

Livestock productivity in the tropics is limited, mainly due to low fertility. Characterizing livestock reproductive traits is thus indispensable for elucidating the reproductive potential of indigenous stock, and thereby enabling the design of appropriate strategies to improve fertility. Body size, testicular traits and sperm attributes are important indicators of the reproductive potential of an animal because of their close association with fertility (Lagerlöf, 1934; Coulter & Foote, 1977; Aziz *et al.*, 1996; Chacon *et al.*, 1999). However, these traits are influenced by breed, nutrition and season, climatic factors and other environmental cues (Bielli *et al.*, 2000; Karagiannidis, Varsakeli & Karatzas, 2000).

The fact that Ethiopia has many different goat breeds, a diverse agro–ecology ranging from cool highlands to hot lowlands and diverse goat production systems indicates that undertaking such characterization under prevalent husbandry patterns is very relevant, as it would provide a benchmark for genetic improvement and biodiversity conservation. Furthermore, as the availability of feed nutrients has an overriding effect on reproductive traits, it is of utmost importance to search for alternative, nutritious, cheap and locally available feed resources and investigate the response of indigenous goats to various nutritional management regimes. As goat production is increasingly gaining importance in developing countries, mainly because of its socio–economic role, goats’ unique adaptive features to harsh tropical environments and growing demand for goat meat in the world market, the present studies (**Papers I–IV**) were considered timely and important.

Prior to this study, information was scarce on the reproductive traits of bucks of indigenous breeds raised under extensive husbandry in Ethiopia. Information was also limited regarding the response of indigenous bucks to different nutritional management regimes utilizing non-conventional feed resources, such as khat leftovers and agro–industrial by-products. The present investigation was, therefore, intended to explore whether the reproductive traits of tropically adapted goats indigenous to Ethiopia were differently influenced by breed and physiological stage (age) under extensive husbandry (**Papers I and II**), and whether and how a single indigenous breed could respond to different nutritional regimes (**Papers III and IV**).

### Effect of breed and age class on reproductive traits

This thesis (**Paper I**) has demonstrated that breed diversity is reflected in scrotal shape and scrotal anatomy in indigenous Ethiopian goats. The fact that the different goat breeds included in this study were distributed and adapted to the diverse agro–ecology prevalent in the country might account for this. Whether the occurrence of higher proportions of ovoid scrotal shape in AB and CH bucks might relate to their adaptation to a cooler highland agro–climate remains to be proven, but it is tempting to speculate in this direction. On the other hand, bucks

of the Afar, WG and Boran breeds, which are more common in hot lowland agro-climates, had high proportions of split scrotum with long ovoid (pendulous) scrotal shapes (**Paper I**). Once again, though confirmation must be provided, such an apparent relationship could be an adaptation mechanism through which indigenous goats help regulate testes temperature in an environment of high ambient temperature, so that normal spermatogenesis can continue (Hafez, 1993). A pendulous scrotum may improve testicular thermoregulation by moving the testes farther away from the body, thus facilitating heat loss from the testicular vascular cone for best sperm production and semen quality (Coulter & Kastelic, 1994; Brito *et al.*, 2002). Variation in scrotal shape influenced another BSE variable, e.g., scrotal circumference (SC), indicating the value of recording both SC and scrotal shape when undertaking BSE.

This thesis demonstrates that the body size of indigenous goats raised under extensive husbandry was differently influenced by breed and age class (**Paper I**). This could be due to differences in breed, agro-climate, production system and nutritional management in the environments to which these breeds adapted. Earlier reports have also found that the live BW of an animal is modified by breed, age, season and nutrition (Tegegne, Dembarga & Kassa, 1992; Bielli, 1999; Karagiannidis, Varsakeli & Karatzas, 2000). Thus, among the bucks of the studied breeds – compared at similar ages – Boran bucks were classed as large, CH, WG and AB bucks as medium, and Afar bucks as small. Such variation in body size is one criterion used in classifying goat breeds (Devendra & Burns, 1983). As a small-sized breed, Afar attained its highest BW early (in the intermediate age class, according to the terms of the present study), while the other studied breeds attained the highest BW later (in the old age class) (**Paper I**). Such divergence in the attainment of peak measured BW could be explained by the physiology of body size of each breed, as large-sized genotypes mature late and continue to grow until they attain mature BW, while small-sized breeds mature early (Taylor, 1971; Al-Ghalban, Tabbaa & Kridli, 2004). Similarly, the growth rate of smaller goats is slower than that of larger ones (Warmington & Kirton, 1990). The fact that the Afar breed is adapted to a hot lowland agro-climate might explain its small body size, as this is one mechanism by which desert goats have adapted to a harsh tropical climate (Silanikove, 2000). Even though Boran bucks had the highest BW in the old age class in the present study, the difference in BW in the old age class was greater than in the intermediate age class (**Paper I**), indicating that this breed is late maturing and might attain its mature BW later than the presently studied “old” age class, probably due to its intrinsic genetic potential. In contrast, the difference in BW declined from the intermediate to the old age class for both WG and AB bucks (**Paper I**), suggesting that these breeds had already approached their mature BW. The fact that the export abattoirs, where the present study was undertaken, have been concerned with relatively younger goats, limited this study to concentrating on an age class of less than 24 months old goats. Thus, the young, intermediate and old age classes used in this study were largely determined by the available age range of the bucks handled by the participating abattoirs. This limitation calls for further studies including relatively older age classes than those examined here, to estimate, for example, mature BW in the Boran breed.

As with BW, breed had noticeable effects on physical linear traits in indigenous bucks (**Paper I**). Physical linear measurements are important in judging the quantitative characteristics of an animal's meat (Islam *et al.*, 1991). The fact that physical linear traits have medium-to-high heritability and are well correlated with BW indicates their importance for effective selection (Magnabosco *et al.*, 2002). As Afar is a small-sized breed, it displayed smaller physical linear measurements than did the medium- and large-sized breeds. By the same token, due to its large body size, Boran displayed, numerically, the highest physical linear measurements (**Paper I**). The occurrence of significantly greater differences in HG, compared to the other physical linear traits, despite existing differences in BW in the present breeds, indicates that this trait might advantageously be used to predict the weight of animals raised under extensive husbandry where weighing scales are not available. It has been demonstrated that HG is highly correlated with BW (Mayaka *et al.*, 1996) and thus could be used as a reliable guide for estimating weight in bucks. However, HW and BL would not be useful guides for estimating weight in adult animals, since linear and vertical growth has wrinkled in adults (Mohammed & Amin, 1996). Afar bucks had a greater measured HG at an intermediate age and the shortest BL at both young and intermediate ages; this resembles its BW pattern and reaffirms the earlier assertion that this breed is small sized (**Paper I**).

Body condition score (BCS) is an important variable measuring body reserves and thus the nutritional status of an animal. It is the most reliable indicator of animal well-being under conditions of extensive production and, together with BW, provides a useful method for assessing reproductive potential (Rekwot, Oyedipe & Ehoche, 1994). In the present study (**Paper I**), the difference in BCS among the studied goat breeds was best explained by differences in agro-climate and feed resource availability. Thus, the lower BCS in CH bucks could be related to inadequate feed supply, as this breed dominates the mixed crop-livestock production system, where supplying year-round feed is a challenge (FARM-AFRICA, 1996). In this system, grazing lands are planted in the cropping season, and goats, together with other livestock, are forced to graze hillsides and communal grazing lands or be tether fed in backyards, where they generally receive inadequate nutrition. Furthermore, the maintenance energy requirement is higher for animals in cooler highlands than in a hot lowland agro-climate, which could explain why CH and AB bucks had lower BCS (Silanikove, 2000). As Boran and WG bucks are adapted to hot lowland agro-climates, their higher BCS indicates a different adipose fat cover, which may help insulate them against the prevailing higher ambient temperature (Van Jones, 1996). Besides, these breeds dominate the pastoral and agro-pastoral production system in which animals can freely browse on vast rangelands. Apart from the fact that goat feed is composed of browses, this feed resource is least affected by drought, indicating that lowland goats might have a steady supply of feed year round (Silanikove, 2000). Furthermore, desert goats have high digestive efficiency and low maintenance energy needs, which indicates the more efficient utilization of available feed nutrients, as reflected in the higher BCS in these animals (Silanikove, 2000). The present study (**Paper I**) was undertaken in the rainy season, which might have improved the natural vegetation in the lowlands; to account for this possible bias, longitudinal studies over a full set of seasons are called for.

The SC is an important trait that is closely associated with the testicular growth and sperm production capacity of domestic animals. Thus, selecting males based on their SC would result in larger testes, potentially with the capacity to produce more spermatozoa (Coulter, Rounsaville & Foote, 1976; Daudu, 1984). Being a highly heritable component of fertility, it is important to include SC when evaluating breeding animals (Bailey *et al.*, 1996). The present study (**Paper I**) has found that SC was differently influenced by breed and age. The higher SC measurements for Boran bucks indicate their higher testicular mass and larger sperm production, as reported earlier (Fernandez *et al.*, 2004). Since larger testicular size indicates a higher volume of seminiferous tubules and hence of seminiferous epithelium (Oldham *et al.*, 1978; Martin, Sutherland & Lindsay, 1987; Abi-Saab *et al.*, 1997), males with larger SC produced ejaculates with greater morphologically normal spermatozoa than did males of the same breed with smaller SC (Coe, 1999). The fact that Boran bucks had the highest SC measurements while Afar bucks had the lowest, compared to those of the other studied breeds, could thus be explained by their different body sizes (Al-Ghalban, Tabbaa & Kridli, 2004) (**Paper I**). On the other hand, the differences seen within and between age classes could be due to environmental factors, such as nutrition. Like BW, the SC in the Boran, WG and AB breeds was largest in the old age class, while in Afar bucks it peaked at an intermediate age, which could be attributed to their stage of maturity (**Paper I**). However, even though SC increases with age, its growth increment is sluggish compared to increases in BW. Earlier studies have demonstrated that SC increases rapidly in young bulls, only gradually in mature bulls and can even decrease as bulls age (Coulter & Foote, 1977; Brito *et al.*, 2002).

Evaluation of the testicular weight (TW) of goat breeds at similar ages is an important criterion for the selection of superior breeding animals (Harder, Lunstra & Johnson, 1995). In this thesis, the TW of indigenous goats raised under extensive husbandry appeared to be influenced by breed and age. The higher TW of Boran bucks, especially in the intermediate and old age classes, might be due to breed differences, as large-sized breeds are expected to increase in size until they mature (**Paper I**). As TW is highly related to sperm production, selection of bucks with larger testes would enable the insemination of more females in any AI programme (Coulter, Larson & Foote, 1975). In contrast, due to their medium size, the TW of the WG, CH and AB breeds could not increase as much as the Boran breed, and due to its small size, the TW of Afar was significantly lower than that of the medium-sized breeds, suggesting that testicular growth is related to BW, since the growth rate declines as maturity approaches (Warmington & Kirton, 1990). This is supported by the present study (**Paper I**), which demonstrated a favourable correlation between TW and BW in indigenous bucks. Despite this, and taking into account its small body size, Afar bucks displayed the highest TWPBW by the time they attain their mature BW at an intermediate age. Despite their heavier BW and larger SC, however, medium- and large-sized breeds had lower TWPBW, which might indicate that testes growth is more variable than BW is when animals are older (Nsoso *et al.*, 2004). It has also been found that TW reaches mature levels earlier than SC or BW does (Coulter & Foote, 1977). Since

a higher TWPBW suggests a higher sperm production per unit of BW, such values are advantageous for semen banking, since maintaining high BW under such hot and harsh environments might be a challenge. The presence of heavy epididymides in Boran bucks relates to their larger BW and higher testicular mass, and indicates high sperm reserves and a better endocrinological basis for stimulating the site for sperm maturation and storage (**Paper I**).

Sperm morphology is one seminal parameter that reflects the degree of normality and maturity of the sperm population in the ejaculate and it is associated with fertility (Rodriguez-Martinez & Barth, 2007). However, analyses of sperm morphology, unlike examinations of sperm motility or sperm concentration, are seldom done, particularly under the extensive management of breeding herds. There are several reasons for this lack, most of which could be grouped under the heading of “inconvenience”, for example, logistical problems in gathering males for collection, difficulties collecting semen via AV or electro-ejaculation and insufficient knowledge of mating frequencies. An alternative way to obtain spermatozoa for morphological analyses is to collect epididymal spermatozoa. This method is a last resort, as it can only be done post-mortem or post-castration, but is still of value when analyzing male populations (Chacon, 2001); indeed, such a strategy was used in the present study (**Papers I and II**). However, there were no major differences in incidence of sperm-head abnormality among the different breeds of bucks included in the present study (**Paper II**). Sperm head morphology has been reported both as being uniform (Brito *et al.*, 2002) or as differing significantly (Söderquist *et al.*, 1991; Dowsett & Knott, 1996) in breeding herds, suggesting that it is the degree of selection of the males and their normality that places them in either category. **Paper II**, however, showed that differences in sperm head morphology could be seen between groups of breeds instead. Comparison of grouped data based on the different agro-climates in which the breeds normally graze revealed that bucks from the lowland agro-climate had significantly more sperm head shape abnormalities than did bucks from the highland agro-climate (**Paper II**). Such results suggest that the higher ambient temperature in the lowlands might have influenced spermatogenesis, as defective testicular thermoregulation has been reported to decrease sperm quality in bulls (Coulter, Cook & Kastelic, 1997; Brito *et al.*, 2004) and goats (Florentino *et al.*, 2003). High ambient temperature has also been considered an important factor affecting ruminant sperm quality (Roca *et al.*, 1992). Other factors, such as malnutrition, can also challenge spermatogenesis. For those breeds that dominate the mixed crop-livestock production system where the supply of year-round feed resources is very variable, inadequate nutrition is also a possible deleterious factor, particularly for sperm maturation defects and acrosomal abnormalities (Barth & Oko, 1989; Dana, Tegegne & Shenkoru, 2000). Other differences were present among the samples, some of which might well be iatrogenic, such as sperm tail defects.

Sperm morphology changes with animal age. For example, the occurrence of abnormal sperm head shapes is reportedly higher at younger and older ages than at around the age of sexual maturity (Barth & Oko, 1989; Amann, Seidel & Mortimer, 2000). In contrast, no differences in terms of major and total sperm

defects due to age were reported in bulls (Brito *et al.*, 2002; Hallap, Jaakma & Rodriguez-Martinez, 2006). Similarly, increased age did not affect sperm abnormalities in bucks (Roca *et al.*, 1992). Small positive associations have been reported between the percentage of morphologically normal spermatozoa and animal age ( $r= 0.26$ ) in bulls, and older bulls often produce greater morphologically normal spermatozoa than do younger sires (Coe, 1999). In the present study, however, the numerical differences in sperm head shape abnormalities between bucks of the different age classes employed did not attain significance (**Paper II**). The fact that the age of the studied animals was predicted from their dentition (large within-age-class variation) and that the present bucks were neither too young nor too old, might explain the lack of significant differences between age classes. Alternatively, the differences in sperm head shapes seen in other species (in bulls in particular) may simply not be found in goats. Differences in the occurrence of other abnormalities, such as the occurrence of more loose sperm heads in young bucks and higher rates of acrosome abnormalities in older bucks, could, however, be considered, although they may have other sources than disturbances in spermatogenesis. The same might apply to the differences in sperm tail defects seen among the present bucks. Looking at the results in light of the relative proportions of morphologically “normal” spermatozoa, bucks of intermediate age had numerically larger proportions of morphologically normal spermatozoa. These differences were not statistically significant, however, indicating that the profile of epididymal spermatozoa was generally within the limits expected for bucks of these ages.

After spermioteleosis from the seminiferous epithelium, the testicular spermatozoa traverse the excurrent ducts; they then enter the epididymal duct, along which they mature and develop the capacity for fertilization before they are stored in the cauda epididymides until ejaculation (Turner, 1995). Thus, the presence of an overwhelming majority of morphologically normal and mature spermatozoa in the caudae epididymides of the examined bucks indicated the normality of their testes and epididymides. The presence of lower proportions of sperm defects in the caudae than in the other segments of the ducti (**Paper II**) indicated the capacity of these segments to remove abnormal spermatozoa (Sutovsky *et al.*, 2001). The occurrence of a higher proportion of distal cytoplasmic droplets and a lower proportion of proximal cytoplasmic droplets in the caudae spermatozoa than in the preceding segments simply confirms that the process of sperm maturation was functional (Rao, Bane & Gustafsson, 1980). However, the inadequate sample size in the old age class for CH bucks limited the comparison of this breed with others of similar age.

### **Effect of nutrition on reproductive traits**

The expression of reproductive traits of domestic animals is highly influenced by nutrition (Robinson, 1996). It is thus essential to provide animals with the required nutrients to maintain their body metabolism, and beyond that, to produce and reproduce effectively. However, the fact that the dominant feed resources available for livestock in the tropics are natural grasslands and crop residues

suggests limited nutritional support of fertility. Undernutrition, via low-quality feed, is an important factor seriously limiting animal production and reproduction (Rekwot, Oyedipe & Ehoche, 1994). An exhaustive search for nutritious but cheaper feed resources is thus vital for economical and sustainable goat production by most low-input smallholder goat producers in the tropics. In the present study (**Papers III and IV**), the nutritional value of non-conventional feed resources (i.e., khat leftovers and agro-industrial by-products) was evaluated along with their effect on the reproductive traits of indigenous Ethiopian bucks. The observed higher feed intake in bucks supplemented with either agro-industrial by-products or khat leftovers (T1–T3) could be due to the higher CP, GE, IVOMD and lower fibre fraction contained in the supplementary feeds, which might have enhanced the efficiency of ruminal digestion (Murray *et al.*, 1990; Mekasha *et al.*, 2002). Several parameters, such as BW, BCS, SC, TW and testicular size, were significantly better in bucks in the T1–T3 groups than in control bucks (**Paper III**), which could be attributed to their higher DMI, CPI, GEI and lower fibre intakes. This finding is in agreement with the results of previous studies reporting higher BW and testicular growth due to improved feed quality, in terms of improved protein supply (Abi-Saab *et al.*, 1997; Fernandez *et al.*, 2004). Improved nutrition is closely associated with increased testicular size (Oldham *et al.*, 1978). Feeding *Bos indicus* bulls at a high plane of nutrition resulted in heavier testes and epididymides than those of counterparts fed at a lower plane of nutrition (Tegegne, Dembarga & Kassa, 1992). The larger body size and better testicular traits in the T3 group than in the other supplementary treatment groups (T1 or T2) could be explained by the higher feed DMI and OMI compared to T1 and the higher CPI compared to T2 (**Paper IV**). The difference can also be explained by the fact that khat leftovers have a higher IVOMD than that of hay, which comprised the basal diet of the T1 and T2 groups; moreover, the khat leftovers were offered *ad libitum*, unlike in the T1 and T2 diets, in which supplements were fed to only 1% of BW. Taken together, the data suggest that T3 bucks might have consumed the largest proportion of digestible organic matter. Furthermore, the GEI of the T3 bucks was the highest of the bucks given supplementary feed (**Paper IV**), indicating that, besides the availability of CP, energy availability might have positively influenced digestion and led to the improvements seen. Energy intake and digestibility are the most important nutritional factors influencing testicular growth (Braden *et al.*, 1974; Murray *et al.*, 1990). The interrelationship between energy intake and reproductive performance and protein feeding and semen quality has also been demonstrated previously (Murray *et al.*, 1990; Fernandez *et al.*, 2004).

Fibre is more slowly digested than cell solubles are and is strongly negatively correlated with feed intake (Van Soest, 1994). Feed resources with a small fibre fraction have higher IVDMD, indicating the existence of a strong negative association (Mekasha *et al.*, 2002). Therefore, the low fibre intake of T3 bucks might have promoted digestibility, the effect of which was ultimately reflected in better performance (**Paper IV**). The lack of differences in BW and SC between T1 and T2 bucks could be due to similarities in nutrient supply. In contrast, the lower nutrient composition and intake of the control bucks resulted in lower body size and poorer testicular traits (**Paper III**). Consequently, bucks on the control diet

lost BW and testicular size over the observation period, as reflected by the negative BW and SC change. Similarly, Almeida *et al.* (2007) found less subcutaneous scrotal fat in unsupplemented bucks fed a basal diet of winter hay than in their supplemented counterparts. Such a BW and testicular tissue loss could be attributed to the active catabolism of tissue reserves, as reported earlier (Ndama, Entwistle & Lindsay, 1983). However, such measurements of scrotal fat were not made in the present study (**Paper III**). Thus, the loss in testicular tissue due to low feed intake should be attributed to the sensitivity of the testicular tissue to nutrition, the inadequate nutrient supply in the control diet explaining the ultimate decline in SC over the observation period (**Paper III**). Feed resources containing under 7% CP do not support optimum rumen fermentation, and animals fed such diets cannot maintain optimum growth (Whiteman, 1980). Feeding a poor-quality diet to Boer goats has resulted in lower BW, SC and TW than those of supplemented bucks (Almeida *et al.*, 2007). In contrast, feeding bucks more nutritious diets, like those of the T1–T3 treatments, not only sustained BW but led to increases in body and testicular size over the observation period (**Paper III**), in agreement with earlier reports (Mukasa-Mugerwa & Ezaz, 1992; Tegegne *et al.*, 1994).

Similar to the above-related effect on body size and testicular growth, supplementing the diets of indigenous Ethiopian bucks with either agro-industrial by-products or khat leftovers improved sperm traits (**Paper III**). The observed higher ejaculate volume, sperm numbers and proportions of motile spermatozoa could be due to the higher nutrient contents of the supplementary feed in treatments T1–T3 compared to controls. Higher protein intake can improve semen characteristics by increasing sperm cell proliferation in the seminiferous tubules (Oldham *et al.*, 1978; Abi-Saab *et al.*, 1997). Supplementing rams with fodder from multi-purpose trees containing higher CP has been found to increase sperm motility and sperm numbers in rams (Dana, Tegegne & Shenkoru, 2000). This indicates that cellular division during spermatogenesis could be positively affected by increasing feeding level, as occurs in other bodily tissues and organs (Fernandez *et al.*, 2004). Improving the plane of nutrition of rams can lead to increased sperm production per day (Oldham *et al.*, 1978). In line with this, the poorer sperm attributes seen in the ejaculates of the control bucks could be due to inadequate nutrition (**Paper III**). Similarly, it has been demonstrated that protein undernutrition severely affects sperm motility (Nsahlai, Byebwa & Bonsi, 2000). Depressed spermatogenesis has been reported in rams with protein deficiency (Oldham *et al.*, 1978). Furthermore, the nutrients contained in the supplements used in the T1–T3 treatments maintained higher sperm production and sperm motility, throughout the observation period, than found in controls (**Paper III**). However, in the T2 bucks, supplementation did not improve semen volume compared to control levels, possibly due to inconsistencies in sample collection arising from the use of electro-ejaculation, or to a lower level of supplementation. The lack of difference in most sperm traits among the different nutritional treatments (T1–T3) might support earlier statements, in that there may be a level of nutrition beyond which no further positive response in sperm production variables is achieved (Mukasa-Mugerwa & Ezaz, 1992). Although the literature is inconsistent on this matter, some studies have demonstrated that sperm traits such

as sperm motility may not be affected when protein is supplied above maintenance requirements (Nsahlai, Byebwa & Bonsi, 2000). However, others have suggested that sperm production can be affected by changes in protein intake, even when these changes take place above maintenance requirements (Fernandez *et al.*, 2004). The effect of protein supplementation on sperm traits could be better evaluated by taking into account the availability of digestible energy and the degradability characteristics of proteinaceous supplements, aspects our experiment was not designed to test.

The effect of nutrition was apparent in improving sperm morphological characteristics in indigenous bucks (**Paper IV**). Thus, through the supply of higher CP, energy and IVOMD, the tested supplements promoted spermatogenesis, as reflected by the production of higher proportions of morphologically normal spermatozoa in treatment than in control bucks, in which the proportion of abnormal sperm head shapes, to cite one parameter, exceeded the 10% threshold (**Paper IV**). Supplementing low-quality veld hay with feedstuff containing more nutrients (e.g., maize, molasses and urea) increased the production of morphologically normal spermatozoa and decreased abnormal sperm population in bucks (Almeida *et al.*, 2007). Cell renewal during spermatogenesis could be positively influenced by increasing the feeding level (Cameron, Murphy & Oldham, 1988). Protein deficiency in ruminant diet can depress the production of morphologically normal spermatozoa (Oldham *et al.*, 1978). The higher nutrition status of the T1–T3 diets might have also promoted the secretion and maintenance of testicular fluids and hormones involved in sperm production and maturation. Higher protein intake increases sperm production and positively influences the neuro–endocrine system, resulting in improved semen characteristics (Abi-Saab *et al.*, 1997). The higher proportion of proximal cytoplasmic droplets in the control than in the treatment bucks indicates that inadequate nutrient supply might have negatively affected the function of the ducti epididymides and impaired sperm maturation; similarly, the occurrence of higher proportions of proximal droplets in Ethiopian highland sheep was previously reported to be due to low-quality diet (Dana, Tegegne & Shenkoru, 2000). However, due to the lower CP and higher fibre fraction, T2 bucks produced spermatozoa with a relatively higher incidence of abnormal morphology than did T1 and T3 bucks (**Paper IV**). Moreover, the existence of relatively more spermatogenic cells in the ejaculate collected from the control bucks indicates that inadequate nutrition might have either led to slight testicular disturbances or challenged the ability of the ductus epididymides to cleanse these foreign cells along their transit.

The high tannin and lignin contents of khat leftovers might have an inhibitory effect on feed intake and digestion in the present study. The adverse effects of tannins from forages include reduced palatability, low rate of evacuation of digesta out of the rumen, gastritis and other toxic effects (Kumar & Singh, 1984; Silanikove *et al.*, 1996). Tannins also bind to the dietary proteins and form an insoluble complex, which decreases digestibility of the protein by the rumen microorganisms (Silanikove *et al.*, 1996). However, the present bucks, especially those fed khat leftovers *ad libitum* (T3), consumed a high quantity as reflected by

their high DMI and performance. With the exception of the control bucks, which had low feed DMI expressed as a percentage of live weight, for the T1–T3 bucks, it was in line with that reported for tropical goats (Devendra & Burns, 1983), indicating that the amount of tannin consumed might have not limited feed intake. This could further be corroborated by earlier research that reported the occurrence of more highly condensed tannin (21.66 abs/g) in field pea hulls commonly used for livestock feed (Mekasha *et al.*, 2002). Besides, the higher feed intake and performance of T3 bucks might be related to goats' better efficiency at digesting tannins. It has been demonstrated that goats fed tannin-containing leaves do not exhibit toxic syndromes, due to their saliva composition and digestive efficiency (Silanikove *et al.*, 1996). Goats are able to consume more tannin-rich browse than sheep can under similar conditions (Silanikove *et al.*, 1996). This could be attributed to the ameliorating function of their rumen (which can be related to binding of tannins to protein and cell walls of plant origin), to endogenous salivary enzymes, or to the detoxification of tannins by microbial enzymes (Silanikove *et al.*, 1996). Despite this, polyethylene glycol (PEG), a tannin-binding agent, has been proposed as an important tool for diminishing the effect of tannins on various digestive functions, thereby increasing feed intake and the digestion of tannin-containing leaves offered to sheep and goats (Barry, Manley & Duncan, 1986).

Khat also contains a naturally occurring alkaloid, cathinone, which is reportedly metabolized into cathine and norephedrine (Adeoya-Osiguwa & Fraser, 2004). The amounts of these chemical constituents were not quantified in the present study. Moreover, their effects on male reproduction are still to be determined in goats as studies done in other contexts and species are inconclusive. While some studies of khat consumption have reported low sperm motility, concentration and poor morphology in humans (El-Shhoura *et al.*, 1995), and degenerative changes in testicular morphology and low levels of plasma testosterone in rats (Islam *et al.*, 1990), others have indicated the up-regulation of plasma testosterone in olive baboons (Mwenda *et al.*, 2006) or improved spermatogenesis, with no effect on testicular morphology, in rabbits (Al-Habori *et al.*, 2002). The present study could not observe any clinical symptoms in the bucks examined. Besides, most of the studies of khat consumption done so far have involved monogastric animals, including human beings, and the mode of administration varied from direct injection to feeding dried leaves (or even chewing fresh leaves in the case of humans). On the other hand, the present study included ruminants in which fermentation in the rumen, the major responsible for decomposition of the ingested vegetation, might have influenced, possibly diminishing or eliminating, the absorption of the alkaloids or their metabolites. For example, goats have a documented unique efficiency at digesting chemical constituents such as tannins, and have the ability to detoxify them (Silanikove *et al.*, 1996). The present study also found that khat leftovers contained moderate to high nutrient levels, as reflected in higher feed DM and nutrient intakes in T3 bucks than in the other treatment groups. According to the design of the present experiment, the higher body size and better reproductive traits of T3 bucks could be mainly related to the better nutritional contents of the khat leftovers consumed. The fate of the cathinone metabolites in the rumen systems of ruminants and the possible effects

of these compounds on nutrient partitioning and on physiological and metabolic pathways in ruminants deserve future investigation.

Securing a year-round feed supply is a major challenge facing livestock production throughout the tropics. As well, khat cultivation is becoming widespread in mixed crop–livestock farming in Ethiopia, its heavy encroachment substantially reducing the availability of grazing land. For these reasons, studying the use of khat leftovers for goat feed might be important to rescuing, or improving, the productivity of native Ethiopian goat breeds.

## General conclusions

- The body weight and testicular traits of extensively managed indigenous Ethiopian male goats were differently influenced by breed and age, while scrotal traits were directly influenced by agro-climatic conditions (Ethiopian lowlands vs. highlands).
- Agro-climate, rather than breed or age, appeared as the major factor influencing sperm head morphology, with bucks raised in the arid lowlands having significantly more sperm head defects.
- Feeding indigenous Ogaden bucks with agro-industrial by-products or with khat (*Catha edulis*) leftovers *ad libitum* resulted in better live body weights, body condition scores, testicular size, sperm production and sperm motility, compared to controls.
- Feeding with publicly available, inexpensive khat leftovers (containing high GE and Ca, moderate CP and IVOMD and a small fibre fraction) improved feed dry matter, nutrient intake and the proportions of morphologically normal spermatozoa in the ejaculate, even more than when using expensive agro-industrial supplements at 1% of body weight.

## Recommendations

- As the present study revealed significant diversity in reproductive traits due to agro-ecology, breed and/or age class, and production system, future plans to improve goat breeding should consider these relationships.
- Khat (*Catha edulis*) leftovers and agro-industrial by-products could be used as alternative feed resources to ameliorate goat productivity by improving reproduction under smallholder farming systems in Ethiopia, provided costs are kept to a minimum.
- As breeding soundness evaluation (BSE) is effective for screening and identifying sub-fertile males, pertinent guidelines should be devised for the procedure, which should be made routine in R&D institutions, large-scale farms and eventually in most smallholdings through provision of appropriate extension services.

## Scope of future research

- The present study could not find any strong disparity in the effect of age on sperm morphological attributes, mainly due to the large within-age-class variation and the use of relatively similar animals. Therefore, further studies should include bucks of extreme ages with minimum within-age-class variation.
- The effect of the studied set of feed resources on goat puberty, sexual maturity, sexual behaviour and semen preservation (cooled and/or frozen) ought to be studied, including interactions with seasonal factors.
- Livestock fertility is improved by applying AI. Therefore, the wider application of this reproductive technology, of proven low cost but high output, is needed. The potential wider use of AI calls for access to data for further studies of male fertility.
- Fertility depends on the availability of superior females and males, prime for reproduction. Since this study focused on evaluating male reproduction, future research should look into female reproductive traits under conditions of both extensive and controlled husbandry, particularly considering the significant benefits achieved by feeding khat leftovers *ad libitum*.

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