GHENT UNIVERSITY

FACULTY OF VETERINARY MEDICINE

Academic year 2016 – 2017

THE EFFECT OF HIGH TANNIN CONCENTRATIONS IN FEED ON PROTEIN DIGESTION: GRAZERS *VERSUS* INTERMEDIATE BROWSERS

by

Jill DERIX

Promotors: Prof. Dr. G.P.J. Janssens
Dr. M. Lourenço

Research in context of the master's dissertation

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ACKNOWLEDGEMENTS

When I decided to choose this subject for my master's dissertation, there were three important things I did not realise. The first thing was, how big impact would be on my personal development, my education and my future prospects. I have learned a lot about research, but also about Ethiopian culture. Of course I expected to learn things, but I think I underestimated how much I would. The second thing is that the trip to Ethiopia, against all odds, made the workload at the clinics feel less heavy. Certain people warned me that I should not go, since the graduation year is hard enough as it is. Indeed, it was hard. However, thanks to this project I have had time to recharge, to write my literature study and to enjoy new experiences. The third thing I did not realise, might be the most important one: it is the fact that I got very lucky. You cannot choose the promoters who come with the project, but if I could, I would not choose anyone other than professor Geert Janssens and doctor Marta Lourenço. To the both of you: thank you so much. Your enthusiasm is inspiring.

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SAMENVATTING (NL)

Doordat in Ethiopië dermate veel dieren op het land grazen, is er een verhoogd risico op overbegrazing, wat kan leiden tot bodem erosie. Dergelijke gevolgen hebben een grote impact op de voedselvoorziening, aangezien er nutritionele tekorten ontstaan in de bodem, planten en hierdoor ook in de dieren. Bovendien bevatten veel van de planten in subtropische regio's hoge tanninegehalten. Planten produceren deze tannines als een natuurlijk verdedigingsmechanisme tegen herbivoren en kunnen het verteringsproces verstoren. Het daadwerkelijke effect hangt echter af van het type tannine en de concentratie in de plant en kan dus voordelig, anti-nutritioneel, toxisch of lethaal zijn. De effecten ontstaan doordat tannines kunnen binden aan eiwitten en enzymen. Er worden complexen gevormd met als gevolg dat de voedselopname afneemt en dat de nutriënten die worden opgenomen, suboptimaal worden benut. Dit leidt tot verminderde groei en productie. Het doel van dit onderzoek is om een beter inzicht te krijgen in de manier waarop tannines interfereren in de vertering van eiwitten en hoe dit verschilt tussen *browsers* en intermediaire *grazers*. Om dit te onderzoeken werden schapen en geiten gevoederd met tanninerijke bladeren van *Milletia ferruginea*, waarna de eiwitvertering werd geëvalueerd op basis van de nutritionele waarde van het geconsumeerde voeder, ruw eiwit gehalten in de digesta en de verteerbaarheid van het eiwit. Er werd verwacht dat de geiten een betere eiwitvertering zouden vertonen dan de schapen, maar deze hypothese werd niet bevestigd. Er bleken weinig verschillen te zijn tussen de species en de schapen vertoonden zelfs betere productiewaarden dan de geiten. Hoewel de resultaten onverwacht zijn, kunnen zij gebruikt worden in toekomstig onderzoek om het benutten van nutriënten te verbeteren voor grazende dieren in Ethiopië.

Sleutelwoorden: Eiwitmetabolisme – Herkauwers – Tannines - Vertering

ABSTRACT (EN)

As Ethiopia hosts over eighty million production animals that mainly depend on free-range diets, there is a high risk of overgrazing, which can lead to soil erosion. This would cause a nutritional depletion and has thereby many important consequences on feed and food supply. Since plants in subtropical regions contain relatively high concentrations of tannins, the digestion process is disrupted. This is due to the fact that tannins form complexes with proteins, thereby hindering the protein absorption and enzymatic functioning in the gastrointestinal tract. The disrupted nutrient digestion, together with the risk of overgrazing, forms a serious problem in animal production rates, especially in grazing animals, as browsing have developed certain adaptive mechanisms through evolution. The aim of this research was to better comprehend how tannins affect protein digestion throughout the whole digestive tract of small ruminants, and how this differs between intermediate browsers and grazers. Depending on the tannin type and concentration, tannins' effects can either be beneficial, anti-nutritional, toxic or lethal. In this experiment, sheep and goats were fed an intermediate browse diet, containing tannin rich plant material. Thereafter, the protein digestion was evaluated by analysing the nutritional value of the feed, crude protein in the digesta and average protein digestibility. The

expectation was that goats would handle the diet significantly better than sheep and that this would be reflected in weight gain, higher feed intake and a better protein digestibility. However, the results dismiss this hypothesis and show little differences in protein digestion between grazers and intermediate browsers. The results even imply that the sheep actually show better animal performance and feed intake. Despite the fact that the results did not meet the hypothesis, they can provide support for further investigation on improving nutrient utilisation by grazing animals in Ethiopia.

Keywords: Digestion – Foraging behaviour – Protein metabolism – Small ruminants – Tannins

ጪርሶ ቀጠለ (AMHARIC)

ኢትዮጵያ ከ 80 ሚሊዮን በላይ የቤት እንስሳት ለቤት ስትሆን፣በዋናነት የተመሠረተውም ዝቅተኛ ጥራት ባላቸው የግጦሽ መሬት ምግቦች/መኖ ላይ ነው። የግጦሽ መሬትን ከመጠን በላይ ጥቅም ላይ የማዋል ሁኔታ ከፍተኛ አደጋ አለው፣ ይህም በአጠቃላይ የአፈር መሸርሸር ሊያስከትል ይችላል.በዚህ ምክንያትይህ የአመጋገብ ሁኔታ እንዲቀንስና በእንስሳት መኖ እና በምግብ አቅርቦቶች ላይ ብዙ አሳሳቢመዘዞችን ያስከትላል። በከፊል ሞቃታማ አካባቢዎች ውስጥ የሚገኙ እጽዋቶች በአንጻራዊነት ከፍተኛ የታኒን ስብስቦች ስለሚኖራቸው የምግብ መፈጨት ሂደትን ይስተጓጎላል። ይህ የሆነው ታኒን ከፕሮቲኖች ጋር ውስብስብነት ስለሚፈጥር ነው፣ በተጨማሪም በምግብ መጫኛ ቦይ ውስጥ ያለውን የፕሮቲን ውስጣዊ ግፊት እና የኢንዛይሞች ተግባርን የሚያደናቅፍ በመሆኑ ነው። በምግብ ማዋሃድ ሂደት ውስጥ በተፈጠረው የተደናቀፈ የንጥረ ምግቦችን ማዋሃድ፣ የአየር ንብረት ለውጥ እና በግጦሽ የመሬት እጦት የመጋለጥ ሁኔታ ጋር ተዳምሮ በእንሰሳት ምርት መጠን በተለይም በግጦሽ እንስሳት ላይ ከባድ የምርት ውጤታማነትችግርን ያስከትላል። በዝግመተ ለውጥ ሂደት አማካይነት አሳሾች/ፍየሎች አንዳንድ የማስተካከያ ዘዴዎችን ይጠቀማሉ። የዚህ ጥናት ዓላማ የታኒን ንጥረ ነገር በፕሮቲን አጠቃቀም ላይ የሚያሳድረውን ተጽእኖ እንዴት እንደሆነ ለመረዳት ነው። ይህም በመካከለኛ አሳሾች(በጎች) እና በአሳሾች(ፍየሎች) መካከል እንዴት እንደሚለያይ የበለጠ ለመረዳት ነው. በታኒን ዓይነት እና መጠን ላይ በመመስረት፣ የታኒንስ ጣዕም ተጽዕኖዎች ጠቃሚ፣ ፀረ-ንጥረ-ምግቦችን፣ መርዛማ ወይም ገዳይ ሊሆን ይችላል. በዚህ ሙከራ የሚጠበቀው ፍየሎች(አሳሾች) ከበጎች(መካከለኛ አሳሾች) ይልቅ በአመጋገብ የተሻለ እንደሚሆኑ እና ይህም እንደ ክብደት መጨመር፣ከፍተኛ የምግብ ፍጆታ ፣የተሻለ የፕሮቲን አወሳሰድ እና አጠቃቀምን እንደሚሆን ይጠብቅ ነበር። ይሁን እንጂ ውጤቶቹ ይሄንን መላምት ያስወግዱ እና የፕሮቲን አወሳሰድ እና አጠቃቀም አሳሾች እና መካከለኛ አሳሾች መካከል ጥቂት ልዩነት አሳይቷል ፣ውጤቱም በጎቹ በትክክል የተሻለ ምርታማነት እናየምግብ አጠቃቀምን አሳይቷል።

ቁልፍ ቃላት: የምግብ መፍጨት - አመጋገብ ባህሪ - ፕሮቲን ሜታቦሊዝም- ትንንሽ እንስሶች(በግና ፍየል) - ታኒን

INTRODUCTION

Several effects of climate change in Ethiopia are highly reflected in the field of agriculture. Due to extreme drought, livestock is negatively affected. Since Ethiopia hosts the highest amount of livestock in Africa, these problems cause a great impact on food supply and economy.

The easiest and cheapest feed resource in Ethiopia is grazing of natural pasture (Alemayehu, 2006). This high dependence on free-range diets can cause a decrease in feed resources and thereby overgrazing, leading to soil erosion (Yisehak *et al*., 2013). Indeed, Abebayehu (2010) has found that land degradation in Ethiopia can have several detrimental consequences: (1) reduction of soil fertility, (2) decrease of agricultural productivity and (3) depletion in soil quality and nutrients.

Another disadvantage of free-range diets is that the natural pasture often does not meet animals' requirements as it is of poor nutritional value, and/or cannot be used optimally (Yisehak *et al*., 2014). An important reason for the latter, is the occurrence of high levels of tannins in certain plant material. These molecules have no primary function in the plant and are therefore categorized as secondary plant metabolites (PSM) (Iason, 2005; Waghorn, 2008). Unfortunately the amount of tannins present in different plants varies widely and is hard to predict. Furthermore, the effects of tannins differ strongly; they can either be beneficial or harmful, mainly depending on the type of tannin that is consumed, its chemical structure and molecular weight, the amount ingested and the animal species involved (Hagerman and Butler, 1981; Waghorn, 2008; Marais, 2012). Such harmful effect is a decrease in feed intake, which leads to a lower nutrient uptake and thereby disturbance of growth and/or performance. The main mechanism by which tannins have been described to be harmful in animal nutrition is the complex forming property that tannins have for proteins and minerals. When complexes are formed, the nutrients are not available for digestion and absorption (Makkar, 2003).

Animals that have to deal with these problems, have developed specific adaptation mechanisms. It is well known that animals from (sub)tropical regions show a better salivary response against dietary tannins than animals from temperate regions (Yisehak and Belay, 2011). Moreover, animal species that naturally consume forage containing high amounts of tannins, are generally better adapted to this type of diet. These kinds of forage are categorized as browses. Browses are herbaceous and woody dicots, such as forbs, shrub leaves and stems, while grasses are monocots (Hofmann and Stewart, 1972). The preference for foraging behaviour determines the difference between browsers, grazers (sheep) and intermediates (goats) (Hofmann, 1985).

Despite the numerous publications on the impact of dietary tannins on animal nutrition and performance, there is still little known about the subsistence of tannin-protein complexes throughout the gastrointestinal tract and how they differ between browsers and grazers. In other words: it is still unclear how these tannin-(salivary) protein complexes behave throughout the gastrointestinal tract and what their role is in the overall protein digestion of the animal. Most studies found in literature have approached this subject *in vitro* (Frutos *et al.*, 2004). In these studies, certain interfering biological factors, for instance the effect of bile salts, were not taken into account. To fully understand the effect of tannins on intestinal digestibility, *in vivo* experiments are required. Only in this way, one will gain insight in the mechanisms that enabled animals to deal with high dietary tannin intake

throughout evolution. With this knowledge, one will be able to achieve a better rangeland management in these regions of the world, and contribute to a more sustainable form of agriculture.

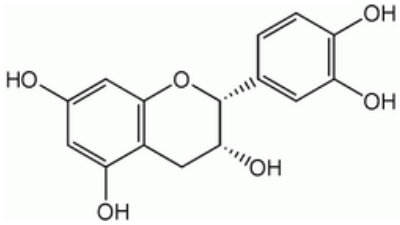
LITERATURE REVIEW

1. TANNINS

1.1. DEFINITION, CLASSIFICATION AND STRUCTURE

Tannins are water soluble polyphenolic compounds that can be divided into two main groups: condensed tannins (CT) and hydrolysable tannins (HT) (Haslam, 1988; Fickel *et al.*, 1997; Frutos *et al.,* 2004). Hydrolysable tannins (Figure 1D) consist of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids. They are polyesters formed out of phenolic acids, mostly gallic acid (Figure 1B), and a sugar molecule. The CT (polyanthocyanidins; Figure 1C) are non-branched polymers of flavonoid phenol units (mostly catechin; Figure 1A), linked by monomers (Mueller-Harvey and McAllan, 1992; Mueller-Harvey, 1999; Seresinhe and Pathirana, 2003) and they belong to the group of secondary plant metabolites (PSM) (Iason, 2005; Waghorn, 2008).

A.



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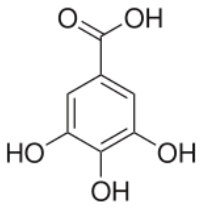

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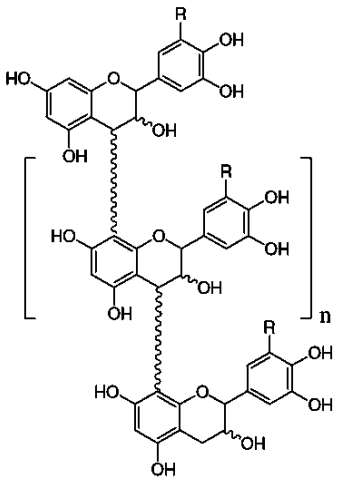

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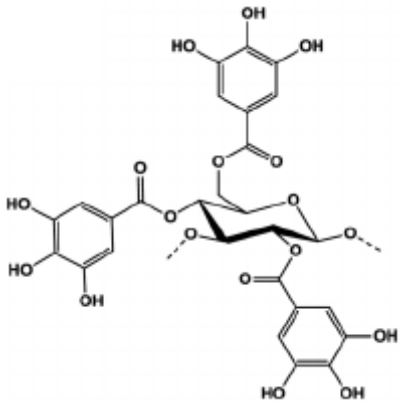

Figure 1. *Molecular structures of condensed tannins with catechin and hydrolysable tannins with gallic acid (based on Seresinhe and Pathirana, 2003).*

Both the HT as the CT are produced by plants in their cytoplasm. This study is mainly focused on the effects of CT, as these are the major tannins present in the natural forage and browses that are used in Ethiopia.

1.2. CHEMICAL PROPERTIES

The physical and chemical properties of tannins differ between the plant species that synthetize them (Mangan, 1988). Therefore, there are various biological results possible (Zucker, 1983). When the leaves are consumed, the dietary tannins are able to bind to amino, hydroxyl and carboxyl groups of different molecules (Kumar and Singh, 1984; Hagerman *et al.*, 1992). Tannins can also bind to the peptide bonds between amino acids (Stern *et al.*, 1996). Due to the possibility to bind to all these different groups, tannins are capable to form reversible and irreversible complexes with proteins, alkaloids, polysaccharides, nucleic acids and minerals (Frutos *et al.*, 2004). Tannins have especially a high affinity for proteins, due to their large amount of phenolic groups, as these provide many points at which bonding may occur with the carbonyl groups of peptides (Mcleod, 1974; Leinmüller *et al.,* 1991; Hagerman *et al.,* 1992). The degree of affinity between tannins and the participating molecules depends on the chemical characteristics of each (Zucker, 1983; Hagerman and Butler, 1981; Fickel *et al*., 1997). The proteins that appear to have the highest affinity for tannins are relatively large and hydrophobic, have an open structure and are rich in proline (Kumar and Singh, 1984; Hagerman and Butler, 1981). Even when there is a high affinity, the complexes are rather unstable. The bonds continually break and reform. In the past, researchers assumed that the bonds in tannin-protein complexes were mainly owed to hydrogen bonds (McLeod, 1974). However, a more recent study from Kumar and Singh (1984) suggested that complexes could form through several types of bonds. According to them, there are four possible bonds: (1) hydrogen bonds, (2) hydrophobic interactions, (3) ionic bonds and (4) covalent bonds. Of these bonds, only the covalent ones are irreversible. Only HT are able to form ionic bonds, as these bonds are formed between the phenolate ion and the cationic site of the protein. Hydrogen bonds are affected by pH-conditions and are approximately stable between pH 3.5 and pH 8 (McLeod, 1974; Watanabe *et al.*, 1981; Makkar, 1993).

All these properties depend on the binding status of the tannin, so the effect of bound tannins on digestion is not necessarily related to that of free tannins. In tree leaves, tannins are normally present in the unbound form (Getachew *et al.,* 1998). Within the digestive system of the animal, Makkar (2003) found that bound CT seem to be inert and do not affect microbial fermentation, as long as they remain bound. Once the tannins become unbound due to microbial action or other environmental factors, they can affect microbial fermentation.

1.3. DISTRIBUTION AND PRODUCTION OF TANNINS

Tannins are widely distributed among trees, shrubs and herbaceous leguminous plants (Mcleod, 1974; Perevolotsky, 1994; Waghorn, 2008). These plants belong mainly to the groups of Gymnosperms or Angiosperms. Within Angiosperms, tannins are more common in Dicotyledons than in Monocotyledons (Haslam, 1989; Giner-Chavez, 1996). In general, tannins are more abundant in new leaves and flowers, presumably since those are the parts that are most valuable to the plant and

are most likely to be consumed by herbivores (Terril *et al.,* 1992; Van Soest, 1994; Alvarez del Pino *et al.,* 2001).

As the synthesis of CT happens from phenylalanine and acetate precursors, there is competition with protein production and the Krebs cycle and thereby the productivity of the plant is reduced (Mueller-Harvey and McAllan, 1992). Therefore, it is likely that high levels of tannins are only produced when the benefits transcend the cost of lower energy and protein availability (Perevolotsky, 1994). According to Rhoades (1979) and Van Soest (1994), tannins are developed in high concentrations under particular circumstances, depending on environmental and seasonal factors. Brief high temperatures, water deprivation, extreme light intensities and poor soil quality increase the tannin content in the cytoplasm of plant cells (Rhoades, 1979**;** Van Soest, 1994; Seresinhe and Pathirana, 2003). The latter could be explained by the fact that the seasonal variation leads to the different demand for nutrients (Iason *et al.,* 1993). The climate factors explain why high tannin concentrations can be most found in plants from (sub)tropical regions. Besides the environmental factors, such as climate and soil fertility, there are some plant-bound factors, such as the stage of growth and the part of the plant (Seresinhe and Pathirana, 2003). Plants produce a lot of biomass during their growth period and at this moment there are few resources available for phenolic compounds. However, during the process of flowering, excess carbon is available for tannin synthesis, since the growth is inhibited at this stage (Perevolotsky, 1994).

In regions where grazing by domesticated livestock is relatively continuous over most of the year, this difference in tannin production by the plants was not observed by Perevolotsky (1994). Most probably because the plant is 'under attack' at a regular basis by the animals, it keeps its tannin production continuously high, as a defence mechanism (Perevolotsky, 1994). This is probably the case in the Ethiopian rangelands, despite the huge differences in water availability in the rainy season compared to the dry season, that could affect tannin production by the plants.

2. EFFECTS OF TANNINS IN RUMINANTS

Plants appear to produce tannins in their cytoplasm as a natural defence mechanism against herbivores (Kumar and Singh, 1984; Waghorn, 2008), though this is merely speculation. Other suggestions for the possible purpose are: defence against pathogens, nitrogen conservation and energy storage. Still, the hypothesis referring to defence against herbivores, seems most likely (Waghorn, 2008). The fact that the tannins bind to proteins, leads in general to an inhibition of the digestion and thereby the utilization of nutrients by the host (Martin *et al.*, 1987; Haslam, 1988; Makkar *et al.*, 1995). The presence of CT has three major consequences: (1) reduction of the content of fluid and fine particles in the rumen, (2) acceleration of the passage of liquid from the abomasum to the small intestine and (3) they can delay the passage of intestinal content in the different parts of the intestine (Frutos *et al.,* 2004). The overall effect of these assets is a delay in the passage of fluid and particulate matter throughout the entire gastrointestinal tract. It is hypothesised that these responses are largely the consequence of the interaction of tannins with digestive enzymes and the epithelium lining the digestive tract (Frutos *et al*., 2004).

The effects of presence of tannins in animal nutrition can differ, though. Their impact may be either positive or negative, depending on the type of tannin that is consumed, its chemical structure and molecular weight, the amount ingested and the animal species involved (Hagerman and Butler, 1981; Waghorn, 2008). High concentrations of tannins reduce voluntary feed intake and nutrient digestibility, whereas low to moderate concentrations may improve the digestive utilization of feed, mainly due to a reduction in protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine. These effects on nutritional utilization are reflected in several forms of animal performance (Frutos *et al*., 2004).

2.1. EFFECTS ON DIGESTION

2.1.1. Effect on feed intake

The effect on feed intake can either be short-term or long-term. Tannins are known to cause astringency on the epithelium of the oral cavity and the oesophagus. These effects are short-term, which means that they last for 20 to 60 minutes. The long-term effects can last for days or weeks and are correlated to a decrease in the levels of ammonia and/or volatile fatty acids (VFA) (Silanikove *et al.*, 2001). Both types of effects cause a reduction on feed intake, depending on the percentage of CT in forage. Opinions on the actual numbers are divided: Provenza (1995) has stated that plant material with CT levels above 3% affect feed intake negatively, while McLeod (1974) has claimed that the anti-nutritional effects only occur at levels of 5% or higher. In a more recent study was found that 2-4% CT leads to beneficial effects, while 5-9% CT works anti-nutritional. At CT levels over 9%, toxic and even lethal effects have been reported by Seresinhe and Pathirana (2003).

The consumption of plant species with high CT contents (> 50 g/kg of dry matter (DM)), significantly reduces voluntary feed intake, while low or medium levels (< 50 g/kg DM) do not seem to affect voluntary feed intake (Barry and Duncan, 1984; Barry and Manley, 1984; Waghorn *et al.*, 1994). Indeed, Barry and McNabb (1999) found negative effects on voluntary feed intake, when animals consumed a variety of *Lotus pedunculatus*, high in CT contents, but not when the animals consumed a variety of *Lotus corniculatus*, low in CT contents.

Hydrolysable tannins can also affect feed intake, and similarly to CT, their effect on voluntary feed intake seems to be variable as well, depending on the quantity that is consumed (McSweeney *et al*., 1988). For both CT and HT, the working mechanisms to explain decreased feed intake seem to be: a) a reduction in feed palatability; b) a reduction in rate of digestion (due to the slower passage rate of digesta in the gut, to give more time to host enzymes to extract nutrients, with the feedback loop for satiety being delayed); or c) the development of accustomed aversions. The latter seems to lead only to a decreased feed intake for tannin-containing feeds, while the first two hypotheses can apply to feed intake in general. A cutback in palatability could be caused by the interaction between the tannins and salivary mucoproteins, or through a direct reaction with the taste receptors, causing astringency (McLeod, 1974).

2.1.2. Effect on protein utilization

By binding to proteins, tannins modify their digestibility. This occurs because of changes in the

ruminal fermentation due to the presence of tannins and/or the presence of tannin-protein complexes, and therefore changes in the intestinal digestibility will occur. In the absence of tannins, degradation of dietary protein would happen in the rumen, so that the amino acids are absorbed in the rumen. Due to the formation of complexes between tannins and dietary protein, ruminal degradation is not possible. Instead, the complexes are passed on to the abomasum where they become soluble as pH decreases below the isoelectric point, so that that the amino acids are passed through to the intestine (Vissers, 2017). There they can be absorbed. In this way, the utilization of dietary essential amino acids is improved (McNabb *et al.*, 1993; Makkar *et al*., 1995). Note that this re-solubilisation is dependent on the type of dietary proteins that are consumed, as different proteins can have different values for the isoelectric point (Hagerman *et al*., 1986). Indeed, Silanikove *et al*. (1994) observed a significant increase in fecal nitrogen excretion, when the dietary tannin content was increased. This could be partly due to the fact that ingestion of high tannin concentrations can evoke an increased secretion of endogenous proteins and digestive enzymes (Mehansho *et al.,* 1987; Waghorn, 1996). According to Frutos *et al*. (2004) this increase in nitrogen excretion would be caused by an increase in metabolic fecal nitrogen. This however, is not necessarily true, as it can also be caused by dietary nitrogen, if the complexes were not dissociated in the gut.

Although CT may have some beneficial properties (in some cases including an increase in digestibility of organic matter) (McSweeney *et al*., 1988), several studies show that tannins can have a negative effect on the absorption of nutrients from the small intestine (Driedger and Hatfield, 1972; Silanikove *et al*., 1994 and 2001; McNabb *et al*., 1998). The presence of CT in browses, further reduces the (already low) protein availability for absorption. Not only by forming complexes with them, but also by limiting ruminal microbial growth and lowering the fractional absorption of amino acids from the intestine (Waghorn, 2008). Tannins are able to block digestive enzymes by forming complexes with them (Kumar and Singh, 1984). This has been confirmed in the study of Silanikove *et al*. (1994), where the authors observed an inhibition in the activity of trypsin and amylase (host digestive enzymes) due to the presence of CT. This, however, is not always the case. After dissociating from proteins in the abomasum, tannins may bind easier again to dietary proteins in the intestine (Mehansho *et al*., 1987; Mole and Waterman, 1987; Blytt *et al.,* 1988). Kumar and Singh (1984) suggest that the inhibition of digestive enzymes can occur when tannin-enzyme complexes are formed in the intestine. Another possibility is that the interaction of tannins with the intestinal mucosa, leads to changes in the rate of intestinal absorption.

2.1.3. Reduction in production of ruminal gasses

When tannins bind to carbohydrates, the fermentation process is delayed and thereby the ruminal gas production is reduced. This leads to a decrease in the production of volatile fatty acids (VFA) (Waghorn and McNabb, 2003). Volatile fatty acids (propionate, butyrate, acetate) are important energy sources for ruminants, so there is less energy available when tannins are consumed. The net energy that is availably may not be sufficient to cover the needs of the animal. When this leads to a negative energy balance (NEB), the animal starts mobilizing its reserves and/or will show a reduced milk yield.

On the upside, methane production is reduced as well, and because of the reduction of gasses, bloat is prevented. The latter can benefit production rates, since less dietary energy is lost in digestion (Silanikove *et al*., 1994; Waghorn and Woodward, 2006).

Research of DSchaak *et al*. (2011) proved that VFA production was reduced, regardless of the level of CT in the forage. Supplementation of CT-extract in the diet led to a decreased ratio of acetate to propionate in high forage diet (a diet with a high forage content in relation to concentrate), while this acetate to propionate ratio increased when CT was added to a low forage diet. On the contrary, Carulla *et al*. (2005) found no effect on total VFA in sheep, after supplementation with different levels of CT-extract, but they did observe a shift in the proportions of VFA: acetate decreased while propionate increased. These effects on the acetate-to-propionate ratio are interesting in context to production levels of milk fat and milk yield, but also for methane emissions, considering less archea bacteria will be present to produce the methane, while propionate producing bacteria are stimulated (DSchaak *et al*., 2011).

2.1.4. Digestibility of organic matter

As tannins are able to form complexes with cell wall carbohydrates, they can reduce cell wall digestibility (Reed *et al.*, 1990). Sheep fed with *Albezia cyanophylla* (high in CT content) showed the lowest digestibility of fibre fractions and organic matter. As earlier mentioned, tannin levels of 5-9% lead to a reduction of the ruminal digestibility of fibre (Reed *et al*., 1985). This occurs because of the inhibiting effect that tannins have on the activity of bacteria and anaerobic fungi (Chesson *et al*., 1982).

2.2. BENEFICIAL EFFECTS

2.2.1. Prevention of pathogen colonisation

An interesting aspect of tannins' properties, is the fact that unbound CT can bind to the brush border of the epithelial layer of the intestine. This could have two possible effects: it can either impair nutrient absorption that occurs via brush borders, or it could prevent colonisation of pathogenic bacteria. If the latter happens, this could form the basis of a method to use tannins as a feed additive to control and prevent problems such as diarrhea caused by *Cryptosporidium parvum*. According to Weyl-Feinstein (2014), more research about this subject is still required, although their study confirmed this hypothesis. After supplementing the milk of neonatal Holstein Friesian calves with 3.75% concentrated pomegranate extract (CPE) (containing 10% DM polyphenols), there was a lower oocyst count and a lower intensity of diarrhea, compared to the control group. These results suggest that it could be very valuable for domestic calves to consume tannins at an early age to establish a significant reduction in the effects of pathogens. The antidiarrheal effect after supplementation of CPE could as well be explained by other factors. Onais *et al.* (2007) mention four possible mechanisms for this: (1) an increase in the reabsorption of water and ions; (2) a reduction in mucosal secretion; (3) inhibition of the intestinal mucosa, leading to a reduction in prostaglandin (PG) release (PG causes an increased net secretion of water and electrolytes in the small intestine (Rode *et al.*, 2013)) and (4) relaxation of intestinal smooth muscles.

2.2.2. Anthelmintic effects

Niezen *et al*. (1998) found less weight loss in animals that were infected with *Trichostrongylus circumcincta* and *T. colubriformis* when these animals were fed with high CT levels (cited from Molan *et al.*, 1999). According to Molan *et al.* (1999) the anthelmintic effect against *T. colubriformis* is due to the fact that CT impairs the development of eggs to L3 larvae and can inhibit eggs from hatching. The effects are proven highest when animals were fed with *Lotus pedunculatus,* compared to *L. corniculatus, Hedysarum coronarium* (sulla) and *Onbrychus vicifolia* (sainfoin). The researchers found that the higher the CT concentration was, the less L3 larvae developed. At a CT concentration of 200µg CT/mL or higher in *L. pedunculatus*, there was no development observed, while in the other plant species, at least 400µg CT/mL was needed to fully inhibit the development. As explained earlier, low CT levels increase protein flow to the jejunum and that way they can enhance the absorption of amino acids in the small intestine (Waghorn *et al*., 1987). Since better a protein metabolism is correlated with higher resistance against *Haemonchus contortus* (Coop and Holmes, 1996; Wallace *et al*., 1996), improving protein digestion is a second (indirect) method of CT to benefit parasitized sheep (Molan *et al.*, 1999).

2.3. PATHOLOGICAL EFFECTS

2.3.1. Tissue damage in the rumen

Hervás *et al*. (2003) studied pathological effects at different concentrations of Quebracho tannin powder, containing 760 g CT/kg. The authors found macroscopically visible lesions (Figure 2) in the ruminal mucosa at a CT concentration of 2.28 g/kg bodyweight. These lesions were small ulcers (approximately 0.5-3mm) that were scattered over the entire ruminal wall (mainly ventral). The ulcers were covered by necrotic material and the edges were congested. No perforation was observed, but the leasions do inhibit the nutrient uptake from the ruminal lumen. This is another indirect way for tannins to affect nutrient absorption.

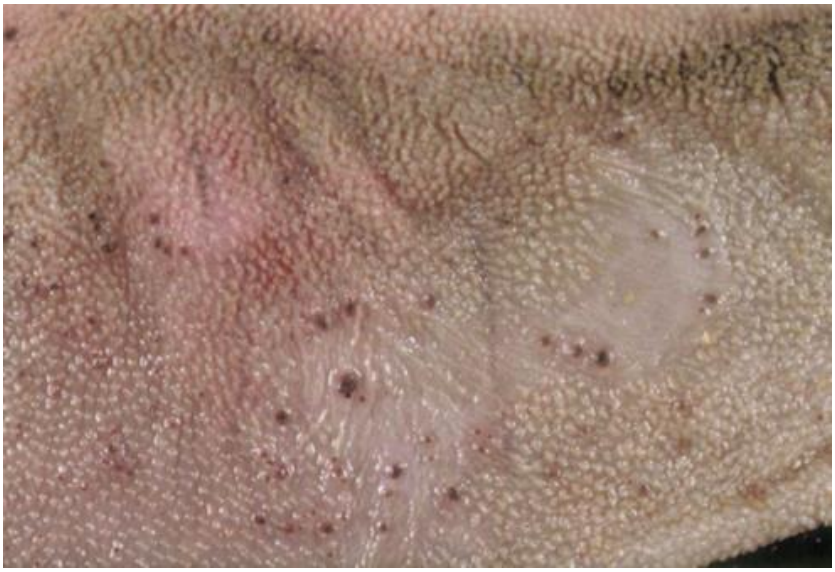

Figure 2. *Macroscopic view of lesions on the ruminal mucosa after supplementing sheep with 2,28 g CT/kg body weight (Hervás et al., 2003).*

2.3.2. Morphological changes in the intestine

According to Brooker *et al.* (2000) tannins are able to inhibit the uptake of nutrients in the abomasum and the intestine of ruminants. Activity of several enzymes are inhibited, such as alkaline phosphatase and aminopeptidase-N. Moreover, the intestinal microvilli structure is damaged (Figure 3). This proves that ruminal microbial interactions are not efficient enough to eliminate CT toxicity.

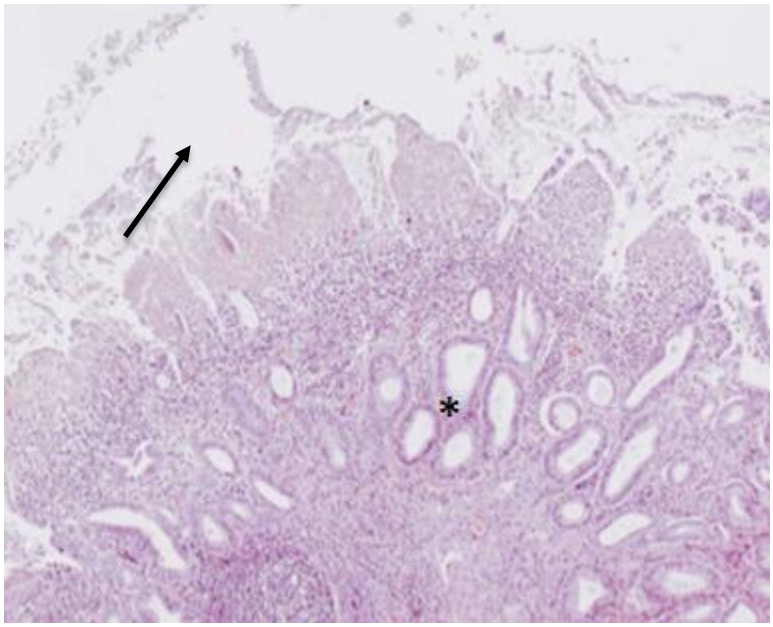

***Figure 3**. Necrosis of the intestinal microvilli in the ileum, mucosa after supplementing sheep with 2,28 g CT/kg bodyweight. At the demarcations there is inflammatory infiltrate shown (*); at the apical side of the epithelium there is loss of cells and dilatation (arrow) (Brooker et al., 2000).*

The small intestine was distended and was filled with watery contents. The mucosa did not show erosions or ulcers. The mucosa of the cecum was thickened and covered with dense mucous material.

2.4. EFFECTS ON RUMINAL FERMENTATON

Since the ruminal pH favours the formation of tannin-protein complexes, proteins are formed into so-called by-pass proteins. This causes a significant reduction in ruminal protein degradation and could lead to higher concentrations of essential amino acids in the small intestine and increase the absorption of these essential amino acids into the blood circulation and tissues (Seresinhe and Pathirana, 2003). This can be the explanation for Waghorn and Shelton (1997) witnessing a higher animal performance when the diet of the animals contained low quantities of tannins. At the same time, the reduction in protein degradation is also associated with a decrease in the production of ammonia nitrogen and an increase of non-ammonia nitrogen (Barry and Manley, 1984; Waghorn *et al.,* 1994; Waghorn, 1996). When tannins bind to proteins, they reduce the degradable fraction and the fractional rate of degradation of the proteins (Aharoni *et al.,* 1998; Frutos *et al.,* 2000; Hervás *et al*., 2000). Since tannins also have an effect on hemicellulose, cellulose, starch and pectins (Barry

and Manley, 1984; Chiquette *et al.,* 1988; Leinmüller *et al.,* 1991; Schofield *et al*., 2001), they can affect the fibre degradation in the rumen (Barry and McNabb, 1999; McSweeney *et al.,* 2001; Hervás *et al.,* 2003).

There are three main hypotheses to explain reduced ruminal digestion in the presence of tannins: (1) substrate deprivation for the ruminal bacteria (Scalbert, 1991; McAllister *et al.*, 1994b; McMahon *et al*., 2000), (2) ruminal bacterial enzyme inhibition (Barry and Manley, 1984; Bae *et al*., 1993) or (3) a direct effect on ruminal microflora (Leinmüller *et al*., 1991; Scalbert, 1991).

The first hypothesis is in agreement with the findings of Chiquette *et al*. (1988) and McAllister *et al*. (1994), where tannins were reported to interfere with the attachment of ruminal microorganisms to the cell walls of plants, and thereby preventing the degradation of the proteins.

2.4.1. Efficiency of ruminal flora

2.4.1.1. Microbial enzymes

Tannins are able to interact with microbial enzymes and to inhibit their activity (Makkar *et al.,* 1988; Mueller-Harvey and McAllan, 1992; McAllister *et al*., 1994; McSweeney *et al.,* 2001). Condensed tannins inhibit the activity of hemicellulases more effectively than they inhibit the activity of cellulases (Waghorn, 1996). This could possibly be explained by the fact that cellulases are associated with the bacterial cell walls, while hemicellulases are extracellular (Van Soest, 1994). Because of the successful inhibition of hemicellulases, several researchers found a higher reduction in the degradability of hemicellulose in presence of certain tannins (Barry and Manley, 1984; Waghorn *et al*., 1994; Hervás *et al.,* 2000). Which type of tannins and at what concentration, is not specified in these publications.

Another mechanism by which tannins could affect the activity of ruminal microorganisms is by altering the permeability of their membranes, leading to membrane disruption and leakage and eventually bacterial death (Leinmüller *et al*., 1991; Scalbert, 1991).

2.4.1.2. Microbial protein synthesis

Makkar *et al.* (1995) found that the microbial protein synthesis *in vitro*, is more efficient in the presence of tannins, even though the tannins lead to a decrease in the availability of nutrients. A higher proportion of available nutrients is channelled to microbial mass synthesis, while less nutrients are used for short-chain fatty acid production. Moreover, the non-protein nitrogen (NPN) is used by the microbes since the protein-nitrogen is not available due to the complexes that are formed (Makkar *et al*., 1997).

2.4. EFFECTS ON ANIMAL PRODUCTION

Since the consumption of tannin-rich plant material can affect the voluntary feed intake and the digestive utilisation of this feed, these effects will definitely reflect on the productivity of the animals that consume tannin rich plant material. For example, Barry (1985) observed a significant reduction in the gain of body mass in lambs that were fed with high CT content (*Lotus pedunculatus*). Besides the reduced accessibility, the digestive physiology can be impaired and the voluntary feed intake is diminished. The quantity of tannins that is consumed, is determinative for the outcoming effect, as

mentioned previously. In moderate amounts (intake of less than 50 g CT kg^{-1} dry matter (DM)), tannins, can have positive effects such as the improved utilization of feed by ruminants, mainly because of a reduction in ruminal protein degradation and, as a consequence, a higher availability of amino acids for absorption in the small intestine (Aerts *et al*., 1999; Barry and McNabb, 1999; Min *et al*., 2003; Waghorn and McNabb, 2003). The latter means that tannins can be associated with the enhancement of animal growth and productivity, and therefore minimizing the effects on the environment (Yisehak, 2013). This happens due to the combination of two abilities of tannins that were discussed in previous sections: (1) when tannins bind to dietary protein, the rate of amino acid absorption in the intestine is increased, because degradation of dietary protein would normally happen in the rumen. Thereby, the utilization of dietary essential amino acids is improved (McNabb *et al.*, 1993; Makkar *et al*., 1995), (2) When tannins bind to carbohydrates, this leads to a reduction in the ruminal gas production.

2.3.1. Carcass weight

Surprisingly, findings of Wang *et al.* (1994) and Montossi *et al.* (1996) showed that grazing of *L. corniculatus* (34 g CT kg^{-1} DM) diminishes feed intake, but increases the gain in body mass and carcass weight, suggesting a somehow improved nutrient utilization by the animals.

2.3.2. Milk production

Wang *et al.* (1996) report an increase of 21% in milk production, during the mid and late lactation in sheep that were fed with *L. corniculatus* at 44.5 g CT kg-1 DM *versus* sheep supplemented with PEG. A second interesting observation is that the efficiency of the milk production is significantly increased with respect to protein and lactose production. The fat percentage of the milk was decreased, but this is due to the dilution effect, since the concentrations of protein and lactose are increased together with the milk yield. The increased concentration of protein is probably caused by the fact that there is a greater availability of intestinal amino acids. This is especially important for the amino acids methionine and lysine, which are assumed to be the limiting factor in milk protein production. The concentration of lactose may be enhanced as a result of the combination of two things: a higher concentration of amino acids leads to an enhanced synthesis of glucose molecules, because amino acids can be used for gluconeogenesis when propionic acid is deficient. Thus, a higher concentration of available amino acids would lead to greater synthesis of glucose molecules. The other thing to cause the increased lactose concentration, is the preference of propionate production over acetate production, as described earlier. Of course propionic acid is still the molecule of preference, since the gluconeogenesis from amino acids is not energy efficient.

2.2.3. Wool production

According to Wang *et al*. (1994) and Min *et al*. (1999, 2003), the wool production increased by 10-14% after the grazing of *L. corniculatus* (30-35 g CT kg-1 DM). The researchers suggest that this increase is due to a bigger absorption of essential amino acids (particularly sulphur-containing amino acids) in the intestine. Further explanation by the authors would be necessary, since this explanation is rather contradictory, as sulphur containing amino acids have more hydroxyl groups available for

tannins to bind to (Makkar, 2003). Possibly, the tannin-protein complexes bind to other things than dietary proteins, such as digestive enzymes, when they become more soluble. An other explanation would be if these amino acids come from by-pass proteins and are therefore not available for the tannins in the rumen. Grazing on *H. lanatus* increased wool production by 10%, even though the CT concentration was much lower (4.2 g CT kg-1 DM) (Montossi *et al*., 1996) compared to the previous studies.

2.2.4. Reproduction

When sheep consume *L. corniculatus* (17 g CT kg-1 DM), the reproductive efficiency is enhanced. Min *et al*. (1999) observed a significant increase of the production of lambs by 25%. This growth happens because of increased rate of ovulations and thereby enhancing the lambing percentage. Min *et al.* (1999) suggested that this is related to an increased efficiency of protein utilization as well.

3. BROWSERS VERSUS GRAZERS

3.1. CLASSIFICATION IN TYPES OF FORAGING BEHAVIOUR

Between different species of ruminants, there are several types of foraging behaviour (Figure 4). Ruminants need to consume large amounts of plant material to maintain their metabolism, growth and reproduction (Gordon and Lascano, 1993; Iason and Villalba, 2006).

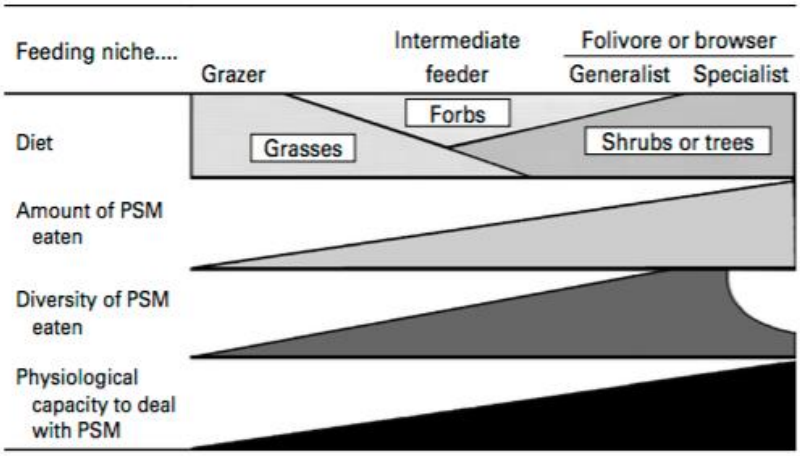

Figure 4. *Types of foraging behaviour in relation to the consumption of plant secondary metabolites (PSM) (Iason, 2005).*

According to Duncan and Gordon (1996), habitat choice varies according to the ability of the animals to eat, digest and detoxify certain feed material. Aligned with this information and based on diet choices and specialized morphology of ruminants, Hofmann (1998) classified the ruminants in three groups of feeders: grazers, browsers and opportunistic/intermediate feeders. Grazers are for example cattle and sheep; their diet is based on high amounts of feed that is rich in cellulose and fibres: mainly grasses. Browsers select foliage, forbs and other dicotyledonous matter; feed that has an high proportion of easily digestible plant material that is relatively rich in energy and protein and contains

less fibre. The disadvantage of the browsers' diet is the high level of tannins that this feed contains (Yisehak, 2013). Opportunistic feeders are an intermediate form of browsers and grazers and can behave in both ways, depending on the availability of different types of feed. This availability is often seasonally dependent. Goats are classified as either opportunistic feeders. However, even within species, there are variations possible in context of feeding behaviour. These intra-species variations are due to environmental differences and to genetic diversity (Pereira *et al.*, 2005; 2006). Herbivores that have a tannin-rich diet, can counteract on these dietary tannins, by certain adaptive mechanisms (Iason, 2005). As goats eat more browsing material compared to sheep, they have the capacity of adapting their feeding behaviour to food items available, and are thereby able to select their diet with the intention of maintaining the ratio between nutrients and tannins relatively constant over time (Figure 4) (Kabaya *et al*., 1998; Iason, 2005). As an animal eats more browses, it consumes an higher amount of PSM and the animal has an higher physiological capacity to deal with these metabolites (Iason, 2005).

Because of their highly adaptive feeding behaviour, goats can perform well in certain environments, where other ruminants would not survive, explains Silanikove (2000). Devendra (1989) adds that sheep use pasture better and are less selective, when the available plant material is of high quality. In less ideal environments (which is often the situation in Ethiopia), goats tend to select their feed more effectively and have in that case higher productivity than sheep.

3.2 ADAPTATIONS

Consuming high levels of tannins can give toxic effects and can even be fatal (Garg *et al*., 1992; Makkar, 2003). Past researches have pointed out that there are certain defence mechanisms against these effects. These mechanisms are usually found in animals that naturally have a relatively tannin-rich diet, like most browsing ruminants do. Several species developed a strategy to circumvent the dietary effects of tannins. These adaptations are genetically determined, but there are researchers that believe that there is also a form of adaptation that can be gained by continued ingestion of tannins. According to them, continued ingestion of tannins would lead to a form of adaptation with attenuation or even exclusion of the negative effects that tannins cause (Barry, 1985; Silanikove, 2000).

3.2.1. Saliva production

The major part of saliva is produced by the parotid glands. These glands are, in relation to bodyweight, significantly larger in browsing herbivores than in grazing herbivores (Hofmann, 1973). The most important strategy to avoid the effects of tannins is by excreting endogenous tannin-binding proteins in their saliva (Hofmann, 1985; Waghorn, 2008).

3.2.2. Proline-rich proteins

An high percentage of the salivary proteins of browsers is rich in proline (Robbins *et al.,* 1987; Austin *et al.*, 1989; McArthur *et al.*, 1995; Foley *et al.*, 1999 (cited from: Frutos *et al*., 2004)). These proline-rich proteins (PRP) have an high capability to bind with tannins and form complexes with them in the oral cavity of herbivores. (Kumar and Singh, 1984; Hagerman and Butler, 1991). There are three

different types of PRPs: the acidic type, the glycosylated type and basic proteins. The head function of acidic PRPs is to control calcium levels, while glycosylated PRPs help the lubrication of food boluses (Bennick, 2002; Charlton *et al*., 2002). The basic proteins seem to have an important role in binding to tannins. However, the specific role has not been completely understood (Bennick, 2002). The complexes that are formed out of tannins and PRPs are stable across the whole pH-range of the digestive tract, while this is probably not the case for other tannin-protein complexes. Because of the stability of the complexes that are formed between PRPs and tannins, the negative effect of tannins on palatability may be cancelled out. Even more important; the stability allows the tannins to pass intact through the gastrointestinal tract and to be excreted (Bennick, 2002; Marais, 2012). Therefore, feed intake will be recovered and the digestion of tannin-rich feeds will be improved (Robbins *et al.*, 1987; Austin *et al.,* 1989; McArthur *et al.,* 1995; Narjisse *et al.,* 1995). Basically most of the tannins adverse effects are neutralized by the complex formation (Marais, 2012). The excretion of the PRPs appears to be an adaptive mechanism from herbivores to enhance the ability to consume plants that have high tannin contents (Robbins *et al.,* 1987; Leinmüller *et al.,* 1991; Hagerman *et al.,* 1992; Narjisse *et al.,* 1995). The PRPs exist mainly out of non-essential amino acids and are endogenously created. They protect the dietary proteins from binding to tannins. That way, the nutritional value of proteins is kept and a qualitative nitrogen saving is ensured (Makkar, 2003; Waghorn, 2008; Marais, 2012). This could explain why the sulphur containing amino acids become more available to benefit the production levels of milk and wool.

Grazing ruminants will normally consume a diet that is relatively free of tannins (grasses, forbs). According to Jansman *et al*. (1994) and Makkar (2003), PRPs are not secreted in the saliva of most grazing animals, like sheep and cattle. However, other salivary proteins were found when these animals are fed with tannin-rich oak leaves. These salivary proteins are not rich in proline, but do have the ability to form soluble complexes with tannins a well (Makkar, 2003). Since these proteins exist in cattle that consume a tannin-free diet, Makkar and Becker (1997, cited from Makkar, 2003) believe that the purpose of them is something other than neutralizing dietary tannins. What the actual purpose may be, remains unclear. Whereas most grazers lack the ability to produce PRP, Robbins *et al.* (1987) and Austin *et al.* (1989) observed that certain types of grazers actually produce PRPs in their saliva, but only when they are consuming plants that are rich in tannins. This is an essential difference between browsers and grazers, since browsing animals secrete their PRPs continually. It remains unclear, however, if browsers are able to increase their PRP production. Robbins *et al*. (1991) found that deer and rats are capable to do this when exposed to CT.

3.2.2. Histatins

Histatins are proteins that have high affinity with both condensed and hydrolyzable tannins as well, but are only found in the saliva of certain primates (Bennick, 2002). They form complexes with tannins, that remain stable in the gut and have antibacterial and antifungal properties. These peptides do not contain proline, but are high in histidine. They appear to precipitate tannins even better than PRPs do. When they bind to tannins, they form insoluble complexes in the stomach and small intestine. These findings suggest that histatins are a defense mechanism against tannins in humans (Naurato *et al.*, 1999: cited from Makkar, 2003). The purpose of histatins in context to tannins in other

species is still unknown.

3.2.3. Microbes

In ruminants that consume high levels of tannin-containing feeds, microbes were found that are resistant to the detrimental effects of CT. These microbes seem to have some adaptive mechanism that enables them to decompose HT faster than tannins can affect the ruminal bacteria, or microbes are able to inhibit tannin activity. How this is achieved by the ruminal microbes remains unclear, but Odenyo *et al*. (1999) suggest it may happen through methylation of the phenolic hydroxyl groups. In this way, the microbes can still work efficiently despite the presence of tannins. Other stated hypotheses for adaptive mechanisms of rumen microbes are: (1) strategic utilization of lipids to protect membrane proteins (Pell *et al.*, 2000), (2) secretion of polysaccharides or glycoproteins with high affinity for binding to tannins (Nicholson *et al.,* 1986; Brooker *et al.,* 2000: cited from Makkar, 2003) and (3) activation of intestinal detoxification by tannins (Miller *et al.*, 1997). Smith *et al*. (2003) studied the effect of CT on bacterial diversity in rats. They found that gram-negative bacteria outweighed the other microbes, when the rats were on a CT containing diet. There was a significant decrease in in *Clostridium leptum*. These microbes were proven to be CT resistant, and increased in CFU in when in presence of CT. Smith *et al*. (2003) could not find an explanation for these findings. Despite all these possible mechanisms of rumen microbes to deal with tannins, it has to be mentioned that there is no evidence so far, proving that these microbes are capable of decomposing the tannin-protein complexes (Brooker *et al.*, 2000; Makkar, 2003). Further research in this area should be encouraged, as knowledge about this subject can help developing strategies to enlarge the proportion of CT resistant microbes in the gastrointestinal tract and to determine how toxic effects of tannins can be reduced (Smith *et al*., 2003).

4. INFLUENCE OF pH ON TANNIN-PROTEIN BONDS

As mentioned earlier, most researchers assume that the affinity between tannins and proteins is dependent on the environmental acidity (Hagerman and Butler, 1981; Stern *et al.*, 1996). However, more recent studies suggest that the affinity may be independent of the pH and that the solubility of the protein is affected. These studies however, have not been published yet. Still, the affinity has shown to be highest when the pH is close to the isoelectric point of the protein (Hagerman *et al.,* 1986; Makkar *et al.,* 1995). Hydrogen bonds are approximately stable between pH 3.5 and pH 8 (McLeod, 1974; Watanabe *et al.*, 1981; Makkar, 1993). This is important, since the pH varies through different parts of the gastrointestinal tract. Consequently, the tannin-protein complexes are stable at rumen pH and would dissociate when the pH falls below 3.5 (typically what is the case in the abomasum, with pH 2,5-3) or rise above 8 (what can be found in the gut of the animals). Therefore, the different pH-levels in the gastrointestinal tract (shown in Figure 5), have a lot of influence on the behaviour of the tannin-protein complexes through the tract (McLeod, 1974; Mangan, 1988; Hagerman *et al.,* 1992; Mueller-Harvey and McAllan, 1992). It is not known whether tannins dissociate from proteins in the abomasum, if these will re-complex when entering the gut and if this would be the case, whether it is to the same extent as before or not. The different parts of the

gastrointestinal tract are shown in Figure 5 with the corresponding pH-values and the resulting effect on the tannin-protein complex. Note that the protein-tannin complexes are protected from degradation in the rumen and that the free tannins inhibit the ruminal fermentation.

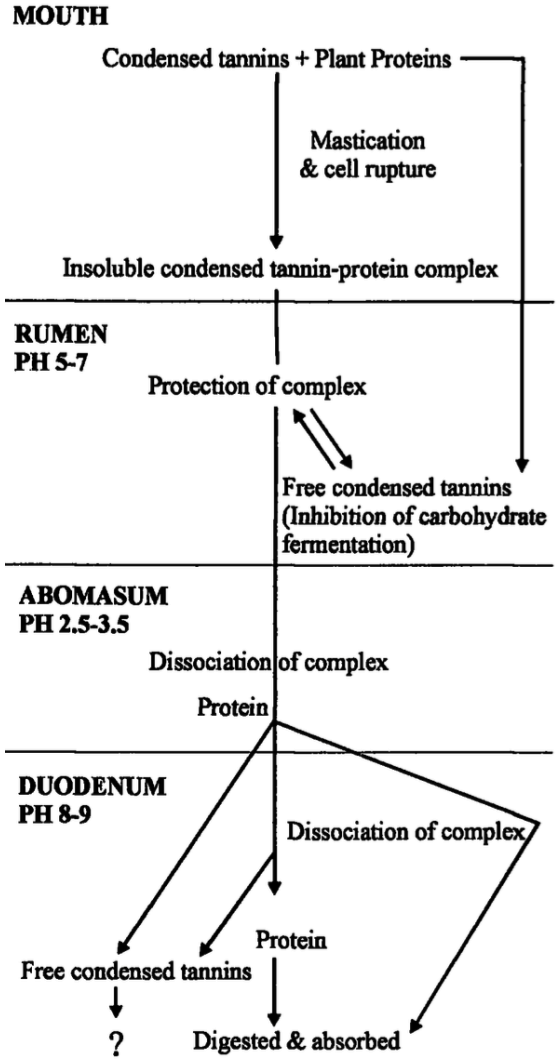

Figure 5. *Different parts of the gastrointestinal tract with corresponding pH-values and the resulting effects on the tannin-protein complexes (Seresinhe and Pathirana, 2003).*

5. RELEVANCE IN CONTEXT OF SUSTAINABLE AGRICULTURE

Ethiopia keeps approximately 25.5 million sheep and 24 million goats (CSA, 2013). The livestock sector is of great importance in this African country for economic development and helps in reduction of poverty. Despite this large livestock population, there is still a higher demand than what can be offered. As the main form of feeding ruminants in Ethiopia is still mostly dependent on free-range grazing, overgrazing is common. Furthermore, grasses from (sub)tropical climates tend to provide a lower protein availability and contain more fibre than grasses from temperate climates (Waghorn, 2008). This does not only apply to Ethiopia. Approximately half of the world's land surface is used for grazing by livestock (Holechek *et al.*, 2011). Still, the amount of grazing area is a limiting factor, and

additional feed resources are necessary to cover animal needs and increase productivity to deliver the much needed nutrients, (in particular protein) to humans. Due to the increasing population, urbanisation and economic improvement, these feed and food demands keep elevating (Salem *et al.*, 2010). Tadesse *et al.* (2015) suggested the use of leaf meal from perennial leguminous trees as a feed additive, as a protein source. This supplementation would be of particular value during the dry season, since that is the time when pasture herbage is not only low in quantity, but also in quality. Salem *et al.* (2010) agree with this and mention that integration of certain browsers (shrubs, trees) could function as additional nutrient resources. Of course, are many browses in these areas contain tannins, one should account for their net effect. Overall, several researches agree that it is necessary to focus more on sustainable, innovative production (Waghorn, 2008).

Waghorn (2008) adds that CT can help against excess nitrogen, which is very beneficial as high nitrogen levels are proven to cause infertility, nitrogen intoxication, bloat and possibly laminitis. Thus, legumes on pasture have two major desired properties: diverting nitrogen towards feces and less need for nitrogen fertiliser on the pasture (Somda *et al*., 1993: cited from Waghorn, 2008). Another asset of CT that could improve sustainability in agriculture, are the anthelmintic effects. As this leads to a decrease in use of anthelmintic drugs and to better animal production rates, Waghorn (2008) and Molan *et al*. (2002) think that this asset of CT, might be the most important one. Finally, the fact that CT in *Lotus major* reduce methane emission by 15% (Waghorn and Woodward, 2006) does not only benefit environment, but also animal production, as less nitrogen is lost during digestion.

RESEARCH AIMS

The specific aim of this research was to compare different coping mechanisms of goats with those of sheep and to learn if the tannin-protein complexes behave similarly in these species to understand the difference between both types of foraging behaviour. As goats are intermediate foragers, it is expected that they will handle the protein digestion more efficiently and that they will show a higher feed intake for the tannin-rich leaves.

Gaining information about these mechanisms can help understanding how dietary strategies can further help animals to increase the extraction of energy and nutrients from the feed and how to modulate the release of tannin-protein bonds in the gut. When such strategies are developed, the feed conversion will be more efficient. That way, less feed gives the same energy and nutrients, following a lower chance of overgrazing.

MATERIAL AND METHODS

1. ANIMALS

For the experiment six male adult goats and six male adult sheep were purchased from a local livestock market in Jimma, Ethiopia. The animals were of comparable body weight (26 – 32 kg) with an average of 26,42 kg for the goats and 30,25 kg for the sheep. All animals had similar body condition scores (BCS) (2.5/5). Their ages ranged approximately from 12 to 19 months, estimated by the fact that all of the animals had two central incisors and six milk teeth (NSW Department of Primary Industries Casburn, 2016). Directly after purchase, the animals were transported by car to the ruminant research and breeding farm of the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), based in Jimma, Ethiopia. Animals were housed in individual pens that were equipped particularly for this experiment. One day after their arrival at the farm, the sheep were clinically examined and found healthy. The goats were examined after five days. Seven days after arrival, all twelve animals were dewormed with an 1cc subcutaneous injection of 1% Ivermectine (Shanghai Tongren Pharmaceutical Co. Ltd.) and were administered 5cc 20% oxytetracycline (® Oxyvic 20) intramuscularly. Animals were housed according to the guidelines for animal health and welfare of JUCAVM, Jimma University, Jimma, Ethiopia.

2. FEED

Throughout the whole trial, all animals were provided with unlimited access to clean water. The feed consisted out of three components: a hay mixture, tannin rich leaves and concentrate. The hay mixture was harvested from a range of JUCAVM in Jimma, Ethiopia, and contained four types of plants, present in equal amounts: (1) *Eleusine coracana,* (2) *Cyperus rotundus,* (3) *Setaria verticillata* and (4) *Phyllanthus amarus*. Adjustments to the amount of feed offered were done based on an a DM intake set at 3% of their body weight. The tannin-rich plant material was composed of sundried leaves of *Milletia ferruginea*.

3. TRIAL

3.1. STARTING PERIOD

After arrival, the animals were adapted to the new environment and to the tannin-rich diet, for ten days. Before the start of the experiment, the animals were individually fed twice a day, to provide hay *ad libitum*, approximately at 8.00 hours and 16.00 hours. During this first period, the animals were given only the hay mixture *ad libitum*. Five days before the actual trial, all animals were equipped with faeces collection bags, to let them adapt to them.

3.2. ADAPTATION PERIOD

To adapt the animals to a tannin rich diet, a small amount of leaves of *M. ferruginea* was added to the feed, while the hay mixture was still provided *ad libitum*. This adaptation period continued for eleven days, to create a steady state in the metabolism of the animals. Concentrate was added to the *M. ferruginea* leaves, to maintain the uptake of crude protein and to improve the palatability of the feed.

3.3. EXPERIMENTAL PERIOD

For each animal the daily amount of hay, leaves and concentrate to be given was assessed, according to their body weight and leftovers. Each day, the leftovers were collected and weighed. Feed was given at 3% of body weight and consisted proportionally of 36% of leaves, 61% of hay and 3% of concentrate, based on a study of Yisehak *et al*. (2013).

At 8.00 hours, every animal was given the calculated daily amount of leaves of *M. ferruginea,* combined with the concentrate. Also, a small amount of salt was added to the leaves, to increase the palatability. After four hours, half of the daily amount of the hay mixture was added. This way, the animals had enough time to eat the leaves and selective feeding was controlled. At 16.00 hours, the second half of the daily amount of hay, was given to the animals.

4. SAMPLE COLLECTION AND PREPARATION

4.1. FEED

The samples of hay mixture and of leaves from *M. ferruginae* were taken randomly from different parts of the plant stocks. Next, the samples were ground in a Wiley Mill to pass through a 1mm screen. Finally the ground material was stored in an airtight container at 25°C, until analysis.

4.2. BLOOD

Blood was collected from each animal at the start and the end of the experimental period. The blood was collected from the jugular vein, using a vacutainer combined with a serum tube and an two heparinised tubes. Each tube contained at least 5cc of blood. The samples in the heparinised tubes were centrifuged at 1500 × g for ten minutes at 25°C and stored at -20°C until further analysis. Serum samples were stored at -20°C until further analysis.

4.3. SALIVA

Saliva was collected from each animal at the start and the end of the experimental period. The saliva was obtained by a swabbing collection technique (Dobson *et al.,* 1960). This technique uses two synthetic sponges of approximately 125cm^3 that are inserted into the oral cavity of each animal to be chewed by the animal for ten minutes in order to soak the sponge

with saliva. The saliva samples were filtered through a sieve (approximately 0,25mm^2) and individually stored at -20°C until further analysis.

4.4. FECES

During the seven days of the experimental period, feces was collected every morning from the fecal collection bags and weighed. Every day, 10% of the total amount per animal, was stored at -20°C until the end of the trial. After the experimental period, the stored samples were pooled per animal. Thereafter, 10% of the frozen mixed samples was oven-dried at 105°C for 24 hours. The dried samples were ground in a Wiley Mill to pass through a 1mm screen. Finally the ground material was stored in an airtight container at 25°C, until analysis.

4.5. DIGESTA

At the end of the experimental period, the animals were fasted overnight and weighed. Thereafter, the animals were slaughtered to collect digesta samples from eight different sites of the gastrointestinal tract: reticulum, rumen, omasum, abomasum, duodenum, jejunum, caecum and colon. All the samples were directly stored at -20°C in plastic bags for approximately twelve hours. The following day, the frozen samples were oven-dried at 45°C for one to three days, until constant weight was reached. The dried samples were ground in a Wiley Mill to pass through a 1mm screen. Finally the ground material was stored in an airtight container at 25°C, until analysis.

5. ANALYSIS

5.1. PROXIMATE ANALYSIS

To gain insight in the nutritional composition of the hay, leaves, concentrate, digesta and feces, the proximate analysis was executed according to the protocol of AOAC (1990). A simplified diagram of this analysis is shown in Figure 6. The feed analyses were done at the Laboratory of Animal Nutrition, Faculty of Veterinary Medicine of Ghent University, Belgium. The digesta and feces were analysed by the International Livestock Research Institute (ILRI), based in Addis Ababa, Ethiopia.

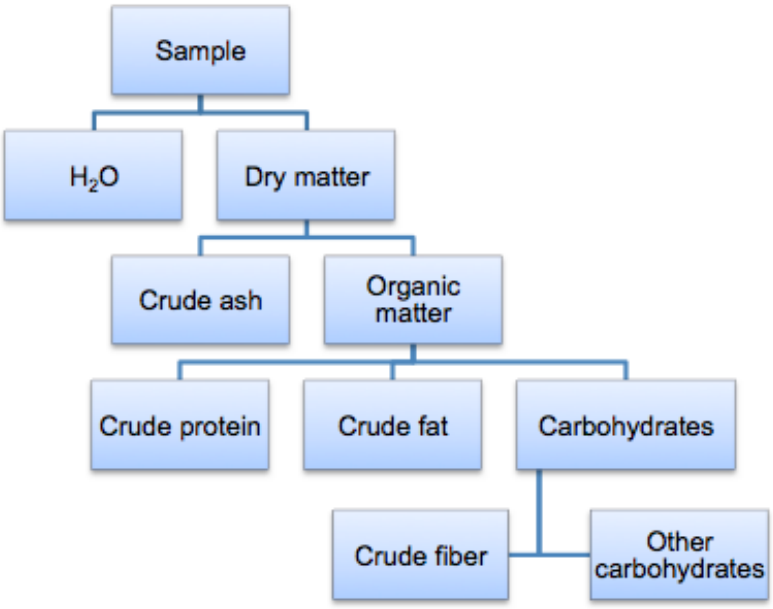

Figure 6. *Diagram showing the different elements of the proximate analysis.*

5.1.1. Dry matter

Dry matter (DM) was determined using the AOAC Official Method 930.15 (AOAC, 1990). To determine the percentage of DM in the sample, 2,0 g (weight 1) of the ground sample was weighed in glass dishes and was oven dried at 135°C for 2 hours. Thereafter, the dishes were covered and were transferred to room temperature to cool. Once the samples were cooled down, they were weighed for the second time (weight 2). Dry matter was calculated by the following formula:

DM = (weight 2 / weight 1) x 100%

5.1.2. Crude ash (mineral content)

Crude ash (CA) was determined in the feed, using the AOAC Official Method 942.05 (AOAC, 1990). To determine the percentage of CA in the sample, 2,0 g (weight 1) of the sample was weighed in a porcelain crucible and placed in a preheated Muffle furnace at 600°C for 2 hours. Thereafter, the covered crucibles were transferred to room temperature to cool. Once the samples were cooled down, they were weighed for the second time (weight 2). Crude ash was calculated by the following formula and expressed on DM basis:

CA = (weight 2 / weight 1) x 100%

5.1.3. Crude protein

To determine the percentage of crude protein (CP) in the sample, the Kjeldahl method was used (AOAC Official Method 976.05; AOAC, 1990). This method determines the amount of nitrogen molecules in the sample and thereby gives an indirect representation of the amount of crude protein.

Firstly, organic bonds are broken down by adding H_2SO_2. By digesting the sample, bound organic nitrogen is converted into ammonium ions.

$$\text{Sample} + H_2SO_2 \rightarrow (NH_4)_2SO_4 + CO_2 + SO_2 + H_2O$$

Secondly, NaOH is added to release ammonia. Finally, ammonia is distilled. Using titration, the total amount of ammonia is determined. The amount of this H_2SO_4 solution that had to be added to see a colour change (T), is directly used to calculate the percentage of nitrogen.
Crude protein was calculated by multiplying the total amount of nitrogen by factor 6.25. This factor is used under the assumption that proteins of animal feeds contain 16% nitrogen on average.

$$CP = \text{total nitrogen} * 6.25$$

5.2. VAN SOEST ANALYSIS – FIBER ANALYSIS

Cell walls of plants contain cellulose, hemicellulose, pectin and lignin, together forming the group of neutral detergent fiber (NDF). The subdivision within this group is shown in Figure 7. To determine the level of NDF in the digesta and feces, the Van Soest method can be used (Van Soest and Wine, 1967; Van Soest, 1994). The sample is boiled in buffered sodium dodecyl sulphate and ethylenediamine tetra-acetate (EDTA). This forms a neutral detergent, dissolving pectin while hemicellulose is retained. Thereafter, acid detergent fiber (ADF) is determined by adding the residue to a weak acid, dissolving hemicellulose. The residue then contains cellulose and lignin. Finally, acid detergent lignin (ADL) is determined by adding H_2SO_4 to the residue, dissolving lignin (Van Soest and Wine, 1967). The amounts of hemicellulose and cellulose are calculated using the following formulas:

$$\text{Hemicellulose} = NDF - ADF$$
$$\text{Cellulose} = ADF - ADL$$

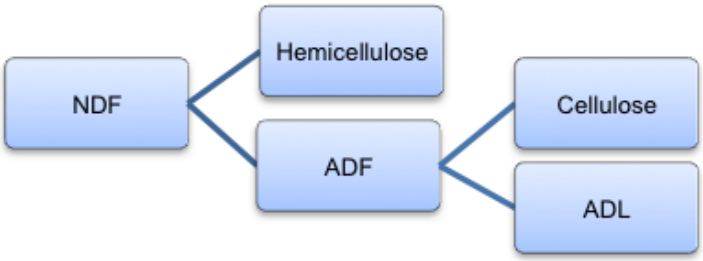

***Figure 7**. Diagram showing the subdivision within neutral detergent fiber.*

5.2.1. Acid detergent fiber

Ash-free ADF and ADL were analysed according to the AOAC Official Method 973.18 (AOAC, 1990). To determine the ADF level in the feed, digesta and feces, 1,0 g of the ground, oven

dried sample (weight 1) is dissolved in 100 mL acid-detergent solution at room temperature. This solution consists of 20 g cetyl trimethylammonium bromide that is dissolved in 1 L 1.00*N* H_2SO_4. The dissolved sample is heated to boiling in five to ten minutes. Next, the solution is refluxed in 60 minutes and then filtered into a glass crucible. The sample is then washed as follows: the glass crucible is filled with H_2O that has a temperature of 90-100°C. Then stirred and soaked for 15-30 minutes and finally dried in a vacuum oven. The washing with H_2O is performed twice. Thereafter, the residue is washed at least twice with acetone, following the same procedure. The acetone washings are repeated until all colour is removed. The residual acetone is removed using the vacuum oven. Finally, the sample is dried for three hours at 100°C in a forced-draft oven. The dried sample is weighed (weight 2). Acid detergent fiber was calculated by the following formula:

ADF	= (weight 2 – weight 1) * 100% / S
S	= weight of sample x weight of oven-dried matter

5.2.2. Acid detergent lignin

To determine the level of acid detergent lignin (ADL), 1,0 g asbestos is added to the dried sample that was used to calculate ADF. The content is covered with cooled (15°C) H_2SO_4 (72%) and stirred. After all lumps are broken down, the crucible is filled halfway with acid and stirred. Next, the crucible is refilled with H_2SO_4 (72%) and stirred every hour as the acid drains, keeping the crucible at 20-23°C. After three hours, the residue is filtered using vacuum filtration and washed with hot H_2O until pH paper indicates that it is acid free. The crucible is oven dried at 100°C, then cooled down in a desiccator over P_2O_5 and finally weighed (weight 3). The crucible is then placed into a Muffle furnace at 500°C for two hours. Next, the sample is oven dried at 100°C for one hour. Finally the crucible is transferred to the desiccator to cool and is weighed (weight 4). The asbestos blank is determined by weighing 1,0 g asbestos into a tared crucible, following the same procedure as the sample. Any weight loss that is recorded, is noted (weight 5). Acid detergent lignin was calculated by the following formula:

ADL	= (weight 3 – weight 4 – weight 5) / S

5.3. PROTEIN DIGESTIBILITY

Protein digestibility was calculated using the following formula:

Digestibility = (CP intake – CP in feces) / CP intake

As there were doubts about the correctness of DM values in feces, a standard value of 33% was used to calculate digestibility. This can lead to a systemic error in the data, so DM values will be revaluated in another experiment of this research.

5.4. pH OF DIGESTA

Before taking digesta samples from the different parts of the gastrointestinal tract, pH of each site was determined using a electronic pH-meter. Due to technical difficulties, only the pH of the organs of sheep 2, 4 and 6 and of goat 2 could be determined in the organ itself. For the other animals, procedure had to be executed *in sacco*, using plastic collection bags.

5.5. BLOOD AND SALIVA

Unfortunately, the blood and saliva samples were not analysed due to organisational difficulties and a limited time window. However, these samples will not go to waste, since they will be analysed and used in another study.

6. STATISTICS

All results were analysed using SPSS 24. To assess differences in the parameters studied between sheep and goats and between sample site, a linear mixed model was used, with animal species and sample site as fixed factors and animal as random factor. The interaction between animal species and sample site was also included in the model. Body weight was compared between species, using a repeated measures ANOVA. An unpaired t-test was performed to explore significant differences between sheep and goats, in regards to digesta pH, feed intake and fiber digestion.

RESULTS

1. FEED

1.1. FEED INGREDIENTS

The three components of the feed were analysed separately. The results of the proximate analysis are shown in Table 1. These ingredients were offered in standard proportions according to body weight (see materials and methods section), leading to a total diet with nutrients divided as shown in figure 8.

Table 1. Proximate analysis of the different feed ingredients: leaves (*M. ferruginea*), hay mixture and concentrate. All values are expressed on DM basis.

	DM	*CA (%)*	*CP (%)*	*EE(%)*	*CF (%)*	*NFE (%)*
Hay	91.9	9.79	6.42	0.98	33.41	49.29
Leaves	92.4	8.33	19.81	3.68	22.73	45.45
Concentrate	90.3	8.86	20.60	5.54	5.65	59.36

Crude ash (CA), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE).

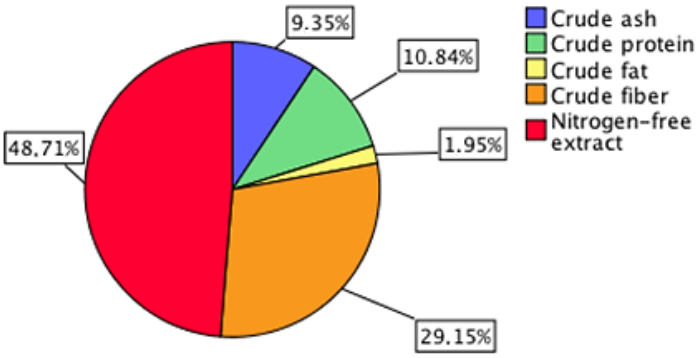

***Figure 8**. Proportional distribution of the nutrients (on FM basis) for total diet (n=3).*

1.2. FEED INTAKE

The intake of hay and leaves was calculated per animal, and expressed proportionally to the amount of feed that was offered daily (Figure 9). On average, the goats consumed significantly less hay and leaves of *M. ferruginea* compared to the sheep. For hay, goats consumed on average 10.9% less hay ($p = 0.038$) and 27.7% less leaves of *M. ferruginea* ($p = 0.01$).

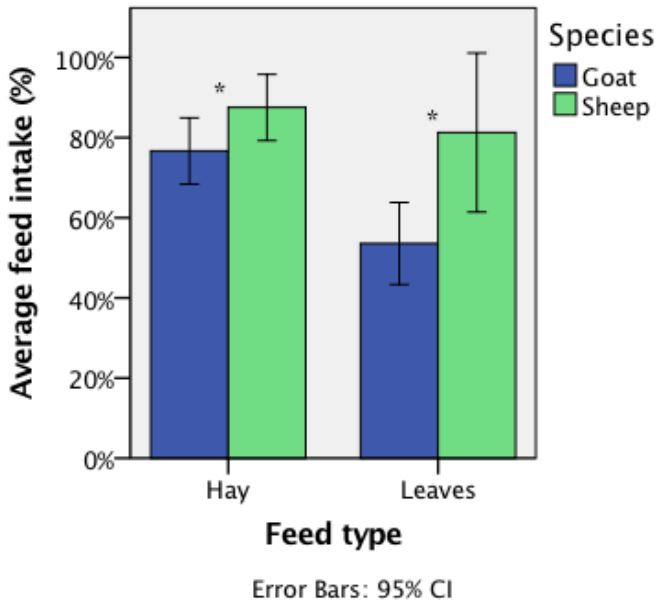

Figure 9. *Average daily feed intake expressed proportionally to the amount offered (%) for both sheep and goats (n=6). Stars indicate a significant difference between species (p<0.05).*

Using MANOVA, an interaction between the animal species and preference on feed ingredient was found (p = 0.017). Due to the different intakes of the different feed ingredients, the nutrient intake was also different between sheep and goats (Table 2), with goats having the lowest intake of feed (total and of individual feed ingredients) and of nutrients.

Table 2. Average amounts (g) of feed and individual feed ingredients ingested both on fresh matter (FM) and dry matter (DM) basis, and nutrient intake for the total diet and the individual feed ingredients (expressed in g of DM) (n=6).

	Diet	FM	DM	CA	CP	EE	CF	NFE
Goats	Hay	393	361	38.5	25.3	3.85	131	194
	Leaves	165	152	13.7	32.6	6.06	37.4	74.8
	Concentrate	26.7	24.1	2.36	5.49	1.48	1.51	15.8
	Total	585	538	54.6	63.4	11.4	170	285
Sheep	Hay	517	475	50.6	33.2	5.06	173	255
	Leaves	284	262	23.7	56.2	10.4	64.5	129
	Concentrate	30.3	27.4	2.69	6.25	1.68	1.71	18.0
	Total	831	765	77.0	95.6	17.2	239	402

Crude ash (CA), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE).

2. DIGESTA AND FECES

Digestive samples from seven sites of the gastrointestinal tract and from the feces were analysed as to their chemical composition, for both sheep and goats.

2.1. FIBER DIGESTION

2.2.1. Acid detergent lignin

Data show that ADL levels (on DM basis) remained rather constant throughout the different gastrointestinal sites, except for the abomasum and the jejunum, where ADL levels were lower for both sheep and goats (Figure 10). It is also clear from Figure 12 that the variation in CP across the gastrointestinal sites was higher than that of the ADL values.

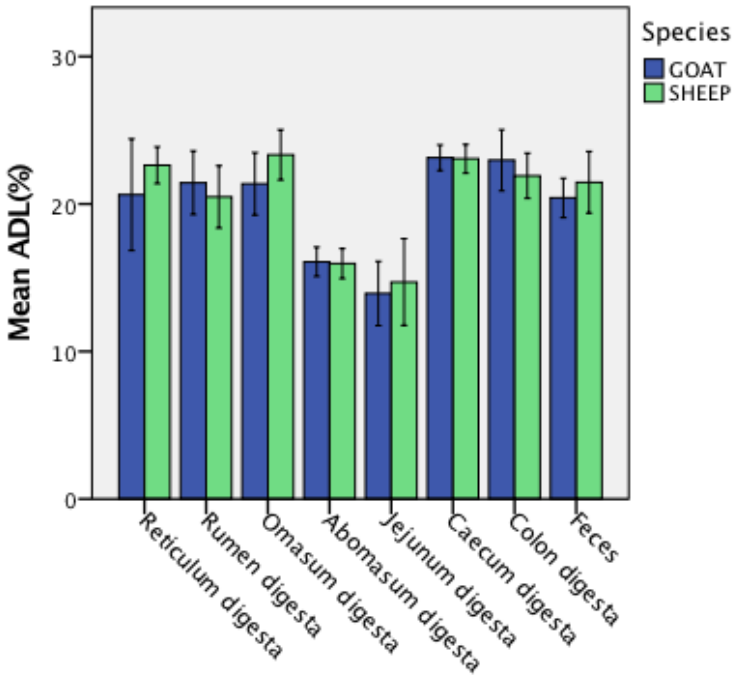

Figure 10. *Average acid detergent lignin (ADL) on DM basis in every segment of the digestive tract in sheep and goats (n=6). The error bars display the 95% confidence interval.*

2.2.2 Acid detergent fiber

To assess fiber digestibility, ADF can be used as an indicator. Individual means of ADF per species and gastrointestinal site are expressed in relative proportion to the total DM of the digesta site and proportionally to the percentage of ADL found for the different gastrointestinal sites (Table 3). To confirm actual fluctuation of ADF, the levels of ADF have to be expressed in relation to the presence of digesta lignin. Acid detergent lignin (ADL) is indigestible for both sheep and goats and is therefore useful as an internal marker, for quantification of nutrient digestibility. The data (on DM basis) show that ADF remained rather constant throughout the gastrointestinal tract with the lowest levels found in the abomasum and jejunum, for both sheep and goats. These results are similar to what was found for the ADL levels. There was found a significant difference between species for ADF in the reticulum ($p = 0.006$), but this result is not very reliable, as there was no difference found in the reticulum, when ADF was expressed in relation of ADL. There were no other significant differences between species

found for ADF, nor for the ADF to ADL ratio. However, over all the digesta, MANOVA showed an interaction between site and species for ADF (p = 0.035). To explore if there was a significant difference between the species in the separate parts of the gastrointestinal tract, a statistical analysis was performed (see materials and methods section) and p-values only are reported in Table 4.

Table 3. Digesta acid detergent fiber (ADF) in the different gastrointestinal sites, expressed on DM basis or proportionally to the amount of acid detergent lignin (ADL) in the digesta (n=6).

	ADF (%)				**ADF / ADL**			
	Goat		Sheep		Goat		Sheep	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Reticulum	48.06*	1.65	52.07 *	1.35	2.34	0.12	2.31	0.11
Rumen	52.07	2.28	52.65	1.62	2.44	0.17	2.59	0.22
Omasum	49.49	2.45	51.55	1.30	2.33	0.15	2.21	0.11
Abomasum	42.02	2.11	42.19	0.63	2.62	0.18	2.65	0.14
Jejunum	35.26	6.03	37.50	6.06	2.53	0.15	2.56	0.10
Caecum	52.22	1.66	51.80	1.19	2.26	0.09	2.25	0.06
Colon	53.51	2.07	51.01	2.65	2.34	0.14	2.33	0.08
Feces	52.65	0.63	53.03	2.34	2.59	0.17	2.48	0.17

** p < 0,05*

2.2.3 Acid detergent fiber in ratio to acid detergent lignin

When expressed relative to the digesta ADL, ADF remained relative constant throughout the gastrointestinal tract and there were no differences between species (Table 3). To explore if there was a significant difference in fiber digestion between the species in the separate parts of the gastrointestinal tract, a statistical analysis was performed (see materials and methods section) and p-values are reported in Table 4. Individual means per species and gastrointestinal site are presented in Table 3.

Table 4. Statistical output from the MANOVA model used to assess differences between gastrointestinal site, animal species and their interaction (n=6).

	p-values		
	Site	*Species*	*Site*species*
ADF (g/100g DM)	<0.001	0.162	0.035
ADL (g:100g DM)	<0.001	0.204	0.225
ADF/ADL ratio	<0.001	0.813	0.658

There was a significant effect of site on all parameters assessed, meaning that at least one gastrointestinal site differed from one or more gastrointestinal sites in levels of ADL, ADF and in the ratio ADF to ADL, whereas animal species had no significant effect on the previous parameters. There was however an interaction between the gastrointestinal site and the animal species for the digesta ADF amount expressed on DM basis (p = 0.035), but not relative to digesta ADL (p = 0.658).

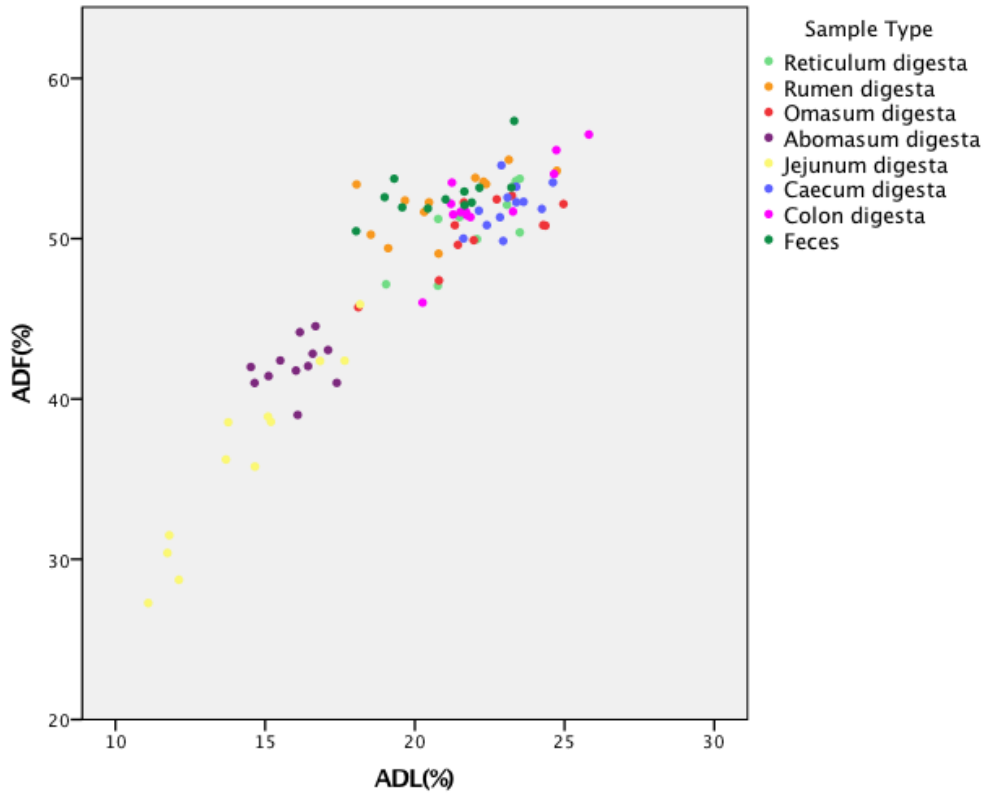

Figure 11. *Scatter plot of the acid detergent fiber (ADF) and acid detergent lignin (ADL) levels relative to the digesta DM for the different gastrointestinal sites sampled (n=12).*

2.2. PROTEIN DIGESTION

Results are expressed in relative proportion to the total DM of the digesta site (Figure 12) and proportionally to the percentage of ADL found for the different gastrointestinal sites (table 5). Based on these data, the total apparent CP digestibility was calculated, assuming a standard DM of 33.00% in the feces. Despite the lower CP intake for the goats, the latter showed an increased apparent CP digestibility (65.12 %) compared to that of the sheep (63.34%).

Abomasum and jejunum showed the highest CP levels compared to the other gastrointestinal sites, but there were no significant differences between species, except for the CP levels found in the rumen, where goats had higher CP than sheep ($p = 0.022$).

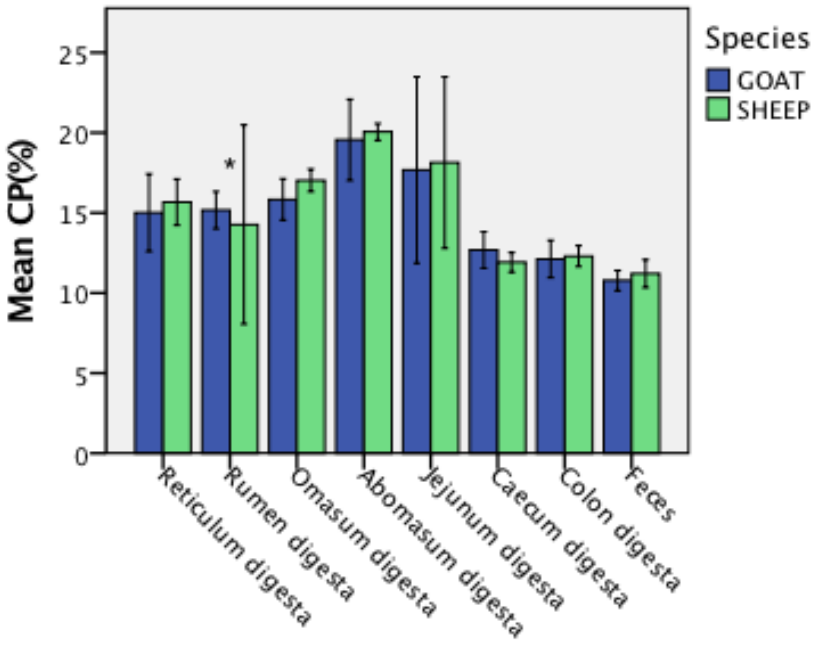

Figure 12. *Average crude protein (CP) on DM basis in every segment of the digestive tract in sheep and goats (n=6). The error bars display the 95% confidence interval and stars indicate a significant difference between species (p<0.005).*

While the CP levels were highest in most gastrointestinal sites for the sheep, in the rumen and cecum goats showed higher values than sheep. To confirm that CP is actually increased, and that this is not just a result of a dilution of nutrients in the digesta due to gastrointestinal fluids or release of nutrients through digestion or fermentation, the levels of CP have to be expressed in relation to the presence of digesta lignin have to be compared. The CP to ADL ratio in relation to the site of the digestive tract, is presented in Figures 11 and 12. As indicated earlier, acid detergent lignin, although part of the fiber component of a feed ingredient, is normally considered indigestible and can be used as an internal marker, for quantification of nutrient digestibility.

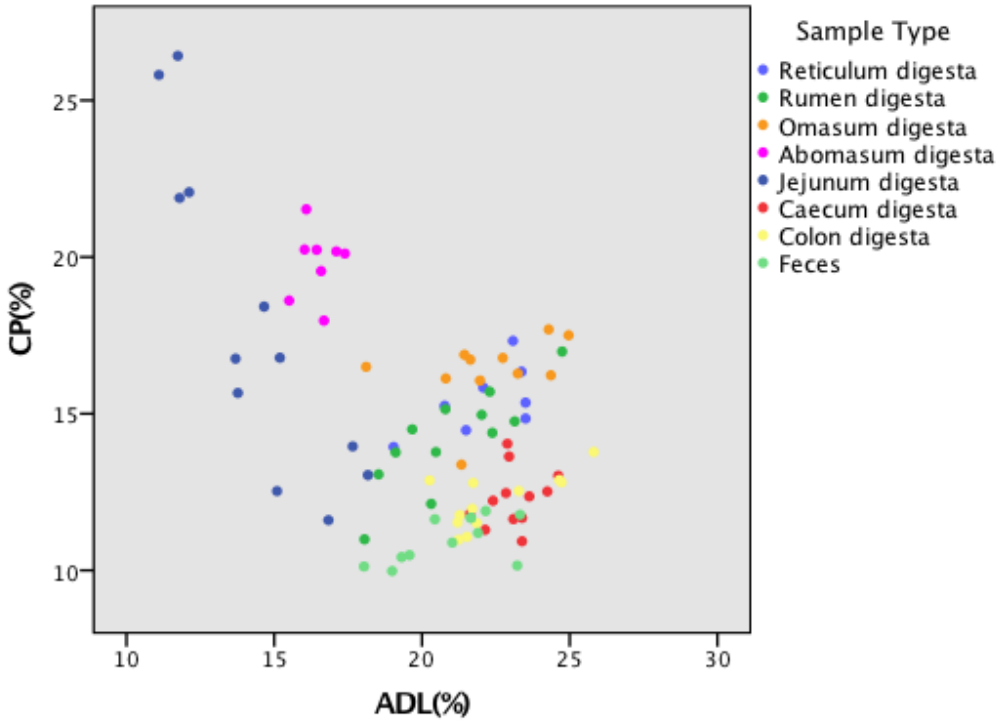

***Figure 13**. Scatter plot of the crude protein (CP) and acid detergent lignin (ADL) levels relative to the digesta DM for the different gastrointestinal sites sampled (n=12).*

When digesta CP is expressed relative to digesta ADL, a similar pattern as that presented in Figure 12, with higher CP to ADL ratios in the abomasum and jejunum compared to the other gastrointestinal sites, for both sheep and goats (Figure 13). The only difference between species for any of the gastrointestinal site, was found in the rumen ($p = 0.006$). Individual means of CP to ADL ratio per species and gastrointestinal site are presented in Table 5.

Table 5. Average crude protein (%) compared to ADL (%) per gastrointestinal site (n=6).

	Goat		Sheep	
	Mean	*SD*	*Mean*	*SD*
Reticulum	0.73	0.01	0.68	0.05
Rumen	0.71*	0.02	0.64*	0.04
Omasum	0.75	0.1	0.73	0.04
Abomasum	1.19	0.11	1.21	0.04
Jejunum	1.34	0.62	1.32	0.61
Caecum	0.55	0.05	0.52	0.03
Colon	0.53	0.01	0.56	0.04
Feces	0.53	0.01	0.52	0.05

** p < 0,05*

To explore if there was a significant difference between the species in the separate parts of the gastrointestinal tract, a statistical analysis was performed (see materials and methods section) and p-values are reported in Table 6.

Table 6. Statistical output from the MANOVA model used to assess differences between gastrointestinal site, animal species and their interaction (n=6).

	p-values		
	Site	*Species*	*Site*species*
CP (g/100g DM)	<0.001	0.854	0.083
CP to ADL ratio	<0.001	0.736	0.063

Since gastrointestinal site was always a significant factor in the model for all the parameters tested, one can conclude that every gastrointestinal site differs from one or more other gastrointestinal sites. While CP differed through the gastrointestinal sites ($p < 0.001$) both expressed on DM or proportionally to ADL, there were no significant differences found between goats and sheep both for the amount of digesta CP ($p = 0.854$) and relative to digesta ADL ($p = 0.736$). Nevertheless, there was a trend for an interaction between the gastrointestinal site and the animal species ($p = 0.083$ and $p = 0.063$, when expressed on DM or relative to ADL), but the differences in CP were too limited (Table 6).

4. BODY WEIGHT

4.1. BODY WEIGHT BEFORE AND AFTER TRIAL

In Table 7, mean body weight before and after the trial, as well as the change in body weight are presented. Note that the body weight of the goats in general has changed significantly more ($p = 0.037$) than the body weight of the sheep. Indeed, goats had an average decrease of 2.08 kg (or 8% of the initial body weight), while the sheep only had an average decrease of 0.33 kg (or 1% of the initial body weight).

Table 7. Average body weight (kg) before and after the experimental period, and the change in body weight throughout the trial expressed both in kg and proportionally to the initial body weight (n=6).

	Goat		Sheep	
	Mean	*SD*	*Mean*	*SD*
Before	26.4	2,15	30.3	1.57
After	24.3	1,60	29.9	1.37
Difference kg (%)	2.08 (8%)	1.53	0.33 (1.00%)	0.61

5. DIGESTA pH

The pH levels were measured in all eight sampled gastrointestinal sites (Figure 14). The lowest pH values were registered for the abomasum as it was expected, as this is the site where HCl is secreted to help the activity of pepsin for enzyme digestion. Surprisingly, pH values for the rumen were above what is normally reported (pH 6.80) for both sheep and goats. For the jejunum, pH values were slightly lower than what is normally reported (pH 7.00) for both sheep and goats. Goats consistently had higher pH values than sheep in all

gastrointestinal sites, except for the abomasum and jejunum. However, the differences were of no significant value (p = 0.334).

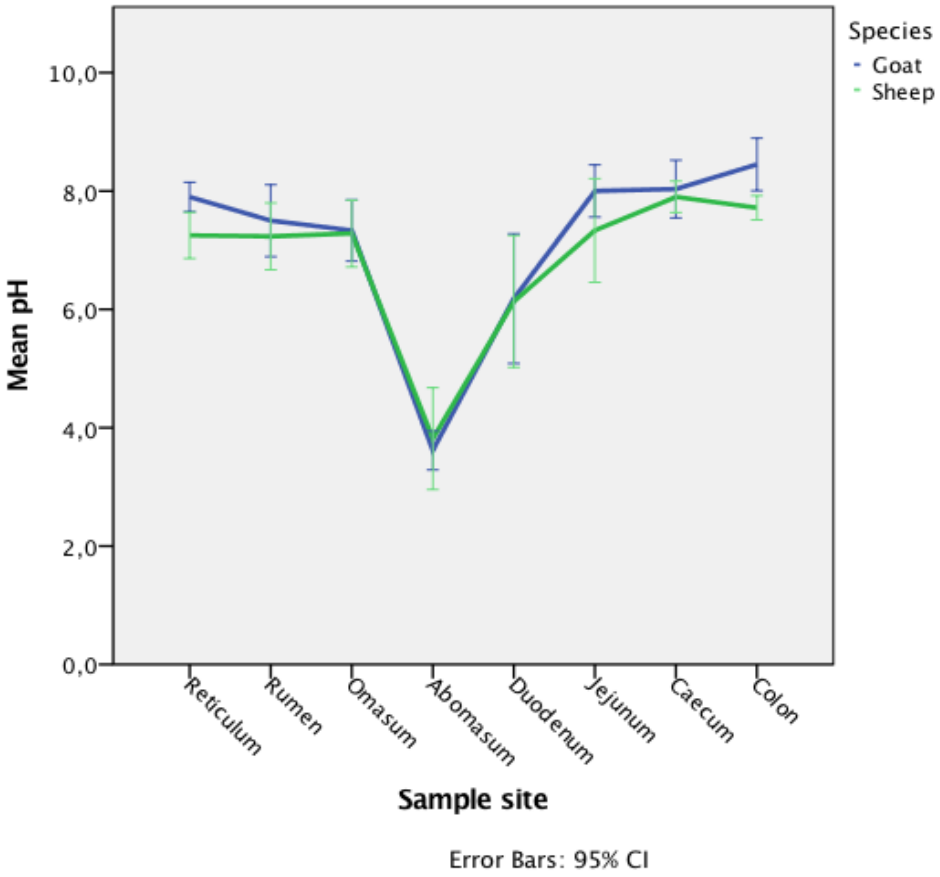

***Figure 14**. pH levels per site of the digestive tract.*

Again, to explore if there was a significant difference between the species in the separate parts of the gastrointestinal tract, a factorial mixed ANOVA was performed (see materials and methods section). The pH differed significantly between the different sites of the gastrointestinal tract ($p < 0.001$). This was of course expected, as every site has its own function and corresponding pH. There was also a significant effect of species for digesta pH ($p = 0.018$). There were no interactions found between animal species and gastrointestinal site ($p = 0.424$).

DISCUSSION

Data showed that goats consumed significantly less feed than sheep. This was observed for both *M. ferruginea* leaves as well as for the hay mixture. The lower feed intake was most probably the leading cause for the relatively large weight loss observed in the goats. It would be interesting to assess why the goats consumed less feed, as these results are in contradiction with the expectations. The hypothesis was that goats, as they are intermediate browsers, would be better adapted to the type of feed offered and therefore would consume more of it, digest it more efficiently and show better production rates. Several studies showed that the protein digestion of (intermediate) browsers follows a longer, more thorough process, compared to grazers' digestion. In other words, the passage rate of (intermediate) browsers is faster than that of grazers (Hofmann, 1998). Others suggested that the passage rate is actually only depending on body size (Illius and Gordon, 1992). Most probably, it is a combination of both (Claus and Lechner-Doll, 2001). When the passage rate is higher, there is a shorter retention of fibrous material in the rumen, causing goats to have a less efficient digestion of fiber. However, as protein digestibility is negatively correlated with fiber digestibility, the impaired fiber digestion implies a more intensive protein digestion in sheep, due to an intensive fermentation process (Varga and Kolver, 1997; Claus and Lechner-Doll, 2001). As described in the literature section, the presence of CT leads to a reduced fluid content in the rumen and an acceleration of the passage rate from abomasum to the small intestine. The CT can delay the overall passage rate throughout the entire gastrointestinal tract (Fruto *et al*., 2004). This would mean (assuming that goats are less affected by CT) that the passage rate in sheep would be even slower, causing a larger difference in passage rate between the species. In a future study, including passage rate as a parameter could be interesting.

When ADF to ADL ratio is explored per site of digestive tract of the goats, fiber digestion showed no changes. Unfortunately, the actual fiber digestibility was not calculated in this study, since ADF levels of the feed were not analysed, due to logistical issues. Therefore, it is not possible to explain the fiber digestion with full certainty. This will definitely be completed in the frame of the project where this study belongs to. Another possible explanation for the low feed intake, might be the fact that all animals were given hay *ad libitum*: a typical grazers' diet during the starting period. This could have put the sheep in advantage at the beginning of the trial, with more body reserves than the goats.

Looking at the protein digestion through the gastrointestinal tract, both the goats and sheep showed a normal physiological evolution. The high levels of CP found in the abomasum and in the jejunum can be explained by the presence of host digestive enzymes in these particular parts of the digestive tract, that contribute to protein digestion and the release of protein from the feed ingredients. In the abomasum there is a high secretion of chymosin and pepsin, while the jejunum contains chymotrypsin, trypsin, colipase and amylase, provided by the pancreatic glands (Guilloteau *et al*., 1984). It was also found that the levels of CP in the pre-absorption sites (reticulum, rumen and omasum), are 15.1% on average, and drop to an average of 11.8% in the post-absorption sites (caecum, colon and feces).

The CP values in the pre-absorption sites are a reflection of the dietary and microbial protein. A part of these proteins is digested and absorbed in the proximal intestines. This causes the CP level to drop after passing the jejunum. Hence, there is indication for protein degradation and absorption. However, when assessing the CP digestibility, the values obtained are relatively low, for both of the species. It is unlikely that these low values were caused by the fact that proteins are bound to tannins, as these complexes are expected to dissolve in the abomasum.

On the other hand, when evaluating the results of the CP levels between species, it turned out that only in the rumen and caecum, the goats show higher CP concentrations than the sheep. This is an noteworthy finding, since these two are the main fermentation sites of the digestive tract. The difference in CP at rumen level was the only significant one. There are two possible things that could cause this difference: (1) the degradation of dietary protein is of inferior intensity, and/or (2), there is an enhancement of the microbial development, leading to more microbial mass in the goats' rumen. The first can be caused by the fact that the tannin-protein complexes first need to dissolve before degradation can occur, or is a result of a shift in the microbial flora, initiated by the offered diet. It is, however, unlikely that an inferior protein degradation is causing the higher CP in the goats' rumen, because then the results were expected to also be higher in the goats' omasum, compared to the sheep (as there is no nutrient absorption in the omasum). However, the CP values in the omasum can also be explained by differences in microbial capacity and/or in the retention time of digesta. The enhanced microbial development, on the other hand, is a plausible cause. It is normally reported that goats, as intermediate browsers are better adapted to digest feed ingredients rich in tannin, opposed to sheep (grazers). This adaptation included the secretion of proline rich proteins that can bind tannins with higher affinity than the dietary protein, and a different ruminal microbes composition or activity or both. This suggests that sheep show low diet digestibility compared to goats. These statements would be true for European sheep and goats. However, it seems that sheep living in tropical countries and challenged with diets rich in tannin and lignin have also adapted to digest these diets. We know about the defense mechanisms against tannins that found in animals that naturally have a tannin-rich diet, but there are researchers that suggest that there is also a form of adaptation that can be gained by continued ingestion of tannins. According to them, continued ingestion of tannins would lead to a form of adaptation with attenuation or even exclusion of the negative effects that tannins cause (Barry, 1985; Silanikove, 2000). Indeed, Teferedegne *et al.* (1999) have reported Ethiopian sheep to have higher total short chain fatty acids (a reflection of ruminal fermentation) production that Scottish sheep when both were fed a tannin rich diet. Authors attributed the different total short chain fatty acid production to a different ruminal microbial population between the two breeds of sheep.

Another surprising result in this study, was the fact that pH values were relatively high over the whole digestive tract. This could be is as a result of microbial growth, but it is also possible that the pH is in fact an indication for another, more adapted microbial population. The large proportion of tough fibrous material in the diet probably stimulated rumination. The more the animal ruminates, the more saliva is produced and swallowed, resulting in more acid being buffered by salivary urea, resulting higher ruminal pH values. This is often the case in subtropical regions, where grasses often tend to

have low nutritional value, as they have high fiber concentrations, low levels of protein and fermentable carbohydrates and the bulk density is higher (Waghorn and McNabb, 2003). Results of Zeitz *et al*. (2016) support this theory, as they proved that grazer diets (grass hay) caused higher pH values in the rumen, than browser diets (dried leaves of *Castanea sativa, Rubus idaeus* and *Populus tremula*). In this study, the browses contained more ADL content and that could have caused a high pH through the digestive tract.

Overall, one should keep in mind that these animals received a challenging diet and this can cause the unexpected results. The differences between sheep and goats are more pronounced in Europe than in Ethiopia, as it seems that sheep have adapted to the tropical diets and can cope with tannins and lignin as well. The CP digestibility was higher in goats than in sheep. This proves that the goats were in fact better in handling the diets as efficient as possible, as the feed that they actually consume, is in fact better absorbed. The goats used a survival strategy, keeping the feed longer in their digestive tract, while it was expected that (1) the sheep would suffer more, and that (2) they would handle this by increasing their feed intake and decreasing the digestive processes. The latter could explain why the sheep had a higher feed intake. Some of the sheep even consumed 100% of their diet. It could be that the nutritional value of the whole feed, did not meet the requirements. To verify this, it would be interesting to compare the digestible protein in the feed to the protein requirements for small ruminants. Dietary crude protein is less informative, because it ignores the microbial function of the rumen. Of course, evaluating only the CP levels on DM can be misleading, since several factors, such as a possible nutrient dilution effect due to the differences in the amount of fluids present throughout the gastrointestinal tract or due to digestion and fermentation sites with increased release of some nutrients relative to others, are not taken into account for.

Generally it can be concluded that the tannin rich feed did not significantly affect the protein digestion in browsers, nor in grazers. This is because the experimental period was relatively short, and it is not possible to presume that the weight loss was a direct result of the tannin rich feed. In addition, there is also no information about the composition and chemical composition of the diet that animals consumed before they were purchased for this trial. To confirm effects of the tannin rich diet, it would be necessary to have a longer trial and to have more information on the diet animals consumed before they are purchased. Although there were little significant differences found, the results were unexpected, leading to new research questions: (1) how does the effect from tannins on microbial flora affect protein digestion (and *vice versa*), (2) how did the sheep get adapted to tannins, and (3), how do tannins influence the passage rate.

Finally, due to logistical issues, it was not possible to complete all the analysis that were planned for this study, like the tannin content of the diet, diet ingredients and digesta, as this would help understand how CP is affected or not by the presence of tannin and how animals cope with the tannin-protein complexes to increase efficiency of diet digestibility. Information on the profile and amount of proline rich proteins in the diet could help explain or not differences in CP digestibility between sheep and goats, and free amino acids in the blood would certainly help understand protein metabolism and possible differences between sheep and goats. For this study, data was not available in time, but in the future this information will be available. If it is shown that Ethiopian sheep are as

good adapted to tannin rich diets as goats, this could be a step forward in the nutrition of sheep in Ethiopia, by giving more browse to sheep and focus research on reducing of the tannin effects on diet digestibility, while helping reduce overgrazing and soil erosion.

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