THE EFFECT OF A NATURAL FEED ADDITIVE, FENUGREEK, ON FEED DIGESTIBILITY AND MILK RESPONSE IN DAIRY GOATS

by

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Animal Science in the Faculty of AgriSciences at Stellenbosch University

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Co-supervisor: Prof CW Cruywagen

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ABSTRACT

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Little research has been done on natural feed additives which enhance milk production in dairy animals. Fenugreek (*Trigonella foenumgraecum*) is a member of the legume family and is found in India, Middle East, North Africa and South Europe. Fenugreek is used as an herb in traditional medicine to promote lactation in lactating women. It also influences the lactation performance in ruminants such as dairy cows, water buffaloes and dairy goats. Diocin is a natural saponin found in Fenugreek and has structural similarity to oestrogen, which leads to an increased release of growth hormone (GH) and ultimately milk production. Three different trials were carried out to investigate Fenugreek's effects. Each trial consisted of three treatment groups where dairy goats were randomly assigned. Nutrifen®, NutrifenPlus® and a control treatment served as the three treatments used in this study. Forty-eight goats per treatment group were used in the first trial where the main objective was to evaluate Fenugreek's effect on milk production and milk composition. The second trial consisted of eight goats per treatment group, where Fenugreek's effect on the *in vivo* and *in vitro* digestibility of the feed served as the main objective of this study. In the final part of the study, growth hormone found in plasma was subsequently investigated using the same goats from trial two. Fenugreek's effect on elevating GH levels was the objective from the third part of the study. The first trial showed promising results in terms of an increase in milk production ($P = 0.01$) from dairy goats using the Nutrifen® treatment and an increase in milk lactose ($P = 0.03$) using the NutrifenPlus® treatment. Blood cholesterol and cholesterol content found in the milk did not differ between treatments used. Apparent digestibility of the total digestible nutrients (TDN) from the feed did not increase and did not differ between treatments and therefore concluded that the dairy goats digested the different treatments with similar efficiency regardless of the additive added to the feed. Growth hormone levels

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found in plasma also did not differ between treatments used in the third part of the study. Variation was found in GH plasma levels and this was expected as GH levels are known to have variation within ruminants. It appears that Fenugreek used as a natural feed additive can increase the milk yield from dairy goats, which would be beneficial to the commercial dairy goat farmer. However, the process on how Fenugreek exerts its effect on milk production still remains unclear.

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UITTREKSEL

DIE EFFEK VAN N NATUURLIKE VOERBYMIDDEL, FENUGREEK, OP DIE VOER VERTEERBAARHEID EN MELKPRODUKSIE VAN MELKBOKKE.

deur

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Tot datum is min navorsing gepubliseer wat die invloed van natuurlike voer bymiddels op melkproduksie aanspreek. Fenugreek (*Trigonella foenumgraecum*) is ‘n peulgewas en kom voor in Indië, die Midde Ooste, Noord Afrika en Suid Europa. Fenugreek word in tradisionele medisyne gebruik om sodoende melkproduksie in lakterende vroue te verhoog. Dit verhoog ook melkprodukise in melkkoeie, waterbuffels en melkbokke. Diocin is ‘n natuurlike saponien, met sterk oestrogeniese strukturele ooreenkomste, wat in Fenugreek voorkom. Diocin lei tot die verhoogde afskeiding van groeihormoon (GH) en uiteindelik ‘n toename in melkproduksie. Drie proewe is uitgevoer ten einde die effek van Fenugreek te ondersoek. Elke proef het bestaan uit drie behandelingsgroepe en melkbokke is ewekansig aan die groepe toegedeel. Nutrifen®, NutrifenPlus® en ‘n kontrole sonder enige additief is gebruik as behandelings. Agt-en-veertig bokke is per behandeling gebruik in die eerste proef. Die doel van hierdie proef was om die invloed van Fenugreek op melkproduksie en melksamestelling te bepaal. Die tweede proef het agt bokke per behandelingsgroep gehad en het ten doel gehad om te bepaal wat die invloed van Fenugreek op die *in vitro* en *in vivo* verteerbaarheid van die voere was. In die derde proef is dieselfde bokke as die in proef twee gebruik en hier is groeihormoon vlakke in sirkulerende bloedplasma gemeet om die invloed van Fenugreek op hierdie parameter te bepaal. Resultate van die eerste proef het getoon dat melkproduksie van bokke wat Nutrifen® ontvang het betekenisvol verhoog het (P = 0.01) terwyl NutrifenPlus® gelei het tot ‘n verhoging (P = 0.03) in melk laktose vlakke. Bloed cholesterol en melk cholesterol vlakke was onveranderd. Skynbare verteerbaarheid van die totale verteerbare voedingstowwe (TVV) van die voer het nie verander (P = 0.34) met die insluiting van Fenugreek nie. Plasma groeihormoonvlakke was nie betekenisvol verskillend (P > 0.05) tussen behandelingsgroepe nie en die gebrek aan verskille kan waarskynlik

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toegeskryf word aan die variasie wat binne behandelings groepe opgemerk is vir hierdie parameter. Sodanige variasie in plasma groeihormoon word as algemeen beskou in herkouers. Gevolglik kan aanvaar word dat die natuurlike voerbymiddel, Fenugreek, gebruik kan word om melkproduksie in lakterende melkbokke te verhoog. Hierdie praktyk behoort voordele in te hou vir die kommersiële melkprodusent. Die proses waardeur hierdie verhoging plaasvind is egter steeds nie duidelik nie.

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NOTES

The language and style used in this thesis are in accordance with the requirements of South African Journal of Animal Science. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has been unavoidable.

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LIST OF ABBREVIATIONS

AA	Amino Acids
BCS	Body Condition Scoring
BUN	Blood Urea Nitrogen
BW	Body Weight
CAEV	Caprine Arthritis-Encephalitis Virus
CCK	Cholecystokinin
CF	Crude Fibre
CMT	California Mastitis Test
CP	Crude Protein
DM	Dry Matter
DMI	Dry Matter Intake
EE	Ether Extract
EDTA	Ethylenediaminetetraacetic Acid
EUN	Endogenous Urinary Nitrogen
GE	Gross Energy
GH	Growth Hormone
GHRH	Growth Hormone Releasing Hormone
GIT	Gastro Intestinal Tract
HDL	High Density Lipoprotein
IVDMD	*In Vitro* Dry Matter Digestibility
IVTD	*In Vitro* True Digestibility
LDL	Low Density Lipoprotein
LS	Least Square
MFN	Metabolic Faecal Nitrogen
MJ	Mega Joules
MUN	Milk Urea Nitrogen
N	Nitrogen
NDF	Neutral Detergent Fibre
NDS	Neutral Detergent Solution
NPN	Non Protein Nitrogen
OM	Organic Matter
RUP	Rumen Undegraded Protein
SD	Standard Deviation
SSC	Somatic Cell Counts

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TDN	Total Digestible Nutrients
UDP	Undegraded Protein
UHT	Ultra High Temperature

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Chapter 1

General Introduction

Dairy goats are used all over the world for their unique milk properties and niche products made from their milk. People often use products from dairy goats and sheep which play an important role in basic human nutrition (Haenlein, 2001). Goats in developing countries in Africa play an important role in sustaining small scale rural communities (Dubeuf *et al.*, 2004). Production from dairy goats seems to be more appropriate than cow milk in these small communities (Donkin, 1998). As the South African population grows, goat milk will become more important for its high nutritional value for children as it is used alternatively to cow milk in some cases where children suffer from allergies.

Nowadays a well-established niche market exists for products, such as a variety of cheeses derived from dairy goat milk. Niche products therefore can play an important role in the sustainability of goat milk production globally as competition exist between other species' milk such as cow, sheep and water-buffalo milk (Dubeuf, 2005). Growing interest in niche products will serve as proof for the industry that a higher demand will exist for milk derived from dairy goats.

Milk production needs to be sustained to supply to a growing demand by consumers. Dairy goats are exclusively used for milk production, and milk production is greatly influenced by nutrition. Researchers and farmers have to come up with ways to ensure an increase in milk that is produced by dairy goats. A popular trend in the world exists to move more towards natural products from plants and plant derivatives to increase milk production. Biological additives (yeast cultures), natural additives (medicinal plants as its seeds) and chemical additives (buffers such as sodium acetate and sodium succinate) is commonly found in animal feeds today (Khattab *et al.*, 2010a).

Natural feed additives have been used all over the world to benefit animals as well as animal production. The use of these natural feed additives can help to improve animal productivity and increase milk production (Khattab *et al.*, 2010a). Fenugreek is such a feed additive and is derived from a plant that belongs to the leguminous family. Fenugreek has been shown to have a positive effect on lactation performance in ruminants such as dairy cows, water buffaloes and dairy goats (El-Alamy *et al.*, 2001; Kholif & El-Gawad, 2001). However, research is still needed to investigate the mechanism by which Fenugreek increases milk production.

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When available literature is consulted, it is evident that relatively little research is conducted on the inclusion of natural feed additives such as Fenugreek to enhance the production of dairy goats in South-Africa. Research is therefore required in this field to ensure maximum production from animals that is adapted to the South African environment.

The purpose of the study was therefore to evaluate the influence of Fenugreek on the production efficiency of dairy goats under South African conditions. In the first part of the study (Chapter 3), the potential effect of a natural feed additive containing Fenugreek on the milk production of dairy goats was investigated. The objectives of this study were to determine whether Fenugreek can increase milk production in dairy goats, and whether Fenugreek can have a positive effect in lowering blood cholesterol in the trial animals, and if this will result in lower blood cholesterol found in the milk. According to Shah and Mir (2004a), blood cholesterol is the main precursor of cholesterol found in milk.

In the second part of the study (Chapter 4), *in vitro* and *in vivo* nutritional trials were conducted to determine the effect of Fenugreek on the overall digestibility of the feed. In the third part of the study (Chapter 5), the influence of the inclusion of Fenugreek in the experimental diets on the plasma growth hormone (GH) levels was investigated. Nutritional supplements that result in an increase in plasma GH levels can potentially result in an increase in milk production (Etherton & Bauman, 1998; Boutinaud *et al*., 2003a). In Chapter 6, findings are summarized, and recommendations made where relevant.

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Chapter 2

Literature review

Introduction

Goats have been domesticated a long time ago. Evidence exist in modern-day Iraq, Iran, Palestine, Jordan and Turkey to support domestication of goats occurred as long ago as 7500 – 7000 BC and apart from the dog, the goat was the first domesticated animal (Wani, 2011). People used a wide variety of products since animal domestication took place. Milk, with its high nutritive value, was used from both goats and sheep (Haenlein, 2007a) to fulfil to the people's nutritive requirements. Goats are used by humans for many different purposes, and hardiness of goats makes it possible for people to keep these animals under various conditions (Dubeuf *et al.*, 2004) where they are able to thrive. Milk is an excellent food source and consumed widely around the world, although it is not the only reason for the domestication of these animals. Meat, skin, fibre, hair, horns and manure seemed valuable by-products of goats and therefore a good reason to domesticate them (Wani, 2011; Sahlu *et al.*, 2004).

Goats are well known for their ability to utilize less favourable feedstuff and adapt to adverse environmental conditions, making it possible for them to thrive all over the world. Goats are able to utilize feed material more efficiently than other domesticated animals, they are more disease tolerant and they have a great reproductive capacity which makes these animals ideal in productive farming systems (Wani, 2011). Goat breeds became superior in terms of their milk- and solids production. Through intense breeding programs, over the last 150 years, and selection for favourable traits together with better feeding strategies, all led to improved productivity on farms (Haenlein, 2007a). People do not only consume milk in liquid form, but also use milk to create a wide variety of other, much different products. An increase market demand, especially in different cheese products, ensure a great potential for evolving both goat and sheep species.

Most goats in the world (67%) are found in Africa and Asia but their hardiness made them adaptable and they thrive almost any place in the world in adverse environmental conditions known to mankind (Wani, 2011). In Africa, goats are considered to play an important role in developing countries as well as sustaining rural communities (Dubeuf *et al.*, 2004). Small village farms, in almost all countries of the world, will farm with small flocks consisting of two

– ten goats (Dubeuf, 2005). Developing countries with poor economies have more goats (94%) than developed countries, and will rear these animals primarily for meat, milk and fibre (Wani, 2011). Goat milk production seems to be more appropriate than milk from cows in small scale farmers for householders from rural communities (Donkin, 1998). In developed countries a niche market exist where goats are farmed with the intention of producing sufficient milk to convert to other milk products, especially cheese.

The dairy goat industry is part of the global milk industry and will always compete with cow, sheep and even water-buffalo milk (Dubeuf, 2005). Dairy goat farms will be in competition with other species and therefore dependant on specific markets and niche products to sustain an on-going farming enterprise (Dubeuf *et al.*, 2004). A growing interest in goats' milk and other products has occurred over the last few years around the world. The industry proof that continuous growth will take place as the demand for niche products also increases (Dubeuf, 2005).

Dairy goats are scarce in South Africa and not a very popular animal to farm with intensively. When they are bred with indigenous goats, breeders attain heterosis and produce hardy animals which are adaptable in South African conditions. The result of this cross-breeding will ensure sufficient milk production to sustain a household or small community (Donkin, 1998). As the South African population grows, milk will become more important in nutrition as a source of high quality protein, especially in rural areas where malnutrition poses a problem in children.

It is estimated that there are six million goats in South Africa and two distinct sectors are identified in South Africa, commercial farmers and the small scale non-commercial farmers (Roets & Kirsten, 2005). Milk production has increased over the last two decades in South Africa and projects are underway to promote better production systems. Projects will aim to increase both the quality and quantity of milk produced by small-scale farmers in order to supply a growing country (Seifu *et al.*, 2004).

Goat breeds

Many different types and variations of breeds exist among small ruminants and especially goats around the world. This is due to selective breeding of these animals to express certain desirable traits and expressing these traits under adverse environmental conditions. Selection is based on consumer demand and related to economically significant and organised sectors (Dubeuf & Boyazoglu, 2009).

It is estimated that 570 different goat breeds exist and of these, only 69 are single dairy types. Within the 69 breeds, it is estimated that 63 originated from Europe, 25 breeds in Asia and about 8 breeds in Africa (Devendra & Haenlein, 2011). Goats are mainly used for meat production in developing countries, but milk is also consumed to a medium to low level. Goats are therefore crossbred to serve as a dual-purpose breed in rural areas. Many breeds of goats were exported to developing countries around the world to upgrade the already existing indigenous breeds (Devendra & Haenlein, 2011).

Selection for meat is more important in certain countries than others (e.g. South Africa, USA and Australia) and is a result of marginal milk production which cannot compete with the already well-established dairy cow sector. Other breeds are also bred and well known for specific traits. The Angora goat is bred for fine mohair fibre where other goats are bred for cashmere and pashmina fibre (Devendra & Haenlein, 2011). Meat and skin from the Barbari goat has led to the evolution and purpose of this breed (Devendra & Haenlein, 2011). Depending on the situation or consumer preferences, different dairy species were selected for desirable traits. Dairy sheep were selected for total solids in milk whereas dairy goats were primarily selected for a high milk yield (Haenlein, 2007a).

Consumption of milk and the demand for other processed dairy products (butter, yogurts and cheeses) increased with time. The principle selection aim was to develop better dairy goat breeds to sustain such a growing demand (Haenlein, 2007a). Switzerland has bred the world's highest leading single purpose dairy goat breeds which led to a high export of these animals to other countries for improving the dairy herds within those countries (Devendra & Haenlein, 2011). Some of these goat breeds that evolved in Switzerland include the Alpine, Saanen, Toggenburg and Oberhalsi (Haenlein, 2007a).

As early as 1886, Sanson first identified and classified goats on the basis of their ear shape (Wani, 2011). Goat breeds can be classified into six different groups according to certain characteristics as can be seen in Table 2.1. Goats are classified as a certain breed when they meet certain requirements and standards which are set aside for each individual breed. Descriptions that these animals have to meet includes: colour, ear size and type, horn size and type, face type, hair coat length, beard, wattles, body weight, and height in adult males and females (Devendra & Haenlein, 2011).

Differences in milk yield exist when exotic breeds are compared to local breeds whereas milk yield are much higher in high producing exotic breeds. However, when these exotic breeds are evaluated in local areas other than their country of origin, their milk yield is lower, in general. The milk yield of exotic breeds would be similar when production systems are more intensive and more closely related to the regions of origin (Serradilla, 2001). The

performances vary depending on various factors like managing programs, climate, environmental conditions and production systems.

Table 2.1: Examples of goats divided into different groups based on certain characteristics.

Group	Characteristics	Example
1. Short eared goats with small/sable horns or none	Resemble the wild bezoar, short hair, prick ears, straight facial profile	Saanen (Switzerland), Malaga (Spain), Creole (West Indies), African Pygmy (West Africa), Small East African (Kenya, Uganda, Tanzania)
2. Short eared goats with twisted (prisca) horns	Horns were selected into a loose spiral to give the prisca horns	Valais Blackneck (Switzerland), Pyrenean (France), Garganica (Italy), Mardi (Nigeria)
3. Pashmina or Cashmere goats	Horns are heteronymous twisted; ears are intermediate between short and drooping	Morghose (Iran), Vatani (Afghanistan), Kaghani (Pakistan), White Himalayan (India), Chungwei (China, Mongolia)
4. Angora goats	Mohair goats with lop ears and spiral horns	Angora goats (Turkey)
5. Lop-eared goats	Larger goats with lop ears; frequently spiral horns	Nubian (Sudan), Benadir (Somalia), Boer goat (South Africa), Bikaneri (India)
6. Long-eared hornless dairy goats	Goats are kept under intensive conditions	Maltese (Malta), Damascus (Syria), Zaraibi (Egypt)

Adapted from Pieters (2007).

Different dairy breeds used in South Africa

Milk production in South Africa is still marginal and only a few big farms exist in the country, which can be considered as high milk producers. For this reason, not a lot of different dairy breeds have evolved and therefore exist in South Africa. As described earlier, a lot of well-defined dairy breeds were imported into the country in earlier years to help improve existing breeds. These breeds acclimatised and adapted quickly in the different environmental conditions found in South Africa. Goats in South Africa, found nowadays, evolved from years of selecting animals that thrived and produced the most milk. This selection gave birth to the modern day herds of dairy goats found in South Africa, which are more tolerant to diseases and parasites than other livestock species (Aziz, 2010).

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Saanen

This breed is widely recognised and known for high milk production. The Saanen is also sometimes referred to as Holstein Friesian amongst goat breeds because of their high level of daily milk yield and relatively low milk fat content. The name of the breed derived from the Saanen valley of central Switzerland, were they originated. This breed is completely white, short haired with occasional black spots on the udder, ears and nose. They are bred for polledness, but goats with horns are also used, because of less infertility problems. As described in Table 2.1, the Saanen's ears are erect and of medium length and they point forward. If horns are present they are saber shaped and point backwards. This breed has two unique external appendices called wattles, but the function of these wattles is still unknown. Beards are also common in this breed as it is in other breeds. Goats are in lactation from 150 – 300 days and produces between 300 to 2000 kg of milk. This may differ from one country to another (Devendra & Haenlein, 2011).

Alpine

Another big breed in terms of milk production, the Alpine, originated from the mountains of Switzerland. This breed however, is known for many colour types and varieties. This led to the formation of new sub breeds which were exported around the world to different countries (Devendra & Haenlein, 2011).

Toggenburg

Like the previous two breeds, the Toggenburg is also renowned for having a high milk yield. This dairy breed has a Swiss origin and gets its name from the Toggenburg valley. The hair coat of this breed may be short or long which makes it an excellent breed for rough climates. They are brown or grey with white on the legs as well as on the area around the base of the tail. Two unique white stripes stretch from the muzzle to the eyes and poll. Erect ears are less than of medium length and polledness is associated with this breed. This breed is also exported around the world because of a high milk yield next to the Saanen and Alpine breeds (Devendra & Haenlein, 2011).

Depending on the farm situation or consumer demand, a certain goat breed will be suitable in meeting the necessary requirements. This demand for different products has led to a great diversity of dairy goat breeds. Some of these breeds are bred for dual-purposes which will include milk and meat products. Hardiness makes goats adapt quickly and therefore these animals are distributed widely across tropical and temperate regions (Devendra & Haenlein, 2011).

Milk production in general

Milk produced from dairy goats is neither the only product nor the only reason that they are kept. People will also keep goats for their meat, fibre and skin (Haenlein, 2001). Limited feed sources together with climate difficulties and rough terrains do not always make it possible for humans to have access to dairy cows. Goats, because of their great ability to adapt, have been known to walk as far as 10 km per day to forage and goats other than sheep and cows can survive on less frequent water intake.

Domestication of animals depended on various different reasons. Some of these reasons include factors such as availability of the animal, ease of milking and organoleptic properties of milk (some milk are unpalatable for human consumption). The position of the udder and of the teats, ability to store milk in the udder, and the quantity of milk stored in the udder all led to humans categorising animals according to a hierarchy based on ease to harvest their milk (Faye & Konuspayeva, 2012).

Around the world a lot of people suffer from poverty and other daily life challenges. People often turn to products from goats and sheep which play an important role in basic human nutrition (Haenlein, 2001). Goat milk is not just used by people for surviving purposes; it also plays an important role in people that suffer from allergies. In some cases children are often able to tolerate goat milk more than cow milk (Wilson *et al.*, 1995).

Many people from poor and developing countries rely on dairy animals for basic nutritional needs. Dairy goats and –products are an important source of basic nutrition of good quality. These animals are not just kept as a source of nutrition, but also serve as some sort of income provided by selling the animals or products produced by them (Haenlein, 1998). Goats make a valuable contribution in developing countries and especially to poor people in rural areas where they are sometimes known as the “cow of the poor”. Goats eat less than cows and occupy a smaller space and still produce sufficient milk to sustain a family, whereas maintaining a cow would be more expensive, making goats more popular as the poor person’s cow (Aziz, 2010).

People often make the mistake to assume all knowledge that exists concerning dairy cattle can be applied to the dairy goat industry. Dairy goats, compared to dairy cows, do not appear to need a dry period for the udder to repair for an optimum milk production in the subsequent lactation (Goetsch *et al.*, 2011). For this reason, goats are often neglected compared to dairy cows and sheep (Aziz, 2010). Both goats and sheep are classified as small ruminants and people often assume that the same rules apply to both species. Extensive research with meat sheep is also implied to dairy goats and sheep (Haenlein,

2001). Goats and sheep are often reared and seen together and called “shoat”, but basic nutrition principles cannot be applied to both (Faye & Konuspayeva, 2012).

A variety of factors influence milk yield and composition which are amongst dietary factors, as well as daily body weight gain (Min *et al.*, 2005a). The number of daily milking on farms is of great importance when milk yield in dairy animals is determined (Salama *et al.*, 2003a). A study conducted by Min *et al.* (2005a) showed that dietary factors like concentrate supply can effect milk yield and milk composition in lactating dairy goats. It is not necessarily important to provide adequate amounts of concentrates to the animals as dairy goats are capable of producing sufficient milk when they forage on pastures (Min *et al.*, 2005a). Severe environmental factors also affect the foraging behaviour of goats which can lead to a decrease in milk production as well as a difference in milk composition (Cannas *et al.*, 2008).

Traditionally goats were milked twice daily, but due to a growing interest in reducing labour costs, producers are now looking at transitioning to milk once a day (Goetsch *et al.*, 2011). In developing countries, the practise of milking only once a day could lead to job losses which can affect a whole community’s economy. Lactation curves for dairy goats are set up in order to predict certain outcomes like milk yield and milk composition. Research on lactation curves are generally related to dairy cows, although the same models and analysis have been done on dairy goats, sheep and other dairy animals (Groenewald & Viljoen, 2003).

Mathematical functions are used to estimate certain parameters in dairy animals. Various functions have been used to study lactation in dairy animals with each function having advantages and disadvantages. Many factors like the breed, first and later parities, season of kidding and level of production effect characteristics of the lactation curve in dairy goats (Gipson & Grossman, 1990).

Lactation curves are not just used to provide a summary of the lactation pattern of an individual animal and allowing comparisons between individual animals and between groups of animals. These curves can also assist in making management decisions as well as pick up any disorders which would affect milk production, long before any clinical signs appear (Groenewald & Viljoen, 2003).

At a global level, milk produced by dairy cows remains the most abundant and preferred product of drinking milk consumed by humans (Faye & Konuspayeva, 2012). The largest amount of goat milk is produced in India, followed by Bangladesh and Sudan. Spain, France and Greece are three countries in Europe that produces a considerable amount of goat milk which makes it profitable for these countries to continue to grow and improve their genetics in order to grow and achieve more (Aziz, 2010).

However, the picture in South Africa is not as clear as in Europe regarding dairy goat milk production because of competition with an already existing big dairy cow industry which makes goat milk production marginal. However, dairy goat production does take place in South Africa to ensure well defined products be delivered to a niche market.

The role of Growth Hormone in milk production

Milk production is influenced by a lot of factors and mechanisms which is regulated by endocrine processes and hormones such as GH (Boutinaud *et al.*, 2003). GH is therefore important in milk production and mammary growth in order for ruminant lactation to take place (Accorsi *et al.*, 2002). As the world population grows, the demand for milk also grows and therefore research is necessary to achieve this increasing demand for milk yield. The administration of exogenous GH was widely used on dairy animals in order to increase milk production. However, this method of increasing milk production is still much discussed (Svennersten-Sjaunja & Olsson, 2005).

Other methods to improve milk production have been exploited and include selection and breeding of animals. Animals have achieved a state where much more milk is produced than needed for their offspring. Milk production has increased but the composition of milk is left unaffected compared to animals not bred for enhanced production. It is therefore no surprise that the demand for nutrients on high producing lactating animals have increased. Problems can occur as a result of this, and an increase in mastitis, hoof problems and metabolic stress are becoming more common in high producing dairy animals (Svennersten-Sjaunja & Olsson, 2005).

The physiology of lactation starts at a very young age in animals as the mammary gland needs to develop properly. The whole period of foetal to adult stage as well as development during pregnancy and lactation has to be taken into consideration. Changes can be noticed in the endocrine system at the onset of pregnancy. Before parturition can take place, the mammary gland needs to grow and this is initiated through the stimulation by GH and prolactin, adrenocortical steroids, oestrogens and progesterone (Svennersten-Sjaunja & Olsson, 2005). Growth hormone secretion is regulated by other hormones such as growth-hormone-releasing hormone (GHRH) and somatostatin (SRIF) (Frohman *et al.*, 1990). A whole orchestra of endocrine processes are clearly involved in milk production.

Hormones such as GH and prolactin play an important role in regulating mammary function in ruminants (Flint & Knight, 1997a) and together with leptin these hormones are important in regulating nutrients to the udder. GH has lipolytic and diabetogenic (blood glucose elevating) properties as well as blood flow are increased by GH (Svennersten-Sjaunja & Olsson,

2005).The mode of action of GH is considered to be indirect, mediated through stimulation of insulin-like growth factor 1 (IGF-1) production (Shkreta *et al.*, 1997; Flint & Knight, 1997b). However, it is not clear whether GH works directly on the mammary gland or if it is indirect via locally produced IGF-1 or via IGF-1 produced in the liver (Flint & Knight, 1997b; Hull & Harvey, 2001).

GHRH and ghrelin stimulates the release of GH and somatostatin inhibits the secretion of GH (Anderson *et al.*, 2004). Ghrelin is a 28-amino acid peptide and identified as an endogenous ligand for the growth hormone secretagogues receptor (GHS-R) and is found to circulate in two distinct forms, acylated ghrelin and unacylated ghrelin (Zhang *et al.*, 2013). It was found that ghrelin may affect milk production in ruminants by stimulating the release of GH from the pituitary (Iqbal *et al.*, 2006; Date *et al.*, 2000).

Dairy goat production and milk recording in South Africa

With an increase in human numbers and the demand for food, population and population growth all over the world, are two major determinants of livestock production (Peacock & Sherman, 2010). Goats can be found in almost any part of the world, even in remote areas where fewer animals are capable of surviving in harsh environments. Goats still have the ability to produce products to sustain a community or larger sector in such environmental conditions. A distribution in goat numbers can be found in Table 2.2, that indicates how widespread they are and that goats can be found in places all over the world.

Table 2.2: The distribution of goats found in different regions of the world (Olivier *et al.*, 2005).

Region	Goat numbers (millions)
Asia	323.64
Africa	217.22
Central America	9.57
China	161.49
Developed world	31.45
Total	743.37

In Africa a lot of goats are kept primarily to sustain small households as a lot of countries in Africa have not yet developed. Goats provide these communities with proper nutrition by providing milk and meat as protein sources. South Africa is known for producing goats with good meat qualities like the Boer goat (Malan, 2000) and for fibre, like the Angora goats found in the semi-Karoo regions of the country (Snyman & Olivier, 1996). Due to an ever

increase in population numbers, people need to find alternative sources for an increase in protein demand and therefore the Boer goat is an excellent animal to produce meat in rural areas where protein is often a limiting factor (Mmbengwa *et al.*, 2000).

A lot of small households, however, milk the Boer goat and consume the milk leaving the dairy sector a very small industry in South Africa, which is a pity considering the dairy goats' high efficiency. Unlike the Boer goat, dairy goats are a limited source of only milk products and do not supply meat that can be consumed by people from small households in rural areas (Casey & Van Niekerk, 1988). Research still needs to be done on dairy goats to see whether or not these species can serve as alternative meat sources. Dairy goat production in South Africa is however still marginal and limited to a niche market depending on consumer demands.

In South Africa there is a growing demand for goat milk as the niche market increases as well as in the tourist sector. Another demand exists in South Africa, as in the rest of the world, for people that suffer from health problems such as allergies that cannot consume any other animal milk. The history of dairy goats in South Africa can be dated back before the arrival of Europeans in the country. Namaqua people had encounters with goats, similar to those of Nubian or Egyptian origin, along the Olifants River (Olivier *et al.*, 2005).

During early years the dairy situation in South Africa was similar and comparable to the situation in a big part of Africa found today. Not a lot of new genetic material was imported into the country in order to improve the genetic diversity and quality of local dairy goats. It is now known that three Saanen bucks and 12 does from (probably) Switzerland were imported in 1898 to South Africa by the Cape Agricultural Department (Olivier *et al.*, 2005). Most of the dairy goats that can be found in South Africa today originated from only two bucks and 15 does that were imported from Switzerland in 1903 (Olivier *et al.*, 2005).

In South Africa, dairy goats as well as dairy cows are included in the National Dairy Animal Improvement Scheme. The milk yield of does is therefore measured over a 300 day lactation period from a ten monthly test of the amount of milk produced at two or more milking per 24h period (Olivier *et al.*, 2005). Only the fat content of milk was initially determined at these monthly tests, but since then, analysis for fat and protein content are determined when milk samples are collected from each doe (Olivier *et al.*, 2005).

Milk yield and milk composition of dairy animals are influenced by a lot of factors. South Africa has a wide range of different environments and climates where both registered and non-registered dairy goats produce milk under these climatic conditions. Goats being

grazers and browsers adapted well to the different regions in South Africa therefore surviving and producing milk under a wide range of production systems (Olivier *et al.*, 2005).

In developing countries it is mentionable that one of the main reasons goats are kept in a production system, is the production of meat, whereas in developed countries, milk production fulfil a more important role. More than 30% of goats in developed countries can be regarded as dairy goats, whereas in the developing countries only 20% are regarded as dairy goats, which suggests that production on a per animal basis is higher in developed countries compared to developing countries (Olivier *et al.*, 2005).

It should be noted that goat producers are unable to influence the price paid for their products through a process of value adding. In order for animal production to be sustainable, animals need to produce more at a lower cost (Olivier *et al.*, 2005). However, this can be achieved, especially with goats, because they are easily adaptable, hardy, can utilize nutrients more sufficient and they have a high survival rate which producers should acknowledge and take full advantage of.

Feeding and goat nutrition

It is important to understand the requirements of the animal in order for them to reach an optimum production level. However, animals cannot achieve this when there are inadequate levels of feed intake. Feed intake seems to be the most important factor determining animal performance (Illius & Jessop, 1996). Goats can maximise feed intake because of their ability to utilize more nutrients derived from bushes rather than grasses and convert it to high-quality products (Cannas *et al.*, 2008).

Goats are known to be both grazers and browsers and they are included in an intermediate class between roughage and grass eaters (Cannas *et al.*, 2008). Goats are therefore able to utilize a wide variety of feed sources and digest a wide range of nutrients much more than other ruminants. Unlike cattle which swallow a large bolus after grazing, the goat will nibble on leaves, rather than biting of the whole plant, which they carefully select and swallow in a small bolus (Wani, 2011).

As a result, goats more than any other species, are able to choose the parts of plants which has the highest protein content and highest digestibility which makes plant material eaten by goats more nutrient rich than the same plants eaten by other ruminants (Provenza *et al.*, 2003). Goats therefore obtain more energy per bite of feed ingested and therefore goats need a smaller amount of food (Wani, 2011). However, research on goat nutrition remains more limited than in cattle and sheep (Morand-Fehr, 2005a).

In South Africa goats are usually found on farms with an extensive production system. A common goal for all farmers is to convert forage to usable animal products (e.g., milk, meat, fibre, skin) (Rankins *et al.*, 2002). A lot of these farmers only farm with goats for bush encroachment, to control different plant species in the veld which is unpalatable to other species of animals. The meat sector is big in South Africa and most of the country's goat population consist of meat type goats like the Boer goat. The small dairy sector that exists in South Africa makes use of both concentrate feeding as well as to allow time during the day for the goats to graze on pastures to optimize milk production.

The mouth of the goat is similar to wild intermediate feeders and with these small mouths and prehensile lips, goats are able to select a wider range of forage to meet their requirements (Cannas *et al.*, 2008). They will readily consume other feedstuffs rather than grasses like flowers, fruits and leaves (Rankins *et al.*, 2002). Goats naturally seek diversity in their ingesta, which help to maintain a rumen environment within a certain microbiological and physiological range (Morand-Fehr, 2005a).

If goats do forage on grasses they tend to select highly digestible portions. Goats will graze, but they are browsers and they prefer to forage at head height, which is also a natural defence mechanism that protects them against some nematode parasites (Rankins *et al.*, 2002). Although goats are highly adaptable, parasites can still affect them negatively and spread throughout the whole herd leading to a decrease in production.

The nutrients from forages are not the only important aspect when it comes to goat nutrition. Sometimes a very important, common nutrient is left out because one assumes it to be readily available in the diet. Water is an extremely important nutrient and it makes up most of an animal's body. If a goat is deprived of feed and thus nutrients available to it, water would be the first limiting factor for the animal to succumb to (Rankins *et al.*, 2002).

Goats are known to graze and browse on pastures but they can easily adapt to a more intensive feeding system. They can tolerate concentrates rich in starch but also a diet high in forages, due to selecting feed and chewing behaviour. In intensive feeding systems, the use of total mixed rations (TMR) is advantageous to supply a balanced feed and to prevent selective feeding. Goats are also able to utilize feed that does not contain a lot of forage, however, feed particle size and fibre level should be carefully balanced (Cannas *et al.*, 2008) to prevent metabolic disorders such as acidosis when feed particles are too small.

Nutrient requirements

It is necessary to understand each individual animal's needs in a production system in order for that animal to produce to its maximum genetic potential. First of all, maintenance requirements have to be met and only then can the physiological as well as production stages be identified and fed accordingly (Cannas *et al.*, 2008). Animals can forage and gain the correct consistency of nutrients themselves in the wild, but due to production systems all over the world, it is the farmer's responsibility to understand the requirements and correctly formulate diets according to the needs of the animals.

In order for the dairy goat to produce a high milk yield, the goat has to take in a lot of dry matter. The intake of energy seems to be the most important dietary factor that affects milk production (Morand-Fehr & Sauvant, 1978). Goats are grazers and are sensitive to diets low in fibre and rich in concentrates, particularly around parturition (Morand-Fehr, 2005a). The feeding and management programmes of dairy goats should be adapted according to their needs, behaviour and physiological status. Only when this is understood and applied can dairy production take place at the lowest inset cost (Morand-Fehr & Sauvant, 1978).

When goats are kept in an all grazing system, the feeding strategies should be adequate to fulfil the animal's needs for both maintenance and production. Time should be given to allow recovery and improvement of the natural pasture as well as the correct management to ensure the natural pasture will have the correct amount and consistency of nutrients in order to prevent nutrient deficits in goats (Cannas *et al.*, 2008).

Proposed feeding systems exist for goats, but estimates are based on research done on other species. Energy and protein are the two main determinates to estimate in goat nutrition and together with maintenance, it is the major component of total requirements. Goats are widespread all around the world which makes it difficult to estimate parameters which makes modelling of their requirements more complex and challenging (Cannas *et al.*, 2008).

Energy

For every animal, energy is probably the first limiting nutrient that effects growth and production in animals. The stage of production will determine the energy requirements needed by the animal to produce to its genetic potential. The level of activity and physiological status also determines energy requirements and can be subscribed to maintenance needs. Depending on high growth rates or milk production, the energy needs can be met with a medium-to-high forage based diet (Rankins *et al.*, 2002). Energy seems to

be the feeding factor that is the most positive correlated with milk production (Morand-Fehr & Sauvant, 1978).

Most dairy goats around the world should have access to pastures at least once a day. Other than pastures, dairy goats can also be given concentrates to meet their requirements especially in the lactating stage where energy is of most importance to sustain milk production. Energy can come from a lot of varieties of choices that are available as feedstuff. Cereal grains are sometimes used such as maize, which contains a lot of energy in the form of starch. Several other feedstuffs are available to supplement forage based diets with energy to meet the animal's requirement depending on their needs (Rankins *et al.*, 2002).

Protein

Ruminants differ from other mammals for having a high functioning rumen. The rumen is home to microorganisms which plays a very important role in ruminant nutrition. Hence nitrogen metabolism differs in ruminants as the microorganisms synthesize most of the protein and serve as a protein source themselves which reaches the small intestine and therefore contribute more than 50% of the total metabolizable protein supply (Cannas *et al.*, 2008). The rest of the protein that reaches the small intestine is undegraded protein found in the feed (Nsahlai *et al.*, 2004).

In order for the animal to achieve optimum productivity there must be a way to get the protein to the lower digestive tract without it being degraded in the rumen. To achieve this, protein must be protected from microbial attack in the rumen to reach the lower digestive tract in a form where it can be absorbed, digested and utilized by the animal.

Animals need certain nutrients for optimal growth. Amino acids (AA) are the building blocks of proteins required for growth, reproduction, lactation and maintenance. When the animal ingests protein, it is first subjected to microbial degradation in the rumen making it difficult to estimate the amount absorbed by the animal. In ruminants, the AA comes from microbial protein and from exogenous sources of protein or AA which are undegradable in the rumen (Kung Jr & Rode, 1996).

Amino acids can be defined as organic substances containing both amino and acid groups. Amino acids can also be divided into two groups, the essential AA end the non-essential AA. The essential AA is defined as the AA which are inadequately synthesized by the body because the rate of utilisation is greater than the rate of synthesis. It should therefore be provided in the diet of the animal to meet the animal's requirements (Wu, 2009).

In order for rumen bacteria to grow and function at an ideal rate, a minimum of 7% dietary crude protein is needed. If the dietary protein drops below this level, forage intake and digestibility will be negatively affected (Rankins *et al.*, 2002). Protein that reaches the small intestine is mostly in the form of bacterial and/or protozoal protein that escaped digestion in the rumen. The quality of this protein is a good source of protein to the animal, whereas protein content of most plants decline with maturity. Stage of production and physiological status determines protein need of the animal just like in the case of energy (Rankins *et al.*, 2002).

Protein should be fed to meet the animal's requirements but not exceed it, as excess protein results in increased feed costs and higher incidence of disease (Rankins *et al.*, 2002). Protein concentration of a diet can be increased in an inexpensive way by supplying the animal with non-protein nitrogen (NPN). The most commonly form of NPN used is urea. Whenever NPN is used in a diet, a sufficient amount of highly fermentable energy should be included in the diet to prevent metabolic problems (Rankins *et al.*, 2002).

Minerals

Mineral requirements of goats have long been believed to be somewhere between the requirements of sheep and cattle (Meschy, 2000). Minerals are used almost everywhere in the body where they exert a certain biological function. We distinguish between macro- and micro minerals, however, this do not reflect the relative importance of each group but rather the amount required as a portion of the diet (Rankins *et al.*, 2002). Seven commonly macro minerals and eight micro minerals are identified.

Calcium and phosphorus are interrelated and can be found in skeletal tissue. A deficiency in these minerals results in poor or retarded growth in young animals and predisposes them to metabolic bone disease. A reduction in calcium uptake can result in a decrease in milk production by the female animal (Rankins *et al.*, 2002).

Sodium and chloride can be found in the body to work as integral components of bodily functions. Salt (NaCl) plays an important role as a carrier for most *ad libitum* mineral supplements. Sodium is an extracellular ion and is important for ion gradients in intracellular and extracellular functions as well as important for water metabolism and acid-base balance inside the body. Chloride plays an important role as it is a component of gastric secretions, which aid in digestion, and for normal osmotic balance (Rankins *et al.*, 2002).

Adequate amounts of magnesium should be ingested in order for the nervous system to function normally as well as magnesium which is required for many enzymatic reactions

(Rankins *et al.*, 2002). Like sodium, potassium also plays a role in normal acid-base balance. It is a component of many enzyme systems and like sodium; it is an important intracellular ion (Rankins *et al.*, 2002).

Sulphur makes up an important component of body proteins. It is usually required in animals that produce wool or mohair because of sulphur-containing amino acids (cysteine and methionine) in keratin (Rankins *et al.*, 2002). Ruminants with their effective rumen have bacteria that produce vitamin B_{12} by using cobalt (Rankins *et al.*, 2002).

Vitamins

As stated previously, ruminants have bacteria available in their rumen that can synthesize most of the B-vitamins. The only vitamins needed by the animals are the fat soluble vitamins (A, D, and E) (Rankins *et al.*, 2002). If an animal suffer from any disease or disorder that effects rumen function that prevents the rumen microbes to synthesize sufficient vitamin B, it would be wise and of value to supplement vitamin B in the diet (Rankins *et al.*, 2002).

Vitamin A is involved in a lot of bodily functions. It helps with normal growth and development, normal reproduction, vision and membrane integrity (Rankins *et al.*, 2002). Vitamin D requirement are adequate when the animal is exposed to sunlight as the animal body can synthesize this vitamin itself. Vitamin D also plays an important integrated role to prevent calcium and phosphorus deficiencies in the body (Haenlein, 1987). Vitamin E plays an integrated role with selenium and acts as an anti-oxidant in the body that plays a major role in cell membrane integrity (Rankins *et al.*, 2002). Vitamin K is necessary when blood needs to clot and it also plays a role in vision (Rankins *et al.*, 2002).

Body condition scoring

Body condition scoring (BCS) is a parameter for assessing nutritional status under various conditions (Morand-Fehr, 2005b) to determine how healthy the animals are and if the animal is using its reserves in order to sustain production. It is almost impossible to maintain a constant body condition throughout the entire herd. Animals are either too thin (under-conditioned) or too fat (over-conditioned). These animals should be taken into account and corrected for their body condition, as a failure would result in decreased fertility, increased disease or internal parasite incidence, decreased milk production, and at the end an increase in cost (Villaquiran *et al.*, 2004a).

Dairy goats, like dairy cows, are able to store access energy in the form of adipose tissue in their body and are able to mobilize these reserves in times of dietary deficiencies (Majele-Sibanda *et al.*, 2000). Reserves are usually mobilized depending on the nutritional status,

physiological stage and availability of adipose tissue (Morand-Fehr, 2005b). It is important to consider managing factors in order for the herd to attain the correct body condition. BCS is therefore an effective managing tool for the farmer to assess his herd (Mendizabal *et al.*, 2011). Goats should be supplemented with energy-rich feeds or concentrates to ensure mobilization of fat reserves do not happen all at once, as this could lead to negative effects such as metabolic disturbances (Eknæs *et al.*, 2006).

Dairy goats, unlike dairy cows, have little subcutaneous adipose tissue and this makes it difficult to work out a BCS method based on these body palpations (Morand-Fehr, 2005a). BCS indicates a lot of what's happening with the animal. If the animal is too fat, it indicates that the animal is not using its body reserves efficient enough to sustain productivity and whereas a low BCS, especially at kidding, would limit tissue mobilisation and restrict milk yield (Goetsch *et al.*, 2011). Body reserves are therefore catabolised when energy is inadequate to supply to the animal's demand in order to sustain production (Majele-Sibanda *et al.*, 2000; Mendizabal *et al.*, 2011).

A five point scale exist that people use to assess body condition. This scale has a range from 1.0 to 5.0 and increments of 0.5. A BCS of 1.0 would indicate an extremely thin goat with no fat reserves whereas a BCS of 5.0 indicates a very fat, over-conditioned animal. Normally healthy goats are indicated with a BCS of 2.5 to 4.0. Goats that have a BCS that do not lie in-between these values should receive attention as this condition can indicate management or even health problems. Goats rarely receive a condition of 4.5 and 5 and these values can be observed and seen as normal in show-goats (Villaquiran *et al.*, 2004a).

A BCS cannot be allocated to an animal by simply looking at it. The animal must be touched and felt in the proper regions to successfully allocate a BCS to an individual. In goats subcutaneous adipose tissue develops and forms around the sternum as well as the lumbar vertebrae (Morand-Fehr, 2005b). Scoring in the lumbar area is based on the amount of muscle (*longissimus dorsi*)(Mendizabal *et al.*, 2011) and fat that can be felt over and around the vertebrae. As indicated in Figure 2.1 the lumbar vertebrae consist out of a vertical protrusion (spinous process) and two horizontal protrusions (transverse process). Both of these processes are used to give a BCS to goats (Villaquiran *et al.*, 2004a). Large amounts of adipose tissue accumulates around the sternum in goats and therefore the sternum is sometimes used as indicated in Figure 2.1, to attain a BCS that indicates a more accurate description of body weight than the lumber area (Mendizabal *et al.*, 2011).

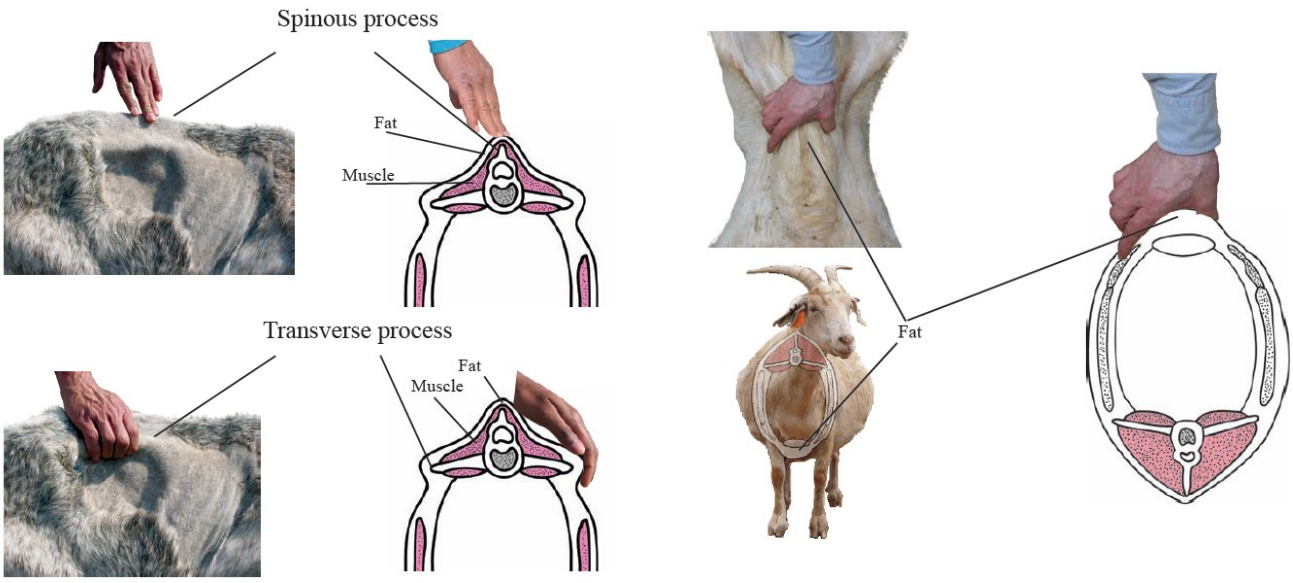

Figure 2.1: Body condition scoring in goats (Villaquiran *et al.*, 2004b).

It is important to develop the proper skills in assessing body condition. When the herd experience a decrease in body weight, pasture rotation is needed, or supplement feeding should be applied and deworming can also make a difference. The same applies when the herd gains a lot of weight and that management should rather restrict supplemental feeding (Villaquiran *et al.*, 2004a). A lot of time, body condition is ignored by people and this led to the problem being carried on until it is too late, resulting in lower production which leads to economic losses for the farmer.

Milk composition and milk solids

A lot of precursors are involved in the formation of milk proteins, lactose and milk fat. These primary precursors include: free amino acids, triacylglycerols, fatty acids, acetic acid and glucose. When any one of these precursors are limiting in the feed, it would have a negative effect on milk production and will change the milk composition as these precursors are interlinked with one another (Khaled *et al.*, 1999b; Jelinek *et al.*, 1996). Physical form of the diet can also effect milk production as well as composition, although the effects are smaller than in dairy cows (Goetsch *et al.*, 2011). Physical form of the diet is known to change milk composition such as the butterfat and protein found in the milk. Fibre plays an important role as fibre digestion results in the production of volatile fatty acids such as acetate and butyrate. Butyrate is converted to beta-hydroxybutyrate in the rumen wall tissue. About half the fat found in milk is synthesised out of acetate and beta-hydroxybutyrate.

It is clear that any changes in nutrition would have an effect on milk composition (Khaled *et al.*, 1999a). This is supported by a study conducted by Min *et al.* (2005b) that confirmed that

when concentrates are supplied to dairy goats, it will affect milk yield and milk composition throughout the lactation period. The effect is larger on milk yield and minimal compared to milk composition. In order for new marketable dairy goat milk products to develop, the milk produced should have a high dry matter content as well as consistent and satisfactory in flavour (Eknæs *et al.*, 2006).

Important mono-unsaturated and medium chain fatty acids are in greater amounts found in goats' milk than in cows' milk (Haenlein, 2001). In Table 2.3, a comparison is given between cow and dairy goat milk. The higher proportion of medium chain fatty acids are known to be anti-bacterial, anti-viral, inhibit development and dissolve cholesterol deposits (Shingfield *et al.*, 2008). Milk content, in terms of fatty acids, can vary greatly depending on the feed source, pasture versus grain supplements, different roughages, different fats and fat contents in the grain supplement, and different roughage to grain ratios. However, milk content in terms of amino acids and minerals are fairly stable, unlike fatty acids (Haenlein, 2001).

At a later stage in lactation, the energy needs of the dairy goat will increase and the animal will find itself in a negative energy balance. Energy is then mobilized from body fat reserves to sustain on-going milk production. Parts of the precursors for milk fat synthesis are mobilized which leads to a different composition in the milk synthesized when sufficient energy is absorbed from the digestive tract (Eknæs *et al.*, 2006).

A lot of similarities exist between goat and cow milk in terms of gross composition. However, goat milk lacks beta-carotene, agglutinin and has less alphas- 1-casein, citric acid, sodium, iron, sulphur, zinc, molybdenum, ribonuclease, alkaline phosphatise, lipase, xanthine oxidase, N-acetylneuraminic acid, orotic acid, pyridoxine, folate, vitamins C and B_{12}, lower freezing point and pH (Haenlein, 2001). Goat milk is higher in calcium, potassium, magnesium, phosphorus, chlorine, manganese, short and medium chain fatty acids, vitamins A and D, Nicotinic acid, choline, and inositol (Haenlein, 2001). It also has a better buffer capacity, alkalinity and digestibility (Park, 1994).

Goat milk contains smaller size fat globules and different casein types and is therefore more easily digested. However, because of this, goat milk often has a softer curd in cheese making, but a lower yield than cows. The amount of carnitine found in both goat and cow milk does not seem to differ (Haenlein, 2001). Lactose; however, is lower in goat milk than in cow milk (Aplocina & Spruzs, 2012). The process of feed pelleting also has an effect on milk composition where increase levels of casein are observed, which could be explained by the slightly greater digestible fat intake (Goetsch *et al.*, 2011).

Table 2.3: Comparison between goat and cow milk (Anon, 2014).

Composition per 100g	Goat	Cow
Protein (g)	3.1	3.2
Fat % (g)	3.5	3.9
Calories/ 100 ml	60	66
Vit A (IU/gram fat)	39	21
Vit B1 (thiamine (UG/100/ml))	68	45
Riboflavin (UG/100/ml)	210	459
Vit C (mg ascorbic acid/100 ml)	2	2
Vit D (IU/gram fat)	0.7	0.7
Calcium %	0.19	0.18
Iron %	0.07	0.06
Phosphorus %	0.27	0.23
Cholesterol (mg/100 ml)	10	14
Sugars (lactose)	4.4	4.8
Saturated fatty acids (g)	2.3	2.4
Monounsaturated fatty acids (g)	0.8	1.1
Polyunsaturated fatty acids (g)	0.1	0.1

Larger protein micelles can be found in goat milk when compared to cow milk (260 nm versus 200nm, respectively). Fat globules are smaller in goat milk and contain more short chain and middle-chain fatty acids. In cow milk, the proportion of unsaturated fatty acids is higher. These properties make it ideal to further process cheese (Faye & Konuspayeva, 2012). Fat and protein levels in milk seem to drop and milk yield increases as the goat advance in lactation. Milk yield decreases when the goat reaches mid- to late lactation but an increase in fat and protein concentrations can be observed (Goetsch *et al.*, 2011).

Proteins found in goat milk have a high value because of all the non-essential amino acids it contains. The content of non-essential AA varies according to the protein fraction. Whey proteins found in goat milk is rich in lysine, threonine, isoleucine and valine. Cysteine and tryptophan makes up a large amount of alpha-lactoalbumin, which has the highest biological value (Bernacka, 2011). Protein content, milk yield and fat vary according to year and season of kidding, herd, lactation, breed and interaction within the herd (Piliena *et al.*, 2012).

Dairy goats seem to be less responsive to the inclusion of rumen undegraded protein (RUP) than dairy cattle (Huston & Hart, 2002). The composition of proteins and their components in both goat and cow milk differs from one another because of the unique differences between

the two species, a wide variety of diverse genetics within each species, stage of lactation, feeding, climate, and subclinical mastitis all affects protein composition (Min *et al.*, 2005a).

When products are made using milk, it should be important to know which milk components are more important than others. Protein is the single most economically important milk component (Min *et al.*, 2005b). Therefore from a processing standpoint, an increase in milk protein concentrations would benefit products of milk origin (cheese, milk powder, evaporated milk, UHT milk) and this benefit would be greater if the casein concentration in milk were to increase (DePeters & Cant, 1992).

At a point where maximum milk potential has been achieved, very little can be done to change the composition of milk; however, milk fat concentration can be increased (Goetsch *et al.*, 2011). Milk fat is affected by forage source independent of energy intake and a diet which has a low dietary fat content will lead to a low milk fat concentration (Goetsch *et al.*, 2011). It is usually when grass forage is young and at an early growth stage that milk production as well as milk fat can rise (Aplocina & Spruzs, 2012). Milk fat plays a role in cheese yield and firmness, where it is involved with colour and flavour of dairy goat products (Chilliard *et al.*, 2003).

A positive correlation exists between amount and concentration of metabolizable energy and either milk protein content or protein yield (Spörndly, 1989; Min *et al.*, 2005b). When goats experience a negative energy balance, the milk they produce tends to be low in dry matter content and this often leads to rancid and tart flavour (Eknæs *et al.*, 2006). Feeding and managing strategies should be adapted and improved to sustain better productivity, which can lead to great differences in milk and fat yield (Haenlein, 2007b). It is clear that dietary characteristics can influence milk yield as well as milk composition (Min *et al.*, 2005b).

Objectives should be in place in order to maximise profitability but still at the lowest cost possible. Certain traits should be more focused on if the aim is to focus on milk yield or milk composition. Both quantity and quality of milk should be produced from healthy animals that are easy to milk. For selecting purposes the following criteria should be considered: If milk yield is the aim, then *volume of milk* is the most important factor and should be measured (Aziz, 2010).

When the aim shifts to processed products, the selection criteria should also change. If f*at* is focussed on, it should be noted that continued selection for fat is undesirable. Cheese making depends on *protein* concentration in the milk and therefore it is a major component in any selection scheme. *Total solids* are a good indicator for selecting fat and protein as it is a

composite sample that represents both. *Cell count* is an indicator of the bacteriological status of milk and should be a management tool rather than a breeding aim (Aziz, 2010).

Somatic cell counts in dairy goat milk

In a lot of countries, somatic cell count (SSC) is used in the dairy industry to monitor udder health and milk quality to prevent abnormal milk from entering human channels (Paape *et al.*, 2007a) as well as to ensure products are safe for human consumption (Haenlein, 2002). It is also a managing tool to do a quick test for the absence of clinical and subclinical mastitis and it is a widely applied health quality standard to accept or reject milk for the industry (Haenlein, 2001). A lot of countries uses SCC as an indication of milk quality and is therefore used as a criterion for milk payment to producers, penalizing them if milk contains more than the set amount (Salama *et al.*, 2003a). Mastitis is referred to as a serious disease of dairy animals when an invasion of an infectious agent has accumulated in the udder (Petzer *et al.*, 2008).

Mastitis causes a huge problem on farms and has deleterious effects on milk production that can lead to serious economic losses as SCC can be used to define milk prices. Abnormal milk and the control thereof are most complex and expensive for the dairy farmer of today (Paape *et al.*, 2007a). There is still a lack of criteria for diagnosing subclinical mastitis, that makes interpretation of SCC in goat milk a grey area, and therefore research still needs to be done (Petzer *et al.*, 2008). It is of critical importance for dairy farmers that adequate research on quality milk standards and procedures which are applicable to dairy goats and the milk they produce are being done (Haenlein, 2002).

Hygiene in milking and milk handling plays an important role in the control of bacterial numbers and SCC (Goetsch *et al.*, 2011). Another way of controlling the level of mastitis and SCC, is to treat the animal with antibiotics at the time of drying off (Poutrel *et al.*, 1997). Milk is produced from the goat's mammary gland by apocrine secretion and more than often does cellular tissue appear in milk in the form of DNA-free particles, similar to the size of leukocytes (Dulin *et al.*, 1983). Intact epithelial cells get sloughed from acini and ducts and ends up in variable numbers in goat milk (Smith & Sherman, 1994). Normal goat milk compared to cow milk, has a higher SCC (700 000 to 1 000 000 cells per ml milk) (Hinckley & Williams, 1981).

Somatic cell counts are used in the dairy cow industry where it is an indication of mastitis when leukocytes or neutrophils (Poutrel *et al.*, 1997) accumulate in milk. An increase in SCC numbers has a positive correlation with mastitic conditions. Goats and cows differ in the way that milk is produced (Zeng & Escobar, 1995). However, non-leukocyte cell particles are

found in goat milk from its apocrine secretion process, which do not have DNA or a nucleus as leukocytes do, and therefore have nothing to do with mastitis (Haenlein, 2002). The loss of part of the secretory gland cell due to the pinching-off of the secretion-filled end of the gland cell, but leaving the nucleus and most of the cytoplasm to recover and repeat the secretion process is referred to as apocrine secretion. This type of secretion in goats leads to high levels of cell fragments which are counted in various methods to indicate udder infection. These cells are not nucleated and are unrelated to leukocytes, which do represent an infection in the mammary gland (Haenlein, 2001). Therefore an increase in SCC in dairy goats is not valid enough to be of any indication that a goat suffers from a mammary infection (Goetsch *et al.*, 2011).

Somatic cell counts can be tested and several methods have been developed to do so. Some of these methods include: the California mastitis test (CMT), which is a quick and inexpensive, widely used test; electronic automated machines, such as the Coulter Counter and Fossomatic, which are used on the base of chemical principles for estimation of SCC (Haenlein, 2002).

Various reasons exist for an increase in SCC. Increasing parity would lead to an increase in SCC (Salama *et al.*, 2003b; Paape *et al.*, 2007b; Zeng *et al.*, 2010), which can be related to increasing bacterial presence or cumulative mammary gland stress (Boscos *et al.*, 1996), as well as lactation advances (Goetsch *et al.*, 2011). Within different breeds of goats, variation is found in the number of somatic cells (Zeng *et al.*, 2010). Mixed herds of different breeds are therefore used for a balanced volume, fat and protein concentration, and SCC to meet the necessary regulatory requirements (Goetsch *et al.*, 2011). Other factors that have an effect on SCC in dairy goats include: farm, breed, age, stage of lactation, oestrus, milk production, management conditions, intramammary infections (Poutrel *et al.*, 1997), and caprine arthritis-encephalitis virus (CAEV) (Paape *et al.*, 2007a).

Somatic cell counts differences do occur in breeds but managing is important in minimising SCC and bacterial numbers. Hygiene should be a priority and therefore it should include sanitation of the farm, animals, and milking parlour, udder sealing, milking equipment maintenance as well as distributing the milk as soon as possible after milking to cooperative storage tanks (Goetsch *et al.*, 2011).

Differences exist between the anatomy of goats and cows. An obvious difference is that a goat's udder only consists out of two halves compared to a cow. Goats therefore have a small udder cistern volume compared with a relative medium volume in cow udders. One should also take into account the much tighter diameter of the teat sphincter of goat udders (Haenlein, 2001).

Producers, researchers and veterinarians assessed this problem in the commercial industry and came to the conclusion that dairy goats differ in SCC from dairy cows and especially in animals without intramammary infection. Further research is also needed to determine how effective post-milking teat disinfections in goats are (Poutrel *et al.*, 1997). Commercial dairy farms struggle to keep the herds' SCC numbers below the legal limit in developed countries especially when lactation advances (Wilson *et al.*, 1995). SCC in under developed countries has much scope and research needed to establish legal and acceptable limits for consumers. South Africa produces goat milk on a marginal basis and therefore a legal limit needs research to be applicable to the countries' own standards. Researchers need to take non-infectious factors that contribute to an increase in SCC into account when establishing legal cell count limits (Paape *et al.*, 2007a).

Feed additives used in animal nutrition

Feed additives have been used widely around the world to benefit both animals and animal production. A lot of rules and regulations prohibit the use of feed additives in many countries due to environmental and health issues of both animals and humans consuming products of animal origin. Certain products are still legal in countries and a lot of varieties and different enzymes, plant metabolites, essential oils, and organic acids are but a few to mention that are still used nowadays. A lot of research has been done in this field to ensure safe products reaches the market which has no side effects and are safe to use on animals. A few products will be mentioned below.

Dietary antibiotics were widely used in animal feeds to improve average daily gain, increase in feed conversion, and reduce losses due to certain diseases. Responses vary from one animal to another and effects depend on the stress of the animal as well as management (Rankins *et al.*, 2002). As the use of antibiotics decreases, new products have to be developed which is beneficial to animal health. Probiotics, prebiotics, some organic acids involved in metabolic pathways, and plant extracts can provide some benefits that antibiotics provides (Jouany & Morgavi, 2007). Probiotics are sometimes used when an animal undergoes a transition from forage-based diets to concentrate rich diets that contains a lot of cereal (Jouany & Morgavi, 2007).

Buffers are salts that help to maintain pH levels in the rumen of ruminants. Some of them include sodium bicarbonate (most widely used), sodium sesquicarbonate, sodium bentonite, and calcium carbonate (Rankins *et al.*, 2002). Buffers are added to high-grain diets which would otherwise cause a drop in ruminal pH, as these feedstuffs are highly fermentable. Buffers do not exert the same effect if the diet contains adequate amounts of fibre when

forage-based diets are offered. In dairy goats, buffers seem to improve milk production, minimize milk fat depression, decrease the incidence of lactic acidosis-rumenitis complex, and improve overall health (Rankins *et al.*, 2002).

Methane emission from animals has raised worldwide awareness and is a very sensitive topic in animal production systems. Researchers and companies are trying to develop ways to decrease ruminal methanogenesis which would lead to a decrease in methane emission. Fungal organisms have been added to ruminant diets for several years (Beev *et al.*, 2007). Yeast cultures are also added to diets in order to minimise ruminal methanogenesis (Patra, 2012). Yeasts have positive effects on nutrient digestibility especially fibre contents, probably by stimulating the cellulolytic microbial populations in the rumen (Patra, 2012). *Saccharomyces cerevisiae* are widely used as a yeast supplement in ruminant nutrition (Newbold & Rode, 2006). However, responses to yeasts are not always observed in ruminant nutrition due to various reasons (Patra, 2012).

Organic acids are also investigated to see their effects and possible uses in ruminant nutrition. Plant extracts have potential, but are mainly limited to the more developing countries (Newbold & Rode, 2006). Choline is used in dairy goat nutrition in order to improve metabolic health especially during the transition period when liver functionality can be impaired. However, choline is degraded in the rumen and therefore do contribute to the choline body pool in ruminants (Savoini *et al.*, 2010). Specific fatty acids are incorporated in dairy animals such as dairy goats, which can improve milk fatty acids profile end eventually the health of these animals. These fatty acids along with choline could have positive effects when animals are stressed and can lead to enriched properties of milk (Savoini *et al.*, 2010).

Microorganisms as feed additives or supplements is not a new concept in ruminant nutrition as researchers and veterinarians have long ago started to use rumen fluid from a healthy animal to inoculate another ruminant that have been deprived of food for a long time to stimulate normal rumen function (Beev *et al.*, 2007). *Lactobacillus* spp. is one of the most common microorganisms used in products that promote microorganisms as feed supplements (Beev *et al.*, 2007).

Dicarboxylic acids (aspirate, fumarate and malate) occur naturally in the citric acid cycle as they are major metabolic intermediates that play an important role in metabolic processes in the animal body. These acids also occur naturally in plant and animal tissues (Jouany & Morgavi, 2007). Plants synthesises a broad range of secondary metabolites for their own protection as these metabolites are not involved in growth, development or reproduction. Saponins are glycosides found in many plants and used because of their potential as

pharmaceutical and nutraceutical agents. Supplementation of saponins in ruminant diets has claimed to improve growth, feed efficiency and health (Mader & Brumm, 1987).

Essential oils are products extracted from plants and used in animal feeds for their beneficial properties on animal production (Jouany & Morgavi, 2007). However, plant extracts used as performance enhancers in animal feeds are limited as these products vary in the quality and quantity of active compounds and therefore difficult to standardise. Other factors which affect quality and quantity are the vegetative state of the plant and whether the plant was stimulated to produce secondary defensive compounds. Antioxidants are lost during the storage of these products and dosage to animals should be carefully monitored as the secondary compounds can have additive, synergistic and antagonistic effects in the animal's body (Cordell, 2000; Burt, 2004).

It is widely accepted that the use of plant extracts are safe as they are produced naturally. However, it should not be forgotten that these products are synthesized by the plant as a defence mechanism against disease and herbivores. Dosage is important as an excess of a product can lead to toxic effects and negative responses in production (Jouany & Morgavi, 2007).

Tannins are secondary metabolites produced by plants as a defence mechanism against herbivores. These plant products are believed to bind to proteins, making them unavailable to digestion. However, tannins included in ruminant feeds showed to have a positive effect on protein digestion (Frankič *et al.*, 2009). Tannins, as stated previously, forms complexes with proteins which makes them undegradeable inside the rumen. These protein complexes pass undigested by the microorganisms into the small intestine where they are successfully utilized by the animal, therefore providing adequate amounts of proteins needed by the animal during different physiological stages such as early lactation and when feed is not of best quality (Waghorn *et al.*, 1990).

Research started to focus on enzymes which can be added to animal feeds to improve efficiency of ruminants. Exogenous enzymes are added to animal feed mainly to assist in the digestion of cell wall carbohydrates in forages. The mode of action still warrants further research and as information become more available, commercial products can evolve and improve to be used by farmers in animal feeds (Jouany & Morgavi, 2007).

All of the above mentioned products which are added to animal feeds are able to act positively on rumen feed digestion and enhance production. These additives are unique and exert their action through different mechanisms. Despite their diversity these products

ultimately affect rumen fermentation metabolic pathways and (or) the digestive microbial ecosystem (Jouany & Morgavi, 2007).

Fenugreek as a natural feed additive

The use of antibiotics in animal feeds raised awareness and in some cases led to the ban of these substances in some countries (Pugh, 2002). Researchers and farmers had to come up with a way to increase production and obtain better production results from farm animals. A more natural way of enhancing performance in farm animals led to the use of plant metabolites and other plant extracts to achieve this. In the last decade the use of feed additives were successful. Biological additives (yeast cultures), natural additives (medicinal plants as its seeds) and chemical additives (buffers such as sodium acetate and sodium succinate) is commonly found in animal feeds today (Khattab *et al.*, 2010a).

Plant extracts can be used in animal feeds as feed, appetite and digestion stimulants. It is also used as stimulants of physiological functions, altering rumen microbial populations in a positive way, as colorants and antioxidants (Frankič *et al.*, 2009). Additives are sometimes used to manipulate rumen fermentation, to increase nutrient digestion and absorption. This is done in such a way that productivity of the animal is increased as well as methanogenesis is decreased (Frankič *et al.*, 2009). The use of these natural additives helps in improving animal productivity and increase milk production (Khattab *et al.*, 2010a).

Fenugreek (*Trigonella foenum-graecum L. Leguminosae*) is a member of the legume family and is found in India, Middle East, North Africa and South Europe. It is well known around the world as a medicinal plant and for its medicinal properties in both humans and animals (Smith, 2003). Productivity of lactating animals can be improved by medicinal plant seeds and hormonal alert effects as circulating levels of prolactin and growth hormone increases in the body. In addition, udder tissues are activated with increasing glucose concentration with a reduction in cholesterol concentration in the blood (Khattab *et al.*, 2010b).

Fenugreek has been shown to have a positive effect on lactation performance in ruminants such as dairy cows, water buffaloes and dairy goats (El-Alamy *et al.*, 2001; Kholif & El-Gawad, 2001). Research done on Fenugreek is not well known and the mechanism by which Fenugreek increases milk yield still remains unclear. There is still research needed to better understand the mechanism by which Fenugreek exerts its effect on milk production (Al-Shaikh *et al.*, 1999).

Fenugreek also seems to play a beneficial role in digesting and absorbing of lipids by enhancing bile acid synthesis in the liver (Frankič *et al.*, 2009). It is also possible that

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saponins found in Fenugreek lowers lipids because these saponins are transformed in the gastrointestinal tract into sapogenins (Smith, 2003).

Diocin is a natural saponin found in Fenugreek and has a structural similarity to oestrogen, which leads to an increased release of growth hormone (GH) by binding to the receptors on pituitary cells that recognise the GH releasing hormone. This, in turn, results in an increase in milk secretion (Graham *et al.*, 2008).

Humans are very aware of healthy foods and prefer food which has a low cholesterol content. Fat from animal sources which is high in cholesterol has been believed to lead to high cholesterol concentrations in humans which can cause heart disease (Shah & Mir, 2004). Increased health benefits are available for consumers if milk cholesterol concentrations can be decreased in milk, improving milk quality and increasing desirable functional fatty acids (Shah & Mir, 2004).

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Chapter 3

The effects of a natural feed additive on the milk production of dairy goats.

Abstract

Relatively little research is done on feed additives to enhance production of dairy goats in South-Africa. There is a need for research on milk production and improved milk quality, especially under adverse environmental conditions. Many feed supplements are available in the wider dairy industry, and therefore a trial was conducted with dairy goats using a natural feed additive, known to improve milk production characteristics in dairy cattle but not tested in dairy goats. Three treatment groups consisting of forty-eight goats each were randomly selected from lactating goats which were comparable in 'days in milk' and received a diet consisting of a 50:50 mixture of lucerne and oat hay as well as a dairy ration (semi-complete feed). Each group were subsequently allocated a different ration; control group which received a regular ration (no additional supplement), Nutrifen® group, which received the normal ration plus 60 grams of Nutrifen® daily and a NutrifenPlus® group, which received the normal ration plus 60 grams of NutrifenPlus® daily. Milk sample collections were made on a weekly basis on the farm Fairview, near Paarl in the Western Cape with the trial duration of 130 days. The main focus of the study was on milk production and milk composition. Blood samples were also collected to monitor BUN. The milk production for the NutrifenPlus® group was significantly higher than the control group (P = 0.01). Milk lactose for the NutrifenPlus® group was significantly higher (P = 0.03) than the other two groups. The somatic cell count did no differ significantly within the three treatment groups. No significant differences were found for LDL and HDL between treatments. These results would imply a more cost effective solution for dairy goat farms if paid according to litres produced and not by the total solids found in milk.

Keywords: Dairy goats, Cholesterol, Fenugreek, Milk solids

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Introduction

Relatively little research is done on feed additives to enhance production of dairy goats in South-Africa and especially to improve production under adverse environmental conditions. Indigenous knowledge is important in the unique South African environment in order to maximize production from dairy animals. Goats are well known for their ability to utilize less favourable feedstuffs and adapt to adverse environmental conditions all over the world. These animals are more likely to utilize feed material efficiently than other domesticated animals, they are more disease tolerant as well as that they have a great reproductive capacity which make these animals ideal in farming systems (Wani, 2011).

Dairy goats are mostly compared to dairy sheep as well as compared to dairy cows as the genetics in milk production within the three species seems similar (El-Abid & Nikhaila, 2010). People often turn to products derived from goats and sheep, which play an important role in basic human nutrition (Haenlein, 2001) whereas milk proteins from dairy goats have certain nutritional advantages in relation to other dairy species. Goat milk is not just used by people for surviving purposes but it also plays an important role in nutrition for people suffering from protein allergies (El-Agamy, 2007), including children, whom are able to tolerate goat milk more than cow milk because of less complex caseins and whey protein structures found in the milk (Wilson *et al*., 1995).

Nutrition plays an important role in milk production from dairy goats. Dietary factors and daily body weight gain are factors which can influence both milk yield and composition (Min *et al*., 2005). It is therefore important to supply dairy goats with adequate amounts of nutrients from good quality feed sources, for them to produce milk to their full potential. Legume are sometimes used in dairy goat nutrition as it confers several advantages. Legume plants are known to contain more protein content and stimulate a high voluntary intake than grasses and cereals (D'mello, 1992).

Herbal galactagogues are known by a lot of human cultures around the world as products which stimulate milk production in lactating women as well as in ruminants (El-Abid & Nikhaila, 2010). Fenugreek (*Trigonella foenumgraecum*) is such an herb and a member of the legume family, found in India, Middle East, North Africa and South Europe. It is used as a natural herbal medicine to promote lactation in lactating women. It has also been shown to have a positive effect on lactation performance in ruminants such as dairy cows, water buffaloes and dairy goats (El-Alamy *et al*., 2001; Kholif & El-Gawad, 2001).

The mode of action is based on its diocin content. Diocin is a natural saponin found in Fenugreek and has structural similarity to oestrogen, which leads to an increased release of

growth hormone (GH) by binding to the receptors on pituitary cells that recognise the GH releasing hormone. This, in turn, results in an increase in milk secretion (Graham *et al*., 2008). Alamer and Basiouni (2005) found that Fenugreek seeds increased milk production in dairy goats, but did not have an effect on milk fat content. Although feed intake was reduced, it did not affect the stimulatory action of this herb on milk yield.

The cholesterol found in milk can be linked to the cholesterol found in blood. Blood cholesterol is expected to be the main precursor of milk cholesterol (Raynal-Ljutovac *et al*., 2008). Any additional feed additive, added to the diet of an animal, which has a positive effect on lowering the blood cholesterol content, could therefore result in a decrease in cholesterol found in milk. This statement is supported by Alamer and Basiouni (2005) that milk cholesterol content was reduced as a result of reduced blood cholesterol concentration. This will lead to increased health benefits to consumers (Raynal-Ljutovac *et al*., 2008).

Leguminous plants, such as Fenugreek, are rich in saponins (Mir *et al*., 1997), and the consumption of these compounds has been proven to reduce cholesterol content in different species like rats and humans *(Petit et al*., 1993; Sharma *et al*., 1990). A study conducted by Shah and Mir (2004b) indicated that blood cholesterol was successfully reduced in dairy cows when fed Fenugreek seeds in their diets, which resulted in lower milk cholesterol content.

Two commercial Fenugreek products, formulated differently but both containing Fenugreek cotyledon concentrate (yellowish powder extracted from seed by the manufacturing company), were selected for the current study. The products used were Nutrifen® (Fenugreek cotyledon concentrate) and NutrifenPlus® (Fenugreek cotyledon concentrate with other ingredients). According to the literature, Nutrifen® improves growth rate and optimizes appetite, feed conversion and ultimately increases milk production in dairy goats (Al-Shaikh *et al*., 1999b). NutrifenPlus® has been documented to provide additional health benefits and increase performance, with a significant enhancement in milk quality in ruminants such as buffaloes and goats (Mirzaei & Hari Venkatesh, 2012).

The main objective of the current study was to evaluate the effects of Fenugreek on milk yield and milk composition of dairy goats.

H_0: *Fenugreek as a feed additive will not affect milk production.*
H_1: *Fenugreek as a feed additive will affect milk production.*

H_0: *Fenugreek as a feed additive will not affect milk composition.*
H_1: *Fenugreek as a feed additive will affect milk composition.*

A secondary objective of this current study was to evaluate the effect of Fenugreek on the cholesterol content in dairy goat milk.

H_0: *Fenugreek as a feed additive will not affect milk cholesterol content.*

H_1: *Fenugreek as a feed additive will affect milk cholesterol content.*

Material and methods

Ethical clearance for animal use

This study complied with accepted standards for the use of animals in research and teaching as reflected in the South African National Standards 10386: 2008; and was completed with ethical clearance from Stellenbosch University Care and Use Committee (SU ACUC), reference number: SU-ACUM12-00033.

Animals used in the study

Dairy goats used in this study were obtained from a commercial farm Fairview (Paarl, Western Cape). A herd of 144 lactating goats, consisting predominantly of Saanen goats and a small number of Toggenburg and British Alpine goats were used in the current trial. All breeds represented the same number in each treatment. The goats were stratified according to days in milk and randomly allocated to three treatment groups of 48 goats per group. All three groups of animals were kept in a well-ventilated shed close to the milking parlour.

Forages and diet characteristics

Goats were maintained on the regular feeding and management program of the Farm Fairview (Paarl, Western Cape), where they received a 50:50 mixture of lucerne and oat hay *ad libitum* for 24 hours of the day.

The goats also received a semi-complete formulated commercial feed (Meadow Feeds Lactating Goat Feed), supplied by the animal feed company, Meadow Feeds (Paarl, South Africa) and offered at 400 g/goat per day. This semi-complete feed served as the basal diet. The chemical composition of the feed is presented in Table 3.1. Goats had access to fresh and clean water every day.

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Table 3.1: Chemical composition of the semi-complete commercial goat feed as is.

Chemical composition	Amount (g/kg)	Inclusion
Protein	150.0	Minimum
**(11.48% derived from [1]NPN)*		
Moisture	120.0	Maximum
Fat	25.0	Minimum
Fat	70.0	Maximum
Fibre	120.0	Minimum
Fibre	200.0	Maximum
Calcium	10.0	Maximum
Phosphorous	4.5	Minimum
Urea	6.0	Maximum

[1]NPN - Non protein nitrogen

Treatments

Two commercial Fenugreek products, formulated differently, both containing cotyledon concentrate were used in this current study. Nutrifen® and NutrifenPlus® are additives which enhance milk production in dairy animals. Different colour tags (three colours) were used to put around the neck of the animals in the trial for ease of identification of the animals receiving the different treatments and to ensure animals get the appropriate treatment.

The composition of the two treatments used in this trial was as follow:

Nutrifen®

Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*)

NutrifenPlus®

Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*)

Fennel seed (*Foeniculum Vulgare*)

Saw Palmetto berries (*Serenoa Repens*)

Brown Kelp *(Laminariales*)

MSM (natural source Methylsulfonylmethane)

White distilled vinegar powder.

The Control group received the basal diet (as mentioned under the heading: Forages and diet characteristics) with no additive added to the basal diet, while the Nutrifen® group received the basal diet plus 60 g of Nutrifen® per goat/day and the NutrifenPlus® group received the basal diet plus 60 g of NutrifenPlus® per goat/day.

Supplementation occurred in the milking parlour twice daily during the morning (05:00) and afternoon (15:00) milkings, where 30g of the respective additive were top-dressed onto 200 g of the semi-complete feed per milking. Refusals, if any, were collected after each goat had been milked and the amount determined.

Duration of the trial

The trial was conducted for 130 days from November 2011 to February 2012, which is summer and associated with the warmest period in Southern Africa and in the Western Cape region, where the farm Fairview (Paarl, Western Cape) is situated. During February, which was the warmest month of the trial, it was decided to collect the total daily milk yield of each goat over a two week period to determine the effect of Fenugreek under hot environmental conditions. This section was additional to the study.

Limitations

As the milking parlour did not allow for easy daily milk recordings, milk production data was collected from the farm according to their schedule as an indication of the average milk production per goat over the duration of the trial. In February, a 14 day accurate collection period was used to determine the effect on milk production.

Milk collection, composition and analysis

Milk samples were collected once a week by hand from the same lactating indicator goats per treatment group. Milk collected was preserved in a milk sampling vial at room temperature (22º - 25ºC) using a small Bronopol capsule which inhibits the growth of bacteria, yeast and mould. For the determination of milk solids (lactose, protein, and fat) and milk urea nitrogen (MUN), milk was collected from ten randomly assigned indicator goats per group of 48 goats on a weekly basis during the afternoon milking. As far as possible, the same ten goats were used every week. Milk samples were transported to the laboratory immediately and were analysed with the aid of a Milk-O-Scan 605 analyzer (Foss Electric, Hillerod, Denmark) at the Dairy Laboratory of the Agricultural Research Council, Elsenburg, Stellenbosch.

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Blood collection and analysis

Blood collections were done after feeding and done on the same ten indicator goats per group as described above. Blood was collected via venipuncture from the jugular vein into EDTA vacutainors and put on ice to keep the samples cold. Blood collection was repeated every second week. Blood was centrifuged for ten minutes at 3000 rpm with a Sigma 4-15 Bench top centrifuge (Wirsam Scientific cc). Serum was pipetted into clean glass tubes and stored in a fridge at 4º - 8ºC. The next day samples were removed from the fridge and analysed on an automated blood chemistry analyser (Vitros 250 blood chemistry analyser, Ortho-Clinical Diagnostics, Johnson & Johnson) and analysed for blood urea nitrogen (BUN) by The Department of Agriculture, Western Cape's Veterinary Services Laboratory, Stellenbosch.

Blood used for cholesterol content (LDL and HDL) was obtained in the exact same method as described previously and collected after feeding. Both HDL and LDL were assayed on serum. A fasting sample was preferred (10 – 12 hours, only water allowed). The blood was collected into an SST tube (tube without any anticoagulants) with gel separator. The tube was well mixed (not shaken) and allowed to clot as it stood for at least 20 minutes. The sample was then centrifuged at 4000 rpm for 15 minutes. The sample was assayed on a Beckman AU analyser at PathCare Veterinary Laboratory (Neels Bothma Str., N1 City, Cape Town, South Africa).

Two week milk production

The same goats in the different treatment groups, as previously described, were used during this collection period to accurately measure daily and total milk production over a two week period. All 48 goats, from the farm Fairview (Paarl, Western Cape) in each treatment group were used over a period of 15 days in the month of February 2012. Milk was recorded in the milking parlour (herringbone design) daily during morning (04:00) and afternoon (15:00) milking. The total daily yield (in Litres) of each goat in a treatment group was determined over the two weeks. The data was captured and analysed for statistical differences.

Statistical Analysis

Data on milk components and total milk yield over the four month collection period were subjected to a one way ANOVA, using Statistica version 10 (Statsoft Data Analysis Software System, 2011). Data pertaining to daily milk yield and lactose content over 14 consecutive days were analysed using the repeated measures ANOVA procedure of Statistica version 10. Milk production was normalised at the onset of the two week continuous collection

period. Treatment means were separated with a Bonferroni test and significance was declared at $P < 0.05$.

Results and Discussion

The use of an additive within different treatment groups showed promising results pertaining to milk yield and composition. The effect of the different Fenugreek products on milk yield and milk composition is presented in Table 3.3. Milk yield over the total experimental period of four months was higher ($P = 0.01$) for goats that received the Nutrifen® based treatment compared to that of goats in the Control treatment, while values for the NutrifenPlus® treatment were intermediate.

It is well known that adding natural feed additives to the diet of dairy animals such as dairy goats has a positive effect on milk production as reported by El-Abid & Nikhaila (2010). Alamer and Basiouni (2005) also observed beneficial effects of Fenugreek on milk yield and reported that the usage of Fenugreek in the diet of dairy goats resulted in a definite increase in milk yield compared to the control group. Fenugreek has a positive effect in dairy goats as well as a positive effect on milk production in sheep fed Fenugreek seeds in the diet, as supported by El-Abid and Nikhaila (2010).

The increase in milk yield over the total experimental period of 130 days may be attributed to Fenugreek's properties to enhance appetite and increase feed intake (El-Abid & Nikhaila, 2010), which will be subsequently investigated in Chapter 4. However, the increase found in milk yield could also occur due to endogenous hormone stimulation caused by supplementing feed with Fenugreek. In a study conducted by El-Abid and Nikhaila (2010) on Sudanese desert sheep, a conclusion was made that an increase in milk yield was due to increased levels of thyroid stimulating hormone and stimulatory prolactin which has an effect on lactation performance. The effect of increasing levels of GH in dairy goats was evaluated and is discussed in Chapter 5.

Treatment had no effect on milk fat or milk protein content of the randomly assigned ten indicator goats. This agrees with a study conducted by Alamer and Basiouni (2005) who also indicated no significant differences found in milk fat percentage and plasma total protein when Fenugreek seed powder was used as a treatment in the experiment. Al-Shaikh *et al.* (1999b) reported an increase in milk fat percentage in dairy goats receiving Fenugreek, which is contradictory to the findings in the current study and the study done by Alamer and Basiouni (2005).

The average gross milk composition as well as the ranges of the main constituents is presented in Table 3.2. Values presented in Table 3.3 from this study are well within the normal range of constituents found in dairy goat milk when compared to Table 3.2. Milk fat from this study was very near to the average value for milk fat. Milk protein and lactose however was lower than the average but still remained well within the normal range. The same observation was made regarding total solids in milk. The lower than average value for total solids found in milk is expected as milk protein and lactose were less than the average.

Table 3.2: Typical gross composition of dairy goat milk.

Item	Average	Range
	(g/kg)	(g/kg)
Fat	41	24.6 - 77.6
Crude protein	35	24.9 - 50.6
Lactose	45	36.2 - 63.0
Total solids	129	99.5 - 215

*Adapted from Amigo & Fontecha (2011)

Table 3.3: Mean milk yield and milk composition of dairy goats that received different Fenugreek products over a four month experimental period.

Item	Treatment			SEM	*P*
	Nutrifen®	NutrifenPlus®	Control		
Milk yield (130 days), kg	595[a]	563[ab]	547[b]	15.4	0.01
Daily milk yield, kg	4.6	4.3	4.2		
Milk fat content, g/kg	41.4	47.5	43.2	0.26	0.25
Milk protein content, g/kg	27.4	27.3	27.7	0.07	0.93
Lactose content, g/kg	38.5[a]	40.0[b]	38.6[a]	0.12	0.03
Total solids content, g/kg	114	122.1	116.7	0.35	0.27
Somatic cell count, x 1000 cells/ml	5689	5784	5669.7	1029.4	0.99
BUN, mM/L	8.92	8.98	8.94	0.17	0.99
MUN, mgN/dl	23.9	24.2	23.4	1.06	0.99

[a,b,c] Means in the same row with different superscripts differ ($P < 0.05$)

Lactose, also known as milk sugar, is the main carbohydrate found in milk. In this study, the lactose content in the milk differed significantly (P = 0.03) between the treatments. The NutrifenPlus® treatment resulted in a higher milk lactose content compared to the Nutrifen® and control treatments. In a study done by Chilliard *et al*. (2006), dairy goats are compared with dairy cows and it was indicated that milk lactose content can be increased by using dietary supplementation of secondary plant metabolites; in the same study, oils were used as the supplement. Once again this serves as proof that milk constituents can be altered by using secondary metabolites as additives to enhance feed. Lactose and lactose derivatives become significant in dairy products when milk is processed into the making and use of sweetened condensed milk, ice-cream, milk powders, pharmaceutical products and for the production of galacto-oligosaccharides which can be used as a low calorie sugar (Yang & Silva, 1995).

NutrifenPlus®, with active ingredient Fenugreek, supports the previous finding by Chilliard *et al*. (2006) which suggests that using a natural feed additive can increase milk lactose content. The milk lactose profile is illustrated and presented in Figure 3.1. Differences in lactose content in the milk were found to be significant at weeks 4, 5 and 11. Apart from anecdotal claims that Fenugreek supplementation results in “sweeter” milk, no documented studies were found to support the claims as such. The total solids content of milk did not differ between the three treatment groups. When Fenugreek was added to the specific diets it showed to have no effect on SCC found in the milk.

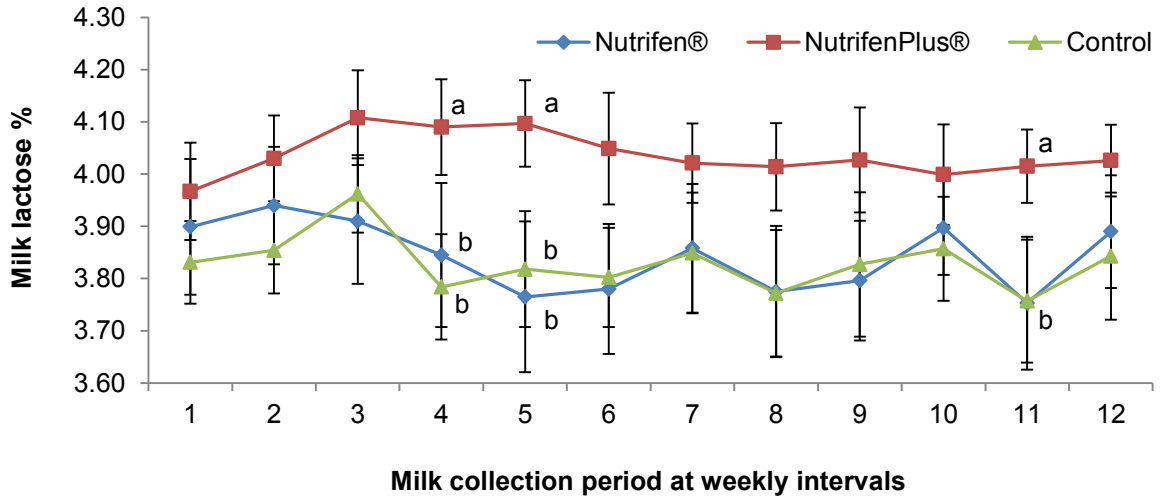

Figure 3.1: Mean lactose content of milk collected from dairy goats (n=10) that received different Fenugreek supplements over 130 days experimental period. Different superscripts indicate differences between treatments, P<0.05.

An increase in neutrophils is a good indication of intra-mammary infection (Zeng & Escobar, 1995). The condition is known as mastitis and can be predicted by the SSC. Milk somatic

cell counts are known to increase with parity and with stage of lactation (Paape *et al*., 2007) due to milk being diluted as lactation progresses (Paape *et al*., 2007). Goats used in the current study, had an age difference. This can explain why no significant differences were found between treatment groups. However, it should be noted that age exceeded the scope of this study.

Fenugreek supplementation had no effect on BUN between the three treatment groups and since BUN is highly correlated with MUN, it can be expected that MUN was not affected. This could lead to the hypothesis that Fenugreek would not impact on ruminal ammonia metabolization and thus utilization. MUN, as predicted, did not differ significantly ($P > 0.05$) between the three treatments.

The two week collection period was done as a separate trial and conducted in February, the warmest month of a year in the Western Cape region of Southern Africa. No treatment effects were observed during these two collection weeks when milk production was recorded twice daily. The effect of the different Fenugreek products on milk yield is presented in Table 3.4. No significant differences ($P = 0.61$) were observed in mean milk yield during this 15 day collection period. However, it was observed over the four month collection period as discussed previously where treatment with Nutrifen® resulted in an increase in milk yield compared to the control group ($P = 0.01$).

Table 3.4: Mean milk yield and total milk of dairy goats that received different Fenugreek products measured accurately over a 15 day collection period.

Item	Treatment			SEM	*P*
	Nutrifen®	NutrifenPlus®	Control		
Milk yield 15 day period, kg/d (n=48)	4.08	3.98	3.91	0.12	0.61
Total yield 15 day period, kg (n=48)	61.26	58.97	58.47	1.07	0.53

The effect of the different treatments on low density lipoprotein (LDL) levels (m*M*/L) found in blood over a period of 130 days is presented in Table 3.5. Although no differences ($P = 0.49$) were found between the different treatments, it is noticeable that all three treatment groups showed a decrease in LDL from the first month compared to the last month. This can be good in terms of healty products, but inconclusive as there was no significant difference between the three groups. The Nutrifen® and NutrifenPlus® treatments showed a greater decrease (0.887 m*M*/L, 0.772 m*M*/L and 0.914 m*M*/L) in LDL when compared between months one and four.

Table 3.5: Low density lipoprotein (LDL) levels over a period of time in goats receiving different treatments.

LDL cholesterol measurement	Treatment			SEM
	Nutrifen® (m*M*/L)	NutrifenPlus® (m*M*/L)	Control (m*M*/L)	
Month 1	1.198	0.916	1.042	0.18
Month 2	1.244	0.975	1.632	0.55
Month 3	1.146	0.898	1.131	0.26
Month 4	0.887	0.772	0.914	0.09

Results found on the high density lipoprotein (HDL) are illustrated in Figure 3.2. Both the Nutrifen® and NutrifenPlus® treatments showed a significant decrease (P = 0.04) in HDL, at the measurement done in month 3, when compared to the control treatment (Figure 3.2). However, there were no significant differences found between the Nutrifen® and NutrifenPlus® treatments. It is also noticeable that HDL decreased for all treatment groups from the first month of measurement up until the measurement taken in the last month.

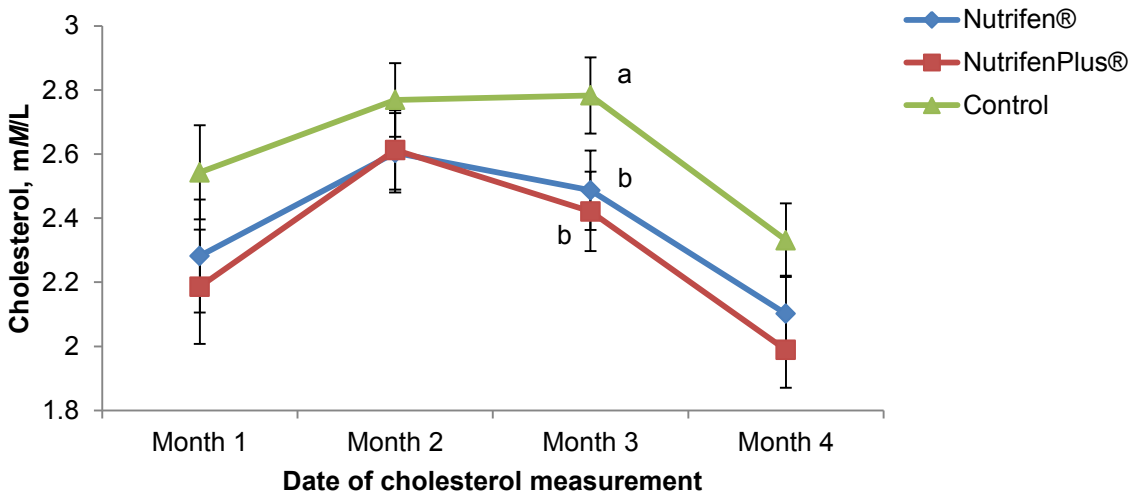

Figure 3.2: Levels of high density lipoprotein (HDL) over a period of time for dairy goats receiving different treatments.

Although a significant difference was found in month three (Figure 3.2) when HDL was analysed, there were no further differences found between treatments when measuring either LDL or HDL. This is with agreement to a study done by Al-Shaikh *et al*. (1999a), who found no differences in cholesterol levels in dairy goats that received Fenugreek as a feed additive in their diet.

Blood cholesterol is known to increase when different feed sources such as fat and oils are added to the diet (Beynen *et al*., 2000). In the current study no differences were found

between treatment groups for LDL and this could be explained as no added sources of fat or oils were used in the different treatments. The feed additives, Nutrifen® and NutrifenPlus® used in the current study does not contain any significant amount of fat or oils (0.9g/100g) which could ultimately lead to an increase in cholesterol levels, in blood plasma. Cholesterol levels, therefore did not change because no alteration was made to their diet apart from adding the natural additives.

Blood cholesterol is the main precursor for cholesterol found in milk (Shah & Mir, 2004a). Lowering the blood cholesterol will therefore have a positive effect on lowering the milk cholesterol and could therefore lead to products that will be healthier for consumers. This claim is supported by Shah and Mir (2004a) who conducted a study on dairy cows and showed that blood cholesterol can be reduced when cows receive Fenugreek seeds in their diet, which ultimately led to a decrease in milk cholesterol. It is possible that future studies with dairy goats can result in different findings using natural feed additives such as Fenugreek to lower the milk cholesterol content.

Conclusion

When used as a supplement, Nutrifen® showed promising results in terms of successfully increasing the milk production of dairy goats; whereas NutrifenPlus® appeared to stimulate milk lactose content. These results warrant further research. It was concluded that Nutrifen® and NutrifenPlus® as Fenugreek supplements have beneficial effects on milk production and milk composition of dairy goats.

The researchers reject the H_0 which stated that "*Fenugreek as a feed additive will not affect milk production"* and accept the H_1 that "*Fenugreek as a feed additive will affect milk production".* The same was concluded as the H_0: *"Fenugreek as a feed additive will not affect milk composition"* is rejected and the H_1: *"Fenugreek as a feed additive will affect milk composition"* was accepted.

A closer look at the secondary objective of this current study to evaluate the effect of Fenugreek on the cholesterol content in dairy goat milk, led the researchers to accept the H_0: *"Fenugreek as a feed additive will not affect milk cholesterol content"* and reject the H_1: "*Fenugreek as a feed additive will affect milk cholesterol content".*

It is difficult to speculate the mechanism on how Fenugreek exerts its effect on increasing milk production by just looking at the blood parameters studied. Further research is needed to investigate the mode of action of Fenugreek to better understand the mechanisms involved with increasing milk production. This objective is addressed in Chapter 4.

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It is questionable that the components found in Fenugreek, such as saponins, may have an effect on digestibility in the gastro-intestinal tract of the animal, making certain nutrients more available to the animal which can directly influence milk production. It is further hypothesised that the milk response observed could be related to effects on GH production and will subsequently be investigated in Chapter 5.

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Chapter 4

Fenugreek as a natural feed additive fed to dairy goats and its effects on feed digestibility.

Abstract

The aim of this study was to compare the effect of two natural feed additives on the digestibility and rumen degradability of a standard ration in dairy goats. Three treatment groups consisting out of eight goats each were randomly compiled from male dairy goats and fitted with faecal collecting harnesses and urine collecting funnels that allowed for quantitative collection of faeces and urine respectively. Goats were maintained on a diet of lucerne and oat hay ad libitum. Two commercial Fenugreek products, formulated differently, both containing cotyledon concentrate were used in this study. Nutrifen® and NutrifenPlus® are supplements that contain Fenugreek, which enhances milk production in dairy animals. Each group were allocated a different ration; control group (no supplement), which received a regular ration; Nutrifen® group, which received the normal ration plus 60 grams of Nutrifen® daily and a NutrifenPlus® group, which received the normal ration plus 60 grams of NutrifenPlus® daily. Ether extract (EE) digestibility for the Nutrifen® treatment group differed significantly (P = 0.02) from the NutrifenPlus® group, but not the control group. Total digestible nutrients (TDN) did not differ between treatments. Dry matter intake (DMI) was the highest for the Nutrifen® group and therefore had a higher energy intake and resulted in the best energy retention, compared to the control group. The same was observed for N retention, where the NutrifenPlus® and the control group showed the lowest N retention and where Nutrifen® had the highest N retention. The in vitro results support the in vivo results that no differences were observed for apparent DM digestibility between the three different treatments. The results from this current study will help us to better understand Fenugreek's effect as a natural feed additive on digestibility of nutrients and ultimately Fenugreek's effect on milk production.

Keywords: Apparent digestibility, Energy retention, *In vitro* digestibility, *In vivo* digestibility, Nitrogen retention, Rumen degradability

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Introduction

Animals in an intensive production system, especially animals producing milk, are in need of a balanced feed source that provides adequate amounts of the correct nutrients in order for animals to sustain production. These animals not only need adequate amounts of nutrients but they also need to be able to degrade and digest the feed as well as absorb most of the nutrients, to meet their requirements. It is only when the requirements are met, that excess nutrients can be used for production.

The challenge exist for nutritionists to find methods and products, not only in the form of additives, but other ways as well to assist with digestion inside the animal, making more nutrients available for microbial degradation, or even by-pass products which can escape rumen degradation and be absorbed by the rest of the gastro intestinal tract. Feed cost make up the most input cost to produce milk in a production system. It is therefore a necessity to introduce products such as feed additives that can assist in helping animals produce more at a lower cost input (Hutjens, 1991).

A big variety of products exist which are added to animal feeds to assist with nutrient digestibility as well as nutrient absorption. Products act through various actions and pathways by acting positively on feed digestion and therefore enhance production. These additives are unique in their content and exert their action through different mechanisms. Despite their diversity, these products ultimately affect rumen fermentation, metabolic pathways and (or) the digestive microbial ecosystem (Jouany & Morgavi, 2007).

Antibiotics is well known in the animal feed industry as feed additives, but the use of antibiotics in animal feeds raised awareness amongst consumers and in some cases led to the ban of these substances in some countries (Pugh, 2002). Researchers and farmers had to come up with ways to increase sustainable production without the use of negative substances that could raise consumer awareness.

It has been considered that additives such as antibiotics will eventually be replaced by natural compounds, which are sustainable and have a positive effect on the environment (Tekeli *et al*., 2007). A trend towards a more natural animal production system has led researchers to explore the benefits of adding products from plant origin, which has positive responses in animals, therefore contributing to sustainability (Tekeli *et al*., 2007).

A more natural way of enhancing performance in farm animals led to encouragement of using natural plant metabolites and other plant extracts to achieve this. These natural occurring plant products have been used as feed additives for the last decade to promote

animal health which led to healthier products in turn (Tekeli *et al*., 2007). Healthier products contain advantages and the use of natural feed additives have positive effects on products, such as reducing cholesterol in milk (Tekeli *et al*., 2007). These products are preferred by consumers struggling with health problems.

Feed additives are popular for their use in animal feeds as ingredients that are responsible for positive responses in animals, which are not from nutrient origin. Additives can be added to feed as dried plants or seeds as well as extracts (Tekeli *et al*., 2007). Feed additives' mode of action usually includes pH shifts, growth and metabolic modifiers (Hutjens, 1991). Products used as feed additives not only act on appetite and as a digestion stimulant, but it can also exert an effect on physiological functions, but moreover act to sustain good health amongst animals therefore promoting welfare and improve animal performance (Tekeli, *et al*., 2007).

It should be noted that although feed additives have a positive response in animals, it is neither a requirement nor a guarantee that all animals, if any, would respond the same way to a certain product (Hutjens, 1991). The response in animals and effect of active compounds in plants and plant extracts depends largely on the dosage provided in the feed given to animals (Tekeli *et al*., 2007). It is also a common misconception that plant products as well as extractions are safe because they are naturally or organically produced (Tekeli *et al*., 2007).

Fenugreek has been shown to have a positive effect on lactation performance in ruminants such as dairy cows, water buffaloes and dairy goats (El-Alamy *et al*., 2001; Kholif & El-Gawad, 2001). Research done on Fenugreek is not well known and the mechanism by which Fenugreek increases milk yield still remains unclear.

Fenugreek also seems to play a beneficial role in digestion and absorption of lipids by enhancing bile acid synthesis in the liver (Frankič *et al*., 2009). Leguminous plants, such as Fenugreek, are rich in saponins (Mir, 1997) and they are found in both leaves and seeds (Wina *et al*., 2005). Diocin is a natural saponin found in Fenugreek and it is possible that saponins found in Fenugreek lowers lipids because these saponins are transformed in the gastrointestinal tract into sapogenins (Smith, 2003). Saponins, found in seeds of Fenugreek have a bitter taste and the nutritional value of these seeds can be further improved and the bitterness reduced through processing methods (Hooda & Jood, 2003).

Two commercial Fenugreek products, formulated differently but both containing Fenugreek cotyledon concentrate, were selected for this study. The products were Nutrifen® (Fenugreek cotyledon concentrate) and NutrifenPlus® (Fenugreek cotyledon concentrate

with other ingredients). As shown in Chapter 3, Fenugreek had an effect on milk production but the mechanism is still not clearly understood. In this chapter the researchers explored the hypothesis that Fenugreek may alter rumen degradation and digestion resulting in more nutrients available to the animal and therefore producing more.

The main objective of the current study was to evaluate the effect of Fenugreek on nutrient digestibility in dairy goats.

H_0: *Fenugreek as a feed additive will not affect total tract digestibility.*

H_1: *Fenugreek as a feed additive will affect total tract digestibility.*

A secondary objective of this study was to evaluate the effect of Fenugreek on rumen degradation.

H_0: *Fenugreek as a feed additive will not affect rumen degradation.*

H_1: *Fenugreek as a feed additive will affect rumen degradation.*

Materials and Methods

Ethical clearance for animal use

This study complied with accepted standards for the use of animals in research and teaching as reflected in the South African National Standards 10386: 2008; and was completed with ethical clearance from Stellenbosch University Care and Use Committee (SU ACUC), reference number: SU-ACUM12-00033.

Animals and experimental design

Twenty four intact buck dairy goats, consisting predominantly of Saanen goats was obtained from the farm, Fairview, Paarl in the Western Cape region of South Africa and used during the investigation of this particular study. Male goats were used in this trial for the ease of collecting faeces and urine samples. Twenty four goats were used in order to have sufficient degrees of freedom for the statistical analysis (n = 8 per treatment). The goats were transported from the farm Fairview to the University of Stellenbosch's experimental farm, Welgevallen. All dairy goats were weighed and initial body weight recorded on arrival at the experimental farm. The average initial body weight (BW) was 53.31 ± 2.77 kg. Goats were housed separately and randomly allocated into individual pens (1 m x 2 m) and was fed indoors, in an adequately ventilated shed with wooden slatted floors. Limitations to the amount of faecal bags and urine funnels led to the division of goats into two main treatment groups A and B. Each group were subdivided into three separate treatment groups of equal

ages. Each subdivided group were randomly assigned to one of two treatments with the third, a control group. The trial was then repeated for group B.

Forages and diet characteristics

Goats were maintained on the regular feeding and management program of the Fairview Farm (Paarl, Western Cape), where they received a 50:50 mixture of lucerne and oat hay. The goats also received a semi-complete formulated commercial feed (Meadow Feeds Lactating Goat Feed), supplied by Meadow Feeds (Paarl, South Africa) and offered at 400 g/goat per day. The semi-complete feed served as the basal diet. The chemical composition of the concentrated feed is presented in Table 4.1.

Table 4.1: Chemical composition of the semi-complete commercial goat feed as is.

Chemical composition	Amount (g/kg)	Inclusion
Protein *(11.48% derived from [1]NPN)*	150.0	Minimum
Moisture	120.0	Maximum
Fat	25.0	Minimum
Fat	70.0	Maximum
Fibre	120.0	Minimum
Fibre	200.0	Maximum
Calcium	10.0	Maximum
Phosphorus	4.5	Minimum
Urea	6.0	Maximum

[1]NPN - Non protein nitrogen

Treatments

Two commercial Fenugreek products, both containing cotyledon concentrate but formulated differently, were used in this study. Nutrifen® and NutrifenPlus® are supplements which enhance milk production in dairy animals. The composition of the two treatments was as follows:

i. Nutrifen®
    a. Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*)

ii. NutrifenPlus®
    a. Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*)

b. Fennel seed (*Foeniculum Vulgare*)
c. Saw Palmetto Berries (*Serenoa Repens*)
d. Brown Kelp *(Laminariales*)
e. MSM (natural source Methylsulfonylmethane)
f. White distilled vinegar powder

The Control group received the regular basal diet with no additive, while the Nutrifen® group received the basal diet plus 60 g of Nutrifen® per goat/day and the NutrifenPlus® group received the basal diet plus 60 g of NutrifenPlus® per goat/day.

Digestibility trial

The *in vivo* trial was conducted in accordance to the description by McDonald *et al*. (2002). Goats were adapted for a period of 14 days onto the various treatments, prior to the trial. The experimental period was conducted over a period of seven days where faeces and urine were collected twice daily, at 08:00 and 16:00 to minimize losses. A representative sub-sample of faeces (10%) and urine (5%) were collected and weighed accurately from each individual goat after the total amount of faeces and urine was measured. Samples were taken twice daily and pooled together with samples taken at the rest of the experimental period to ensure a more representative sample throughout the trial. Representative faecal and urine samples were frozen until later analysis were done in the laboratory. Excess faeces and urine, which were left over after measurements, were disposed of.

Goats had access to clean water and feed every day during adaptation as well as during the experimental period of the trial. The goats were fed twice daily to minimize feed losses. An allocated amount of feed containing hay, a pelleted feed (Meadow Feeds Lactating Goat Feed) and specific treatment (Nutrifen®/NutrifenPlus®) were hand mixed and given to the goats, at 08:00 and 16:00 and feed refusals were weighed back prior to the next feeding. Goats were weighed upon arrival and before the start of the experimental period as well as at the end of the experimental period to determine any drastic changes in weight that might occur.

Methane gas production (MJ/day) was calculated as 8% of the gross energy intake as described by McDonald *et al*. (2002). Nitrogen retention had to be corrected for both endogenous urinary N (EUN) and metabolic faecal N (MFN) and was done as described by McDonald *et al*. (2002):

EUN (g) = 0.18 g N/kg $BW^{0.75}$/day
MFN (g) = 5 g N/kg dry matter intake
N retention (g N/kg $BW^{0.75}$/day) = [N_{intake} – (N_{faeces} – MFN) – (N_{urine} – EUN)]/$BW^{0.75}$/days

Substances excreted in faeces that are not of food origin leads to an underestimation of the proportion of food that is actually absorbed by the animal. For this reason it should be noted that all digestible values mentioned in this chapter are apparent digestibility coefficients and not true digestibility coefficients (McDonald *et al*., 2002).

Analytical Methods

Feed and Faecal analyses

Faeces samples were dried in an oven at 60^0C over a 96h period, air-equilibrated and weighed. Feed and faeces were ground through a 2 mm screen with a Scientec Hammer mill (Scientec, Cape Town, RSA) and analysed according to the AOAC International (2002) official methods of analysis (17^{th} edition) for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF) and gross energy (GE) content.

Moisture

Moisture content of the samples was determined as prescribed by the AOAC (2002a) method 934.041. Labelled porcelain crucibles were thoroughly washed with warm, soapy water and left in a 100^0C oven for two hours (or overnight) to dry. Crucibles were removed from the oven and placed into a desiccator to cool down for 30 minutes. Moisture free crucibles were removed out of the desiccator and placed on a four decimal accurate scale to determine individual crucible weight. The scale was zeroed and a feed or faecal sample of 2 g were accurately weighed into the crucible and recorded to the fourth decimal. Crucibles containing the feed/faecal sample were then placed in a 100^0C oven for 24 hours to dry. Once more the crucibles were placed in a desiccator to cool down for 30 minutes and then weighed back with the weight recorded of the dried sample.

Ash

Ash content of the samples was determined as prescribed by the AOAC (2002b) method 942.05. Two grams of moisture free sample was weighed into a moisture-free porcelain crucible and the weight recorded. The crucibles were then placed into a furnace set at 500^0C for six hours. After six hours, the furnace was switched off and the crucibles were allowed to cool down inside the furnace for two hours before the crucibles were taken out and placed in a desiccator for 30 minutes before weighing back. The weight of the dried sample was recorded to the fourth decimal.

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Crude Fibre (CF)

Feed and faecal crude fibre (CF) content was determined using the method described by Robertson and van Soest (1991) with the aid of a Velp Fibre Extractor (VELP Scientifica, Via Stazione, 16 20865 Usmate Velate (MB); Milan, Italy) instrument.

An acid solution (solution 1) consisted out of 0.128 M H_2SO_4 and was made up to 1 L with distilled water. A second alkali solution (solution 2) consisted of 0.313 M NaOH and was also made up to 1 L with distilled water.

Sentered glass crucibles were washed with warm water and placed overnight in a 100^0C dry oven to remove all moisture. Crucibles were removed from the oven and placed into a desiccator to cool down for 30 minutes. The scale was zeroed and a feed or faecal sample of 1 g were accurately weighed (WS) into the sentered glass crucibles and recorded to the fourth decimal. Crucibles containing the samples were carefully placed onto the instrument and 150 ml of solution 1 was added and heated to 100^0C until boiled where after the temperature was reduced to 65^0C.

The samples were left to boil for 30 minutes. Samples were washed three times with boiling distilled water and solution 2 was added and left to boil another 30 minutes. At the end, crucibles were rinsed out with acetone to ensure the entire sample collected in the crucibles.

Crucibles containing the feed/faecal sample were then placed in a 100^0C oven for 24 hours to dry. After 24 hours, the crucibles were removed from the oven and placed into a desiccator to cool down for 30 minutes. Moisture free crucibles containing the samples were removed out of the desiccator and placed on a four decimal accurate scale to determine individual crucible weight (W1). The crucibles were then placed into a furnace set at 500^0C for six hours. After six hours, the furnace was switched off and the crucibles were allowed to cool down inside the furnace for two hours before the crucibles were taken out and placed in a desiccator for 30 minutes before weighing back (W2).

Neutral Detergent Fibre (NDF)

Feed and faecal neutral detergent fibre (NDF) content was determined using the method described by Robertson and van Soest (1981) with the aid of a Velp Fibre Extractor (VELP Scientifica, Via Stazione, 16 20865 Usmate Velate (MB); Milan, Italy) instrument.

A neutral detergent solution (NDS) was prepared by dissolving 30 g of Sodium-lauryl-sulphate in 500 ml of distilled water with the addition of 10 ml 2-ethoxytehanol. This solution was stirred with a magnetic stirrer until all particles have dissolved. In a different beaker, a solution was made up of 18.61 g of EDTA ($Na_2EDTA.2H_2O$) and 6.81 g of Sodium-borate

decahydrate ($Na_2B_4O_7.10H_2O$) with 200 ml of distilled water and heated to dissolve all particles where after it was added to the Sodium-lauryl-sulphate solution. Another solution was prepared by dissolving 4.56 g of Di-sodium-hydrogen-phosphate (Na_2HPO_4) in 100 ml of distilled water and then added to the mixture. The beaker containing the mixture was then made up to 1 L with distilled water.

Sentered glass crucibles were washed with warm water and placed overnight in a 100^0C dry oven to remove all moisture. Crucibles were removed from the oven and placed into a desiccator to cool down for 30 minutes. The scale was zeroed and a feed or faecal sample of 1 g were accurately weighed (WS) into the sentered glass crucibles and recorded to the fourth decimal. Crucibles containing the samples were carefully placed onto the instrument and 100 ml of cold NDS was added and heated to 100^0C until boiled where after the temperature was reduced to 65^0C. 100 ml of α-amylase (Sigma #A3306) was also added. The samples were left to boil for one hour. Samples were three times washed with boiling distilled water and at the end rinsed out with acetone to ensure the entire sample collected in the crucibles.

Crucibles containing the feed/faecal sample were then placed in a 100^0C oven for 24 hours to dry. After 24 hours, the crucibles were removed from the oven and placed into a desiccator to cool down for 30 minutes. Moisture free crucibles containing the samples were removed out of the desiccator and placed on a four decimal accurate scale to determine individual crucible weight (W1). The crucibles were then placed into a furnace set at 500^0C for six hours. After six hours, the furnace was switched off and the crucibles were allowed to cool down inside the furnace for two hours before the crucibles were taken out and placed in a desiccator for 30 minutes before weighing back (W2).

The percentage NDF was calculated using the following formula:

$$a = \frac{[W1\ (g) - W2\ (g)]}{WS\ (g)} \times 100$$

Where:

a = neutral detergent fibre (%)
W1 = moisture free crucible containing the sample
W2 = moisture free crucible containing the sample after ash
WS = sample weight

Ether extract

An Ether extract (EE) method, prescribed by the AOAC (method 920.39, 2002d) was used to measure fat percentage within the feed samples. The Tecator Soxtec System HT 1043 Extraction unit was used with diethyl ether as reagent.

Method for Fat (crude) or Ether extract in animal feed:

i. Clean aluminium cups (soxhlet flasks) was placed overnight in a 100^0C dry oven to remove all moisture. The aluminium cups are then placed in a desiccator for 30 minutes to cool down.
ii. The weight of the aluminium cups were recorded to the fourth decimal on an accurate scale.
iii. Two grams of dry feed or faecal sample were weighed into a thimble and the weight recorded. A small piece of defatted cotton-wool was placed inside the thimble to prevent any sample from spilling out during the extraction process.
iv. Aluminium cups were filled with 50 ml of diethyl ether.
v. The water tap was opened before switching on the heat. The oil bath and cooling system is also switched on.
vi. When the “ready” light show the thimbles can be placed in the extraction tubes in the correct position and the soxhlet cups on the Tecator Soxtec’s element with the corresponding thimble. The Tecator Soxtec’s handle was pulled down thus sealing the joints of the extraction apparatus.
vii. Thimbles were lowered into the diethyl ether and left to boil for 15 minutes.
viii. Thimbles were then lifted from the boiling ether and left to rinse for 30 minutes.
ix. The small valves were then closed to capture and collect the ether for 15 minutes.
x. The aluminium cups were then removed from the apparatus and placed into a 100^0C dry oven for two hours to allow all ether to evaporate.
xi. Aluminium cups were then placed in a desiccator for 30 minutes to cool down.
xii. The cooled, moisture-free aluminium cups were then weighed and the weight recorded.

Fat content was then calculated as percentage of DM.

Crude protein

Crude protein (CP) content of samples was determined by the Dumas combustion method 990.03 prescribed by AOAC (2002c).

Apparatus: LECO-FP528, Protein/Nitrogen Analyzer (Leco® Corporation, St. Joseph, USA)

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Accessories:	502-186 Tin Foil Cups
Sample weight:	0.1 g
Calibration Standard:	Alfalfa
Furnace temperature:	850^0C
Protein Factor:	6.25

It is important to calibrate the Leco® before starting with analysis. Refer to manufacture instructions while completing the appropriate actions needed in order to calibrate the Leco®. The samples were weighed accurately and placed in a tin foil cup (tin foil cup has been zeroed on an accurate scale) and folded in the shape of a teardrop where after the final weight was recorded. The shape however, should be able to easily pass and fall into the Leco® without any obstructions. The samples are then placed into the Leco® and analysed for nitrogen %.

The crude protein (CP) content was calculated as percentage of DM as follows:

$$\% CP = \% N \times 6.25$$

Gross Energy

Gross energy of the feed and faeces samples was determined using an IKA C2000 basic Bomb Calorimeter System (IKA Works, Inc., 2635 North Chase Pkwy SE, Wilmington, NC 28405-7419 www.ika.net).

Samples were accurately weighed off on a scale and pressed into a pill of (± s.d.) 0.4 g ± 0.1 g. The samples were carefully placed on a melting wire attached to two electrode points and suspended above a crucible that forms part of the electrode. The electrode was placed into a bomb. The bomb was sealed properly and filled up with oxygen until a pressure of 3000 kPa was reached. The bomb was then carefully placed in the Bomb Calorimeter and afterwards filled with water. The sample weight was put into the Bomb Calorimeter and combustion took place after ignition. Results obtained were given in MJ/kg as is.

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In vitro DM digestibility (IVDMD) and Ankom *in vitro* true digestibility (IVTD)

Experimental design

The three experimental diets, Nutrifen®, NutrifenPlus® and the control diet, were placed in a 60º - 80ºC oven for 24 hours to dry. The feed samples were ground through a 2 mm screen with a Scientec Hammer mill (Scientec, Cape Town, RSA) and subsequently sieved through a 106 µm screen to remove all dust and small particles which could escape from the filter bags during the incubation period which can lead to an over estimate of results (Cruywagen *et al*., 2003).

Each treatment were accurately measured into triplicate F57 filter bags (Ankom® F57 filter bag; Ankom® Technology Corp., Fairport, NY, USA) allowing for triplicate measurement of each treatment. This led to nine sample filled bags together with a blank bag that resulted in ten bags per incubating jar. Six incubating jars were used to ensure an experimental repetition of six times.

Rumen samples

Rumen inoculum was collected from six different donor cannulated dairy cows on the experimental farm Welgevallen (Stellenbosch, South-Africa). The diet of each cow consisted out of 26 kg Scientific Bovine Semi Complete (R1109P) and 3 - 4 kg of Lucerne hay. Ingredients for the semi complete feed included processed sunflower oilcake, blood meal, maize, barley, wheat bran, dried apple pulp, molasses, lucerne, urea and ammoniated wheat straw. The chemical composition of the semi complete feed is given in Table 4.2. Rumen inoculum were squeezed through two layers of cheesecloth and poured into preheated (39°C) thermo flasks. The thermo flasks containing the rumen fluid were transported to the *in vitro* laboratory of the Department of Animal Sciences (Stellenbosch University, South Africa), immediately after the inoculum was obtained.

Table 4.2: Chemical composition of the semi complete commercial dairy feed on a dry matter basis.

Chemical composition	Amount (%)
Crude protein (CP)	14.3
Undegraded protein (UDP)	
**as a percentage of CP*	31.5
Energy	9.8
Calcium	2
Phosphorus	2
Urea	0.5

Method and buffer solution

The method used in this trial in determining the *in vitro* true digestibility of the different experimental treatments, was that of Ankom® DAISY^{II} *in vitro* fermentation system (Ankom® Technology Corp., Fairport, NY, USA) and done according to the protocol as described by the manufacturers. Slight alterations were made to this specific study conducted. The buffer solution described in the Ankom® protocol was modified and based on the buffer solution described by Goering & Van Soest (1970).

The components found in the buffer solution described by Goering & Van Soest (1970) can be found in Table 4.3.

Sufficient quantities of the rumen buffer solution, macro-mineral and micro-mineral solution were prepared in advance just before commencement of the experiment. Care was taken with the micro-mineral solution as to its' UV-sensitivity and was therefore placed in a dark glass bottle to ensure the quality of the solution. Solutions were mixed together according to their appropriate amounts as shown in Table 4.3. Trypticase as well as prepared resazurin were added to the mixture on commencement of the experiment.

The correct amount of deionised water, buffer solution, macro and mineral solutions were mixed with trypticase and previously prepared resazurin. The reducing agent were freshly prepared and only added to the mixture once all the chemicals had dissolved. Rumen inoculum collected, were squeezed through two layers of cheesecloth and poured into previously heated (39°C) thermo flasks. Thermo flasks were filled to the brim and capped as quickly as possible to ensure anaerobic conditions. Rumen inoculum containing thermo flasks were transported to the *in vitro* laboratory (Department of Animal Sciences, Stellenbosch, South Africa), immediately after inoculum was obtained.

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Table 4.3: Composition of the buffer solution used for the *in vitro* digestibility trial (Goering & Van Soest, 1970).

Reagent	Per litre
Rumen buffer solution	
Deionized water	2.0 L
NH_4HCO_3	8.0 g
$NaHCO_3$	70.0 g
Macro-mineral solution	
Deionized water	2.0 L
Na_2HPO_4 (anhydrous)	11.4 g
KH_2PO_4 (anhydrous)	12.4 g
$MgSO_4.7H_2O$	1.17 g
Micro-mineral solution	
Deionized water	100 ml
$CaCl_2.2H_2O$	13.2 g
$MnCl_2.4H_2O$	10.0 g
$CoCl_2.6H_2O$	1.0 g
$FeCl_3.6H_2O$	8.0 g
Reducing solution	
Deionized water	48 ml
Cysteine hydrochloride	312 mg
1 N NaOH	20 ml
$Na_2S.9H_2O$	312 mg
Complete buffer medium	
Deionized water	500 ml
Rumen buffer solution	250 ml
Macro-mineral solution	250 ml
Resazurin (0.2% w/v)	2 ml
Micro-mineral solution	0.12 ml
Trypticase	1.25 g
Reducing agent	53 ml

A preheated industrial blender ensured minimal losses to microorganisms, when rumen inoculum was added to the industrial blender to separate microorganisms from other rumen materials. After rumen inoculum was added to the industrial blender, the surface was purged with CO_2 to ensure anaerobic conditions. The blended rumen inoculum collected and separated from other rumen materials (400 ml per jar) was poured into Ankom® DAISY^{II}

incubator jars and purged with CO_2 for a time period of 30 seconds. Rumen inoculum was mixed with 1600 ml of pre-heated (39°C) reduced buffer solution as described by Goering and Van Soest (1970), with slight moderations.

As described by the manufactures, F57 filter bags (Ankom® F57 filter bag; Ankom® Technology Corp., Fairport, NY, USA) were used in this experiment and pre-rinsed with acetone to remove the layer of surfactant covering the bag which would otherwise prevent microbial entering into the bag and inhibit sample digestion. F57 filter bags were dried in a 100^0C oven for 24 hours before weighed. The moisture free bags' weight was recorded where after a feed sample of 0.5 g ± 0.05 was individually and accurately weighed into a filter bag and heat sealed with an impact heat sealer (Ankom® 1915/1920 Heat Sealer; Ankom® Technology Corp., Fairport, NY, USA). A blank bag was also prepared, containing no sample, which served as a correction factor which indicated any changes in weight that might occur during the incubation process due to microbial activity. The sealed bags were placed and evenly distributed inside the Ankom® DAISY^{II} on both sides of the separator inside the jar to incubate for 48 hours. Bags were removed 48 hours and washed in cold water to stop microbial activity and to remove any microbial debris left.

The filter bags were then placed in a 60^0C oven and allowed to dry for 72 hours. After the bags were completely dry, the weight was recorded to determine the DM disappearance of the filter bags. The values obtained were used in calculating *in vitro* DM digestibility. The NDF procedure was done after DM weight was recorded using the ANKOM$^{200/220}$ (Ankom® Technology Corp., Fairport, NY, USA) Fibre Analyzer as described by the manufacturers to allow for the calculation of IVTD. The calculations used to determine both values are shown below:

Ankom *In vitro* dry matter digestibility (%)

$$a = 100 - (\frac{b - \left(c \times \frac{d}{e}\right)}{f} \times 100)$$

Where:

- *a* = *in vitro* dry matter digestibility (%)
- *b* = dried bag weight (post-incubation) (g)
- *c* = original bag tare weight (g)
- *d* = blank bag weight (post-incubation) (g)
- *e* = blank bag tare weight (g)
- *f* = sample dry matter weight (g)

In vitro true digestibility (%)

$$a = 100 - \frac{b - (c \times d)}{e \times DM} \times 100$$

Where:

a = *in vitro* true digestibility (%)
b = final dried bag weight and NDF treatment (post-incubation) (g)
c = original bag tare weight (g)
d = blank bag correction (post-incubation) (g)
e = sample dry matter weight (g)

Statistical Analysis

The mean and standard error for the chemical compositions as well as for the energy- and nitrogen retention were calculated. Feed digestibility data were subjected to a one way ANOVA, using Statistica version 10 (Statsoft Data Analysis Software System, 2011). The assumptions of homoscedasticity and normality were also tested on the data. Least square means were separated with a Bonferroni test and significance was declared at $P < 0.05$.

The *in vivo* data were analyzed using Statistica version 10 (Statsoft Data Analysis Software System, 2011). The assumptions of homoscedasticity and normality were tested on the data and found the data was not normally distributed. Transformation of the data did not improve normality and therefore Bootstrap data was subjected to a one way ANOVA procedure of Statistica version 10. Significance was declared at $P < 0.05$.

Results and Discussion

In vivo digestibility study

The results obtained from the *in vivo* digestibility study where Nutrifen®, NutrifenPlus® and a control were used as treatments, are presented in Tables 4.4, 4.5 and 4.6, respectively. The results were subjected to calculations and then compared in Table 4.7. Calculations were made using the total amount of nutrients consumed and the total amount of nutrients excreted, together with proximate values obtained in the laboratory, to determine nutrient digestibility as described by McDonald *et al*. (2002). Digestibility coefficients for the three different treatments were also calculated and are presented in Tables 4.4, 4.5 and 4.6. Not only does Table 4.7 represent a comparative summary of the *in vivo* digestibility trial, but it also gives the chemical composition of the feed as well as the faeces. The apparent

digestibility (%) of the treatments and the total digestible nutrients (TDN) are also presented in Table 4.7.

Neutral detergent fibre (NDF) is used to evaluate digestion efficiency as NDF is the primary chemical component of feed that determines the rate of digestion (McDonald *et al*., 2002). In the current study the control treatment had the highest NDF fraction in the feed (408.9 g/kg) followed by the Nutrifen® and NutrifenPlus® treatments, which had the lowest fractions of NDF in the feed (364.3 and 363.4 g/kg, respectively). It was expected that the higher the NDF fraction, the lower the digestibility of the feed. However, there were no significant differences (P = 0.60) found between Nutrifen®, NutrifenPlus® or control treatments for dry matter (DM) digestibility (68.5%, 65.7% and 68.0%, respectively). This indicates that the dairy goats digested the various treatments with similar efficiency and suggests that the composition of microbial population was relatively similar (Lu *et al*., 2005).

Ether extract content was the only chemical component that had a significant difference (P = 0.02) where the control treatment did not differ from the other two treatments, but the Nutrifen® treatment had a higher digestibility (91.1%) compared to the NutrifenPlus® treatment (87.7%). Frankič *et al*. (2009) suggested that Fenugreek also seemed to play a beneficial role in digestion and absorption of lipids by enhancing bile acid synthesis in the liver. In the current study a significant difference (P < 0.01) was found in the faeces in terms of EE content, between treatments containing the Fenugreek, compared to the control treatment. The previous result can be supported by a study conducted by Muraki *et al*. (2011) which showed that Fenugreek given to rats can increase the amount of lipid excreted in the faeces.

Nutrifen® and NutrifenPlus® were supplements used in this study to supply Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*). However, the Nutrifen® and NutrifenPlus® treatments which both contain Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*), apart from each other, did not differ from the control treatment pertaining to apparent digestibility for EE. It would therefore be invalid to draw a conclusion on the results based on the current study. Furthermore it is known that ether-extractable substances are also found in faeces, partly originate from metabolic origin and not necessarily a direct result of digestion (McDonald *et al*., 2002).

The digestibility of organic matter, crude protein and total digestible nutrients, did not differ significantly between various treatments and it was noticed that treatment had no effect. It was expected that both crude fibre and NDF digestibility would not differ between treatments as the levels of fibre were not increased nor decreased in the different diets and treatments have not been reported before for such effects.

Table 4.4: Mean (±SD) *in vivo* digestibility (DM basis) results for dairy goats receiving the Nutrifen® treatment.

Nutrifen®	Dry matter	Organic matter	Crude protein	Crude Fibre	Ether extract	Neutral detergent fibre
Feed (g/kg)		926.0	193.6	205.7	40.1	364.3
Faces (g/kg)		869.2 ± 9.6	135.2 ± 13.4	355.1 ± 31.7	11.4 ± 1.5	636.9 ± 24.1
Total ingested (g/week)	5311.6 ± 667.4	4918.8 ± 618.0	1028.1 ± 129.2	1092.7 ± 137.3	213.2 ± 26.8	1935.1 ± 243.1
Total excreted (g/week)	1668.1 ± 390.7	1449.8 ± 339.4	222.6 ± 41.5	601.0 ± 180.1	18.7 ± 3.5	1066.5 ± 268.4
Digested (g/week)	3643.5 ± 625.7	3469.0 ± 570.6	805.6 ± 129.6	491.7 ± 168.4	194.4 ± 27.3	868.6 ± 271.4
Digestibility coefficients	0.68 ± 0.07	0.70 ± 0.06	0.78 ± 0.04	0.45 ± 0.14	0.91 ± 0.02	0.45 ± 0.12

Table 4.5: Mean (±SD) *in vivo* digestibility (DM basis) parameters for dairy goats receiving the NutrifenPlus® treatment.

NutrifenPlus®	Dry matter	Organic matter	Crude protein	Crude Fibre	Ether extract	Neutral detergent fibre
Feed (g/kg)		924.2	193.8	206.8	33.2	363.4
Faces (g/kg)		852.4 ± 13.0	143.3 ± 6.6	329.8 ± 14.6	11.9 ± 1.2	620.0 ± 21.7
Total ingested (g/week)	4822.5 ± 296.4	4457.0 ± 273.9	934.6 ± 57.4	960.2 ± 59.0	160.0 ± 9.8	1752.7 ± 107.7
Total excreted (g/week)	1651.5 ± 135.5	1408.6 ± 129.1	237.0 ± 26.6	544.9 ± 54.7	19.6 ± 2.1	1024.2 ± 96.1
Digested (g/week)	3171.0 ± 315.7	3048.4 ± 295.6	697.6 ± 62.7	415.3 ± 73.3	140.4 ± 10.9	728.4 ± 137.8
Digestibility coefficients	0.66 ± 0.03	0.68 ± 0.03	0.75 ± 0.03	0.45 ± 0.06	0.88 ± 0.02	0.41 ± 0.06

Table 4.6: Mean (±SD) *in vivo* digestibility (DM basis) parameters for dairy goats receiving the Control treatment.

Control	Dry matter	Organic matter	Crude protein	Crude Fibre	Ether extract	Neutral detergent fibre
Feed (g/kg)		917.8	193.4	220.9	26.4	408.9
Faces (g/kg)		852.5 ± 6.1	131.4 ± 5.2	342.1 ± 18.9	8.7 ± 2.9	628.0 ± 15.8
Total ingested (g/week)	4459.3 ± 452.9	4092.7 ± 415.6	727.5 ± 73.9	985.2 ± 100.1	117.6 ± 11.9	1823.4 ± 185.2
Total excreted (g/week)	1419.4 ± 320.4	1209.3 ± 269.6	186.0 ± 40.8	488.9 ± 125.1	12.0 ± 3.7	892.8 ± 204.8
Digested (g/week)	3039.9 ± 499.2	2883.4 ± 448.3	541.5 ± 77.9	496.4 ± 146.2	105.5 ± 10.8	930.7 ± 254.5
Digestibility coefficients	0.68 ± 0.07	0.70 ± 0.07	0.78 ± 0.06	0.50 ± 0.13	0.90 ± 0.03	0.51 ± 0.11

**Chemical composition of the semi-complete commercial goat feed can be found under the heading: Forages and diet characteristics.*

Table 4.7: Comparison between results of the *in vivo* digestibility trial from three different treatments given to dairy goats over a seven day period.

Feed chemical composition (g/kg)	Treatment		
	Nutrifen®	NutrifenPlus®	Control
Dry matter	880.6	879.6	881.2
Organic matter	926.0	924.2	917.8
Crude protein	193.6	193.8	193.4
Crude fibre	205.7	206.8	220.9
Ether extract	40.1	33.2	26.4
Neutral detergent fibre	364.3	363.4	408.9

Faecal chemical composition (g/kg)	Treatment			SEM	*P*
	Nutrifen®	NutrifenPlus®	Control		
Dry matter	959.1	959.8	961.4	1.66	0.85
Organic matter	869.2^{a}	852.4^{b}	852.5^{b}	2.55	< 0.01
Crude protein	135.2^{ab}	143.3^{a}	131.4^{b}	2.06	0.04
Crude fibre	355.1	329.8	342.1	4.96	0.11
Ether extract	11.4^{a}	11.9^{a}	8.7^{b}	0.49	< 0.01
Neutral detergent fibre	636.9	620.0	628.0	4.31	0.29

Apparent digestibility of the chemical constituents (%)	Treatment			SEM	*P*
	Nutrifen®	NutrifenPlus®	Control		
Dry matter	68.5	65.7	68.0	1.18	0.60
Organic matter	70.4	68.3	70.3	1.09	0.69
Crude protein	78.1	74.6	78.3	0.96	0.19
Crude fibre	45.1	45.2	50.1	0.48	0.47
Ether extract	91.1^{a}	87.7^{b}	89.8^{ab}	0.52	0.02
Neutral detergent fibre	44.8	41.4	50.7	2.15	0.21
Total digestible nutrients (TDN)	40.5	39.2	40.6	0.45	0.34

^{a,b,c} LS Means in the same row with different superscripts differ ($P < 0.05$)

Energy intake (MJ/day), excretion (MJ/day), retention (MJ/day) and metabolizable energy content (ME, MJ/kg) of the different treatments used in the *in vivo* digestibility study are presented in Table 4.8. The two treatments, Nutrifen® and NutrifenPlus®, apart from the control contain Fenugreek. Dry matter intake is very important in ruminant nutrition as more nutrients are available for production and other metabolic purposes when an animal ingest

more feed. Fenugreek has been shown in other species to increase the animal's appetite which results in an increase feed intake (Petit *et al*., 1993). Significant differences ($P < 0.05$) were found with DMI (g/day) and therefore differences ($P < 0.05$) were also observed for energy intake (MJ/day). Ghrelin is known to regulate feed intake as plasma ghrelin levels increase with an increase in feed intake (Roche *et al*., 2008). The increase in ghrelin levels is probably caused by physiological means to increase feed intake in response to a higher demand for energy by a high producing animal (Abizaid *et al*., 2008). Increased levels of plasma ghrelin are also known to increase levels of plasma growth hormone (Nass *et al*., 2008) and will subsequently be investigated in the next chapter.

Energy excreted in the faeces (MJ/day) showed no difference between treatments. Total energy excreted (MJ/day) had significant differences ($P < 0.05$) between treatments and the same was observed for energy retention (MJ/day) where different treatments had differed significantly ($P < 0.05$).

Table 4.8: Energy metabolism (mean) of dairy goats given three different treatments.

Item	Treatment			SEM	*P*
	Nutrifen®	NutrifenPlus®	Control		
Dry matter intake (g/day)	826.87[a]	751.67[ab]	694.96[b]	19.07	0.01
Gross energy (MJ/kg)	19.293	19.122	18.751		
Energy intake (MJ/day)	15.95[a]	14.37[ab]	13.03[b]	0.39	< 0.01
Faecal energy (MJ/day)	4.36	4.28	3.64	0.16	0.13
Methane gas production (MJ/day)*	1.28[a]	1.15[ab]	1.04[b]	0.03	< 0.01
Total energy excreted (MJ/day)	6.43[a]	6.15[ab]	5.33[b]	0.19	0.04
Faecal energy (% of energy intake)	27.43	29.89	28.04	0.97	0.58
Total energy excreted (% of energy intake)	40.43	42.89	41.04	0.97	0.58
Energy retention (MJ/day)	9.52[a]	8.22[ab]	7.70[b]	0.29	0.03
Energy retention (% of energy intake)	59.57	57.11	58.96	0.97	0.58
Metabolizable energy (MJ/kg)	11.49	10.92	11.06	0.19	0.45

[a,b,c] LS Means in the same row with different superscripts differ ($P < 0.05$)
Dry matter intake and secretion was quantified over a seven day period.
*Methane gas production was calculated as 8% of the gross energy intake.

Nitrogen metabolism and retention of the different treatments used in the *in vivo* digestibility study are presented in Table 4.9. Dry matter intake (g/day) and therefore nitrogen intake (g/day) as well as nitrogen intake expressed as g N/kg $BW^{0.75}$ /day all had significant differences ($P < 0.05$) between treatments tested. Nitrogen excreted in the faeces (g/day) differed significantly ($P < 0.05$) between treatments which indicate that nitrogen is

metabolised at different rates between treatment groups. No differences (P = 0.11) were found in the amount of nitrogen excreted (g/day) in the urine as well as no differences (P = 0.05) in the total amount of nitrogen excreted (g/day). However, total nitrogen excreted as a percentage of nitrogen intake showed to have significant differences (P < 0.05) between treatments used. Significant differences (P < 0.05) were found between treatments for nitrogen retention (g N/kg $BW^{0.75}$/day) as well as nitrogen retention as a percentage of nitrogen intake.

Table 4.9: Nitrogen metabolism (mean) of dairy goats given three different treatments.

Item	Treatment			SEM	*P*
	Nutrifen®	NutrifenPlus®	Control		
Dry matter intake (g/day)	826.87^{a}	751.67^{ab}	694.96^{b}	19.07	0.01
Nitrogen intake (g/day)	22.55^{a}	20.50^{ab}	15.98^{b}	0.70	< 0.01
Nitrogen intake (g N/kg $BW^{0.75}$ /day)	1.15^{a}	1.14^{ab}	0.85^{b}	0.05	0.01
Faecal nitrogen (g/day)	5.09^{ab}	5.42^{a}	4.25^{b}	0.19	0.03
Urinary nitrogen (g/day)	9.95	14.91	10.46	1.06	0.11
Total nitrogen excreted (g/day)	15.04	20.32	14.71	1.07	0.05
Faecal nitrogen (% of nitrogen intake)	22.81	26.51	26.74	0.99	0.19
Urinary nitrogen (% of nitrogen intake)	44.40	73.00	65.06	5.39	0.07
Total nitrogen excreted (% of nitrogen intake)	67.21^{b}	99.51^{a}	91.80^{ab}	5.41	0.03
Metabolic faecal nitrogen (g/day)	4.13^{a}	3.76^{ab}	3.47^{b}	0.10	0.01
Endogenous urinary nitrogen (g/day)	3.61	3.38	3.45	0.13	0.77
Nitrogen retention (g N/kg $BW^{0.75}$/day)	0.75^{a}	0.42^{b}	0.43^{ab}	0.06	0.02
Nitrogen retention (% of nitrogen intake)	67.21^{a}	35.32^{b}	51.74^{ab}	5.37	0.04

^{a,b,c} LS Means in the same row with different superscripts differ (P < 0.05)
Dry matter intake and secretion was quantified over a seven day period.

In vitro digestibility study

Rumen degradability of feedstuffs can be determined with either *in sacco* or with *in vitro* studies. *In sacco* studies can be costly and difficult to carry out experiments with live animals in terms of animal health and welfare. In this current study, *in vitro* digestibility of treatments was used to determine dry matter digestibility as well as true digestibility (Ankom® Technology Corp., Fairport, NY, USA).

The *in vitro* dry matter digestibility (IVDMD) results of the three different treatments are depicted in Table 4.10. *In vitro* dry matter digestibility had no significant difference between the three different treatment groups (P = 0.07). This is in agreement with the results found previously in this chapter in Table 4.7 where the *in vivo* study also showed no significant

differences between the different treatments. *In vitro* dry matter digestibility was higher for the NutrifenPlus® treatment, followed by the Nutrifen® treatment and then the control treatment. However, no significant differences were found between the different treatments.

Table 4.10: *In vitro* dry matter digestibility (IVDMD) of feed for dairy goats given three different additives over a period of 48 hours.

Treatment	*In vitro* DM digestibility (%)	SEM*
Nutrifen®	77.91	0.87
NutrifenPlus®	78.38	1.12
Control	75.7	0.85

*SEM – Standard Error of the Mean

The *in vitro* true digestibility (IVTD) results of the three different treatments are depicted in Table 4.11. *In vitro* true digestibility (P = 0.08) had no significant difference between the three different treatment groups as expected because no differences were found for *in vitro* dry matter digestibility. The same were observed for *in vitro* true digestibility as for *in vitro* dry matter digestibility, where the NutrifenPlus® treatment had higher rumen degradability, followed by the Nutrifen® treatment and then the control treatment.

Table 4.11: *In vitro* true digestibility (IVTD) of feed for dairy goats given three different additives over a period of 48 hours.

Treatment	*In vitro* true digestibility (%)	SEM*
Nutrifen®	88.86	0.94
NutrifenPlus®	89.98	0.99
Control	88.31	0.87

*SEM – Standard Error of the Mean

Similarly to the *in vivo* results, it is possible to argue that because there were no differences found between treatments regarding NDF digestibility, no significant differences were found in the *in vitro* true digestibility between treatments as NDF is closely related to the digestibility of a feed (McDonald *et al*., 2002).

Conclusion

The effects the different treatments had on the digestibility of the feed were investigated and the following could be concluded. The amount of NDF had in some cases been found to be

closely related to the digestibility of a feed. The results from the *in vivo* study would suggest that the control treatment, having the highest NDF level, would have the lowest digestibility. However, no significant differences were found between the different treatments regardless of the additive added to the feed. This could suggest that the dairy goats digested the different treatments with similar efficiency as they would have digested the feed without any additive added to the feed. Ether extract was the only nutritional (chemical) component that differed significantly between the Nutrifen® and NutrifenPlus® treatments but it is inconclusive for lipid metabolism as both Nutrifen® and NutrifenPlus® treatments contained Fenugreek. No significant differences were found between treatments for organic matter, crude protein and total digestible nutrients. Crude fibre content did not differ as was expected as no alterations were made to the fibre content of the different treatments.

Dry matter intake was the highest for the Nutrifen® and NutrifenPlus® treatments, supporting Fenugreeks' claims to enhance appetite and stimulate feed intake. The Nutrifen® treatment had the highest energy retention where it differed significantly from the control treatment. The Nutrifen® treatment differed from the NutrifenPlus® treatments and had the highest N retention.

The *in vitro* results support the *in vivo* results in that no differences were observed in apparent digestibility between the three different treatments.

The researchers reject the H_1 for the main objective in the current study which stated that "*Fenugreek as a feed additive will affect total tract digestibility",* and accept the H_0 that "*Fenugreek as a feed additive will not affect total tract digestibility".* The same was concluded for the secondary objective for the current study as the H_1: *"Fenugreek as a feed additive will affect rumen degradation"* is rejected and the H_0: *"Fenugreek as a feed additive will not affect rumen degradation"* is accepted.

From the current study it is evident that the two treatments containing Fenugreek may enhance appetite and therefore result in an increase in feed intake. It can therefore be hypothesised that more nutrients are consumed and enters the body which are available for milk production, which can help explain the findings in Chapter 3. Specific hormones are involved in the process of milk production and therefore led the researchers to the hypothesis to evaluate this mechanism in helping to explain an increase in milk production as found in Chapter 3. The effects of a natural feed additive, Fenugreek, on Growth Hormone production will subsequently be investigated in Chapter 5.

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Chapter 5

The effect of a natural feed additive, Fenugreek, on growth hormone levels in dairy goats.

Abstract

Nutrition plays an important role in milk production but it is not the only contributor. Growth hormone (GH) is well-known for its importance in stimulating milk production. The aim of this study was to determine whether or not a Fenugreek containing feed additive can increase the natural levels of growth hormone in the dairy goat. Three treatment groups consisting out of eight male goats each were used. Each group were allocated a different ration; control group (no supplement), which received a regular ration; Nutrifen® group, which received the normal ration plus 60 grams of Nutrifen® daily and a NutrifenPlus® group, which received the normal ration plus 60 grams of NutrifenPlus® daily. Blood samples were collected via venipuncture from the jugular vein and blood analysis was done using a Goat Growth Hormone (GH) ELISA kit. The main focus of this study was to test different Fenugreek containing treatments and its effect on plasma GH concentration levels. No statistical differences were found in plasma GH concentrations between the three different treatment groups. The result from this study indicated that there are more complex processes involved in milk production than just GH alone.

Keywords: Diocin, Ghrelin, Hormones, Milk production

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Introduction

In South Africa there is a growing demand for goat milk as the niche market increases as well as in the tourist sector. Another demand exists in South Africa, as in the rest of the world, for people that suffer from health problems such as allergies that cannot consume any other milk of animals (Wilson *et al*., 1995). Relatively little research is done on feed additives to enhance production of Dairy goats in South-Africa and especially to improve production under adverse environmental conditions.

Nutrition plays an important role in milk production from dairy goats where a lot of factors influence milk yield and composition such as dietary factors, as well as daily body weight gain (Min *et al*., 2005). It is therefore important to supply dairy goats with adequate amounts of nutrients, from good quality sources, in order for them to produce milk to their full potential.

As the world population grows, the demand for milk also grows and therefore research is necessary to achieve this increasing demand for milk yield. Selection of good genetics and breeding in dairy animals has led to an increase in milk production far beyond from the needs of their offspring. Although an increase in milk production took place, the concentration and milk composition remained the same. Selection for a higher milk yield had increased the demand of nutrients due to the high metabolic demand by the animal to produce more milk (Svennersten-Sjaunja & Olsson, 2005b).

Lactation in animals involves their adaptation to the environment as well as an orchestra of endocrine and metabolic processes, which undergo dramatic changes to ensure the onset of milk production (Svennersten-Sjaunja & Olsson, 2005b). It should be noted that milk production cannot be improved further by means of nutrition if genetic potential is a limiting factor (Morand-Fehr *et al*., 2007).

Before parturition can take place, the mammary gland needs to develop and this is initiated through the stimulation by GH and prolactin, adrenocorticoal steroids, oestrogens and progesterone (Svennersten-Sjaunja & Olsson, 2005a). The whole period of foetal to adult stage as well as development during pregnancy and lactation has to be taken into consideration. Changes in hormone levels throughout the body are noticed in the endocrine system at the onset of pregnancy.

A cocktail of hormones stimulate mammary gland synthesis to develop normally and function accordingly. These hormones include: growth hormone, prolactin, oestrogen and progesterone, adrenocortical steroids, gastrin, CCK and secretin produced by the

gastrointestinal tract (Svennersten-Sjaunja & Olsson, 2005b). An increase in blood flow throughout the body is observed to ensure an increase of hormones and nutrients that reaches the udder for milk synthesis (Svennersten-Sjaunja & Olsson, 2005b).

Hormones are a necessity throughout lactation as it controls the persistency of lactation where a positive relationship exist between GH concentrations found in the plasma and milk yield, in dairy cows (Sorensen & Knight, 2002) and dairy goats (Disenhaus *et al*., 1995). Hormones are sometimes divided into categories and GH can be classified as a metabolic hormone, where the main role is to regulate metabolic responses to nutrient intake (Neville *et al*., 2002).

Hormones such as GH and prolactin play an important role in regulating mammary function in ruminants (Flint & Knight, 1997a) and together with leptin these hormones are important in regulating nutrients to the udder. Prolactin and growth hormone are the two hormones that control milk production at the onset of milk secretion (lactogenesis) as well as maintaining milk production (galactopoiesis) throughout lactation. In ruminants during galactopoiesis, GH dominates prolactin (Flint & Knight, 1997c). Flint and Knight (1997d) also suggested that prolactin is at least as important as GH in producing milk in caprine species.

GH, also known as somatotropin, is a hormone secreted from the anterior pituitary gland (Deuben & Meites, 1964). GH secretion is regulated by two hypothalamic peptides where growth hormone releasing factor (GRF) stimulates the release of GH and somatostatin inhibits the release of GH from the anterior pituitary gland (Tuggle & Trenkle, 1996).

GH has lipolytic and diabetogenic (blood glucose elevating) properties. Blood flow is increased by GH (Svennersten-Sjaunja & Olsson, 2005). The mode of action of GH is considered to be indirect and mediated through stimulation of insulin-like growth factor 1 (IGF-1) (Shkreta *et al*., 1997; Flint & Knight, 1997b). However, it is not clear whether GH works directly on the mammary gland or if it is indirect via locally produced IGF-1 or via IGF-1 produced in the liver (Flint & Knight, 1997; Hull & Harvey, 2001).

GH, as the name indicates, is responsible for growth stimulation but it became apparent that GH did much more than just stimulate growth. This hormone's effect then became a popular subject of interest and trials were conducted to conclude its positive effect on milk production in various species (Etherton & Bauman, 1998; Asdell, 1932). The use of GH as a treatment is usually limited to farm animals, where this hormone has a positive response on milk production when administrated to pigs, sheep, goats and cows (Etherton & Bauman, 1998; Boutinaud *et al*., 2003b). However, this method of increasing milk production is still much debated (Svennersten-Sjaunja & Olsson, 2005a).

The administration of exogenous growth hormone increases milk production but it does not work its effect on milk production through an increase in feed intake. It is thought that GH, administrated as an exogenous compound, have an effect on increasing metabolism that results in mobilization of stored body reserves (Rose & Obara, 2000). The mechanism on how GH functions is not yet fully understood, but it is believed that the response could be due to nutrient flux to the udder or GH can have a direct effect on the luminal epithelium (Neville *et al*., 2002).

Leguminous plants, such as Fenugreek, are rich in saponins (Mir *et al*., 1997) and they are found in both leaves and seeds (Wina *et al*., 2005a). Saponins are secondary plant metaboloites (glycosides) and they are known to have antimicrobial properties within the rumen as well as alter rumen fermentation in a positive way which improves nutrient utilization (Hristov *et al*., 1999; Wang *et al*., 2000). Diocin is a natural saponin found in Fenugreek and has structural similarity to oestrogen, which leads to an increased release of GH by binding to the receptors on the pituitary cells that recognise the GH releasing hormone. This, in turn, results in an increase in milk secretion (Graham *et al*., 2008).

The main objective of the current study was to evaluate the effect of feeding two commercial Fenugreek products, formulated differently, on plasma growth hormone levels. The result of this current study will help in the process of understanding the mechanism of how Fenugreek exerts its positive effect on milk production. This will also allow for a better understanding between Fenugreek and growth hormone and their relationship within dairy animals.

H_0: *Fenugreek as a feed additive will not affect plasma growth hormone levels.*

H_1: *Fenugreek as a feed additive will affect plasma growth hormone levels.*

Materials and Methods

Ethical clearance for animal use

This study complied with accepted standards for the use of animals in research and teaching as reflected in the South African National Standards 10386: 2008; and was completed with ethical clearance from Stellenbosch University Care and Use Committee (SU ACUC), reference number: SU-ACUM12-00033.

Animals used in this study

Twenty four intact buck* dairy goats, consisting predominantly of Saanen goats and a small number of Toggenburg and British Alpine goats, were obtained from the farm Fairview, Paarl in the Western Cape region of South Africa and used during the investigation of this

particular study. Twenty four goats were used in order to have sufficient degrees of freedom for the statistical analysis (n = 8 animals/treatment). The goats were transported from the farm Fairview to the University of Stellenbosch's experimental farm, Welgevallen. Goats were housed separately and randomly allocated into individual pens (1 m x 2 m) and was fed indoors, in an adequately ventilated shed with wooden slatted floors. Each treatment group were randomly assigned to one of two treatments with the third, a control group.

Bucks were chosen for the study as they were already adapted on the farm to the various treatments. Female goats were difficult to obtain as they were part of a commercial herd and the stress of blood collecting could have added different negative effects associated with a loss in production. It should therefore be clearly stated at this point that only the effect of Fenugreek on GH levels were investigated. The results could then be interpreted and a link made between elevated GH levels and an increase in milk production in female goats as GH is well known for its effect in milk production in the female animal.

Forages and diet characteristics

Goats were maintained on the regular feeding and management program of the Fairview Farm (Paarl, Western Cape), where they received a 50:50 mixture of lucerne and oat hay. The goats also received a semi-complete formulated commercial feed (Meadow Feeds Lactating Goat Feed), supplied by Meadow Feeds (Paarl, South Africa) and offered at 400 g/goat per day. The semi-complete feed served as the basal diet. The composition of the feed is presented in Table 5.1.

Table 5.1: Chemical composition of the semi-complete commercial goat feed as is.

Chemical composition	Amount (g/kg)	Inclusion
Protein **(11.48% derived from [1]NPN)*	150.0	Minimum
Moisture	120.0	Maximum
Fat	25.0	Minimum
Fat	70.0	Maximum
Fibre	120.0	Minimum
Fibre	200.0	Maximum
Calcium	10.0	Maximum
Phosphorus	4.5	Minimum
Urea	6.0	Maximum

[1]NPN - Non protein nitrogen

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Treatments

Two commercial Fenugreek products, formulated differently, both containing cotyledon concentrate were used in this study and were the same as in the digestibility study. Nutrifen® and NutrifenPlus® are supplements which enhance milk production in dairy animals. The composition of the two treatments was as follows:

iii. Nutrifen®

a. Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*)

iv. NutrifenPlus®

a. Fenugreek cotyledon concentrate (*Trigonella Foenum-Graecum*)
b. Fennel seed (*Foeniculum Vulgare*)
c. Saw Palmetto Berries (*Serenoa Repens*)
d. Brown Kelp *(Laminariales*)
e. MSM (natural source Methylsulfonylmethane)
f. White distilled vinegar powder

The Control group received the basal diet (as mentioned under the heading: Forages and diet characteristics) with no additive, while the Nutrifen® group received the basal diet plus 60 g of Nutrifen® per goat/day and the NutrifenPlus® group received the basal diet plus 60 g of NutrifenPlus® per goat/day. Supplementation occurred in the shed twice daily during the morning (09:00) and afternoon (16:00) feedings, where 30g of the respective additive were top-dressed onto 200 g of the semi-complete feed per milking. Refusals, if any, were collected after each goat had finished eating and the amount determined.

Duration of the trial

Goats were adapted for a period of two weeks to the various treatments and received the treatments for another week during the digestibility trial. The blood collection period was conducted over one day, just after commencement of the digestibility trial.

Blood collection and analysis

Blood was collected on the day of arrival to serve as a baseline. Blood was collected via venipuncture from the jugular vein into EDTA vacutainors and put on ice to keep the samples cold. Blood samples were transported to the university's laboratory. The baseline samples were centrifuged with a Sigma 2-16K centrifuge (Supplied by Wirsam Scientific, Cape Town) at 1000 x *g* for 15 mins, where after the collected plasma samples were kept

frozen (-20°C) until further analysis. On day eight and after commencement of the digestibility study, blood was once again collected, shortly after the morning feeding, using the same venipuncture from the jugular vein. The protocol for blood collection over time was adapted from that as suggested by Trenkle (1989). Catheters were installed in the jugular vein by a veterinarian technician to aid with multiple collections from the same goat. Blood was collected at time intervals of half an hour and started at 09:30 until 15:30. Blood samples were put on ice to keep them cold until the samples were transported to the university's laboratory. The samples were centrifuged with a Sigma 2-16K centrifuge (Supplied by Wirsam Scientific, Cape Town) at 1000 *g* for 15 mins, where after the collected plasma samples were kept frozen (-20°C) until further to analysis.

Blood analyses were performed using a quantitative CUSABIO® Goat Growth Hormone (GH) competitive enzyme-linked immunosorbent assay (ELISA) kit (American Research Products Inc., catalogue # CSB-E13275G, Waltham, Massachusetts, USA), according to the instructions of the manufacturer. This ELISA kit has a range of detection of 6.25 $ng.ml^{-1}$ to 100 $ng.ml^{-1}$ goat GH. Plasma was separated from the blood samples by centrifugation at 1000 *g* for 15 mins in a Prism™ microcentrifuge (Labnet, catalogue # C2500, supplied by Whitehead Scientific, Cape Town, South Africa), where after the collected plasma samples were kept frozen (-20°C) until further to analysis.

At the time of analysis, plasma samples were thawed at room temperature and were re-centrifuged (1000 *g*, 15 mins). All ELISA kit reagents were brought to room temperature before use. Aliquots of 50 µl of each sample and supplied standard (in duplicate) were transferred to marked wells of the ELISA plate, leaving one 'blank' well without any solution. Thereafter, 50 µl of conjugate was added to each well containing sample or standard, excluding the 'blank' well, and the plate was covered and incubated at 37°C for 60 mins. The wells were subsequently aspirated and washed three times with diluted wash buffer (supplied with the kit) using an automated plate washer (BioTek, catalogue # ELx50, supplied by Analytical and Diagnostic Products, Cape Town, South Africa). HRP-avidin (50 µl) was added to each well (excluding the 'blank' well) and the plate was again incubated at 37°C for 30 mins. Following re-washing of the wells as previously described, 50 µl of each of the supplied substrates A and B were added to the wells (including the 'blank' well), followed by incubation in the dark at 37°C for 15 mins. Lastly, 50 µl of stop solution was transferred to each well and the contents were mixed by gently sliding the plate back and forth. The optical density of each well was immediately determined at 450 nm in an Anthos 2010 microplate reader (Biochrom Ltd., catalogue # GF1755011, Cambridge, UK). The duplicate readings for each standard and sample were averaged and the optical density reading of the 'blank' was subtracted from each of the aforementioned values. Final results were calculated from a

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standard curve created with the commercial curve analysis software (Curve Expert version 1.3, Hixson, Tennessee, USA) by plotting the concentration of each standard on the X-axis against the mean absorbance value of each standard on the Y-axis and drawing a best fit curve through the points on the graph.

Statistical Analysis

The effect treatment had on growth hormone (GH) levels in the dairy goats, were subjected to a repeated measures ANOVA, where the main effect of treatment were tested, using Statistica version 10 (Statsoft Data Analysis Software System, 2011). The assumptions of homoscedasticity and normality were tested on the data. Least square means were separated with a Bonferroni test and significance was declared at $P < 0.05$.

Results and Discussion

The results from Chapter 4 showed statistical differences in an increase in dry matter intake for the Nutrifen® treatment. Feed intake and nutrient distribution to the udder before the onset of milk production are regulated by hormones (Svennersten-Sjaunja & Olsson, 2005b). An increase in plasma levels of ghrelin were observed when intake increased and therefore (Roche *et al*., 2008) reported that ghrelin can regulate feed intake. The increased levels of plasma ghrelin are known to increase plasma GH levels (Nass *et al*., 2008). This is also supported by previous studies which suggested that ghrelin may affect GH secretion from the pituitary leading to an increase in milk production in ruminants (Iqbal *et al*., 2006; Date *et al*., 2000). The previous statements, however is contradictory to Takaya *et al*. (2000) whom suggested that ghrelin is not specific for GH release.

It could be speculated that the Nutrifen® treatment increased feed intake which resulted in an increase in plasma ghrelin and therefore in plasma GH levels, which were observed for the Nutrifen® treatment in the current study, although not statistical significant from the other two treatments. Furthermore the results obtained from Chapter 3 showed a significant increase in milk production and could be a result of the increased plasma GH levels in the current study or it may also be due to an increase in feed intake.

The results that treatment had on GH levels of dairy goats fed different treatments are presented in Table 5.2 and illustrated in Figure 5.1. In order to provide a reference for GH levels in the trial animals, blood samples that served as baseline samples were taken prior to the trial period from each group. The avg. GH levels for the treatment groups Nutrifen®, NutrifenPlus® and control were 11.1, 9.1, 9.5 ng/ml respectively. Different time intervals (09:30 – 15:30) were used, for collection every half an hour on the day of the trial, to collect

blood samples for plasma GH analysis to create a profile of GH. Different time intervals were used because in mammals, secretion of GH is pulsatile and influenced by various factors such as stress and feeding (Dutour *et al*., 1997). No significant differences were found ($P > 0.05$) in GH concentration between the different treatments used.

Table 5.2: Growth hormone concentration (mean) of dairy goats given three different treatments over a six hour period.

Time intervals (hours)	Treatment (GH concentration, ng/ml)			SEM
	Nutrifen®	NutrifenPlus®	Control	
09:30	9.3	10.7	11.6	1.249
10:00	10.6	9.3	11.5	1.392
10:30	10.4	10.1	12.5	1.744
11:00	9.2	9.9	13.2	2.039
11:30	10.5	9.1	11.7	1.677
14:30	12.0	10.1	10.7	2.184
15:30	13.0	11.3	11.3	1.892

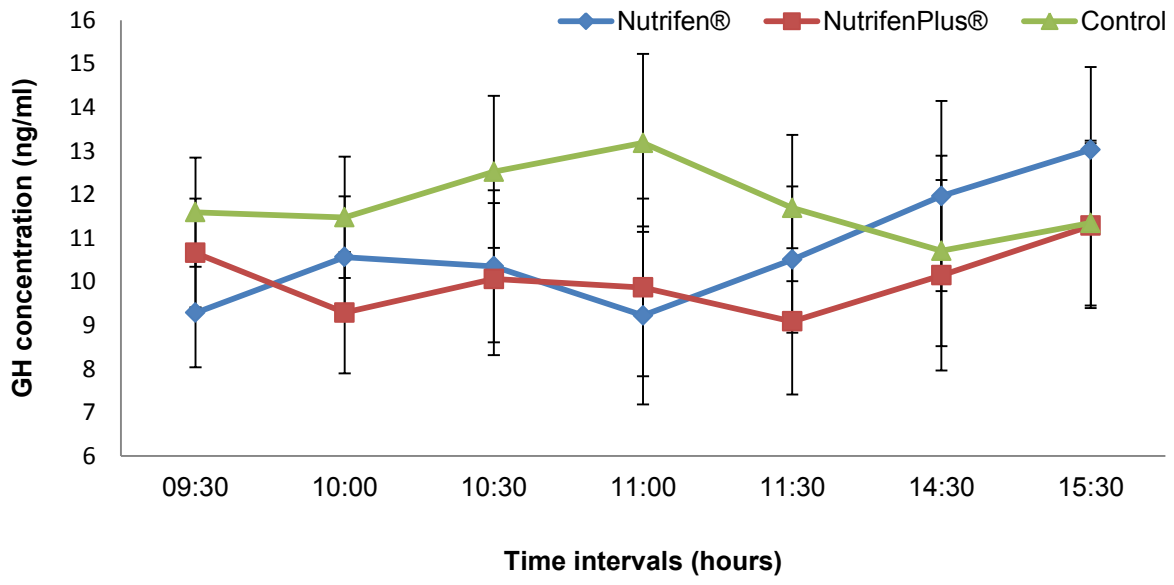

Figure 5.1: The effect of three different treatments, fed to dairy goats, on GH plasma levels (mean) over a six hour period.

Growth Hormone is secreted episodically and regular patterns are also more common in rats, which differ completely from ruminants, due to stricter environmental control (Tannenbaum & Martin, 1976). Variation in GH concentration occurs commonly in ruminants

and is due to various reasons such as stress and feeding (Driver & Forbes, 1981). This variation has been described in goats (Tindal *et al*., 1978a) as episodic, erratic and comprising irregularly spaced episodes. This supports the current findings as clearly no definite pattern could be observed from Figure 5.1 between all three different treatments used on dairy goats to enhance plasma GH concentration.

A decrease level in GH concentration can be due to nonspecific stress in animals as showed in rats (Schalch & Reichlin, 1968). Animals should be customized to stress factors such as blood sampling several days before commencement of experimentation to minimize any stress which might lead to a decrease in plasma GH concentrations (Edén, 1979). Heat is another stress factor. However Hart and Buttle (1975) as well as Tindal *et al*. (1978b) suggested that blood GH concentration in goats are independent of thermal stress. Other experiments that involved sheep have shown a decrease in plasma GH levels just after the morning feeding (Trenkle, 1978; Hove & Blom, 1976). This is similar to current findings of this study where plasma GH levels were low just after the morning feeding and where levels of plasma GH increased with time for both the Nutrifen® and NutrifenPlus® treatments. This is also supported by Bocquier *et al*. (1990) who showed low levels of plasma GH after morning feeding with a regular increase in GH concentration towards afternoon feeding in ewes. The low mean GH concentrations found at morning feeding for the treatment groups can further be explained using the depressive anticipative effect suggested by Trenkle (1989) who reported low plasma GH concentrations in ewes that waited for meal distribution at morning feedings.

A study done by Trenkle (1976) showed that when exogenous GH was administrated to sheep, it had a half-life from 9.2 to 13.4 min. In the current study, GH concentrations were only sampled at intervals of 30 min and sampling started 4.5 h after the morning feeding. It appeared that GH levels started to increase linearly from six to seven hours after Fenugreek ingestion. In hindsight, it could have been more informative had blood samples been taken for a much longer period. Perhaps GH plasma levels should be taken over a few days and not just at different hours in one day. If the increasing trend continued, then one could have speculated that the effect of Fenugreek on milk yield may relate to an increase in GH production. A study by Driver and Forbes (1981) indicated that GH levels were low when sheep were in anticipation of “expected” feeding. The goats in this study were given food at the same time for 15 days and therefore it may be possible that the goats also were “expecting” feeding. The GH content of the two Fenugreek treatments started to increase from 11:30 (two and a half hours after supplementing). This may suggest that an increase in GH is triggered much later than what was expected. Unfortunately, because of the limited time window of blood collections in the current trial, the full temporal effect of treatment on

GH blood profiles are not available. Trenkle (1976) also suggested that if there is no noticeable change in hormone concentration in plasma, it does not necessarily indicate that there is no change in secretion, when there is a corresponding change in rate of removal from circulation.

Growth hormone's mode of action is not a simple endocrine process but rather a complex process of other hormones and events involved in regulating GH's effect at the onset of milk production (Svennersten-Sjaunja & Olsson, 2005c; Boutinaud *et al*., 2003a). Ghrelin and GH-releasing hormone stimulates GH secretion whereas somatomedin inhibits GH secretion (Anderson *et al*., 2004). A study conducted on sheep by El-Abid & Nikhaila (2010) concluded that an increase in milk yield was a result of increased levels of thyroid stimulating hormone and stimulatory prolactin which had an effect on lactation performance. This, once again suggests that GH is not solely responsible for the onset of milk production. Hormones such as GH and prolactin regulate mammary function in ruminants (Flint & Knight, 1997b) and together with leptin regulate nutrient distribution to the udder.

It should be noted that the greatest physiological stimulus for milk production is not in fact a result of some cocktail of exogenous hormones, growth factors, receptors agonists/antagonists, or gene therapies, but the main stimulus remains to be pregnancy (Svennersten-Sjaunja & Olsson, 2005c).

Conclusion

No differences were found in plasma GH concentrations between the three different treatments tested in the current study. The GH concentrations were only measured over a six hour period. The fact that Fenugreek resulted in higher milk yields in lactating dairy goats in previous studies, and the fact that GH started to increase approximately six hours after feeding in this study, may suggest that the increased milk yields were indeed a result of increases in GH. However, this is only speculative as male goats were used in the current study. This study was done as an explorative study to justify if further studies using lactating dairy goats are warranted. Growth hormone's effect on milk production does not involve simple endocrine processes, but rather an orchestra of organised events and hormones involved in regulating milk production by dairy animals. It would make sense to test for other hormones involved in milk production to increase the credibility of Fenugreek's effect on increasing plasma concentrations of GH. Also, the effect of Fenugreek on plasma GH levels over a 24 h period, instead of a 6 h period, warrants further research.

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Chapter 6

General Conclusion

The modern consumer with higher expendable income demands products produced from farming enterprises utilizing natural products. It is well known that people are starting to move away from inorganic components and antibiotics used in animal feeds. Producers started focusing more on natural alternatives to replace so-called 'harmful' substances that may be found in animal feeds. In the current study, two commercial products containing a natural feed additive (Fenugreek) were used to observe their effects on milk production from dairy goats. Nutrifen® and NutrifenPlus® were the two products tested.

The first objective of the study was to test whether or not the products containing Fenugreek could improve milk production by dairy goats. Nutrifen® showed promising results to increase milk production and lactose content.

A decrease in milk cholesterol content could have potential regarding consumer demand for healthier products. However, in the current study, treatments did not result in a decreased blood cholesterol content. These results are in accordance with the research of other groups on the same topic.

The second objective of the study focussed on nutrient digestibility. Fenugreek is known to stimulate feed intake. It also contains natural components, such as saponins, which may have an effect on digestibility. In the current study, however, no significant differences in digestibility values were observed between the three different treatments. Dry matter intake was the highest for the Nutrifen® and NutrifenPlus® treatments, which support Fenugreek's claims to enhance appetite. The *in vitro* results from the study supported the *in vivo* results that no differences were found between treatments regarding feed digestibility.

The lack of response in feed digestibility was the reason for investigating endocrinological processes involved in milk production. It was decided to analyse the blood for plasma growth hormone content, as growth hormone affects milk production. In the current study, no differences in plasma GH levels were observed between treatments. However, it should be mentioned that it was not initially planned to do any blood analyses. In the current study, GH concentrations were sampled at intervals of 30 min; sampling started 4.5 h after the morning feeding and continued for only 10.5 hours after feeding. It appeared that GH levels started to increase linearly, although not significantly, from six to seven hours after Fenugreek

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ingestion. In hindsight, it could have been more informative had blood samples been taken over a much longer period. If the increasing trend continued, then one could have speculated that the effect of Fenugreek on milk yield may relate to an increase in GH production.

To conclude, Fenugreek can successfully be included as a natural feed additive in the diet of dairy goats to increase milk production. However, the mode of action still remains somewhat unclear and needs to be addressed in future studies. Milk production involves various complex processes. Therefore, further research is needed to investigate Fenugreek's effect on milk production, as the results of the current study cannot support all the claims regarding Fenugreek's effects on milk production in dairy goats.