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The role of dietary plant secondary compounds on diet selection, performance, milk quality and milk fatty acids in different ruminating livestock species

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List of Abbreviations

ALA	α-linoleic acid
AOAC	Official Methods of Analysis
ASALS	Arid and Semi-Arid lands
Ca	Calcium
CLA	Conjugated Linoleic Acid
CT	Condensed Tannins
DHA	Docosahexaenoic acid
DM	Dry Matter
DMI	Dry matter Intake
EPA	Eicosapentaenoic acid
FA	Fatty Acid
FAO	Food and Agriculture Organization of the United Nations
FG	Functional Group
FI	Feed Intake
GDP	Gross Domestic Product
HCl	Hydrochloric Acid
HT	Hydrolysable Tannins
KNBS	Kenya National Bureau of Statistics
Mn	Manganese
MUFA	Mono-unsaturated Fatty Acid(s)
MY	Milk Yield
NDF	Neutral Detergent Fiber
P	Phosphorous
PRPs	Proline Rich Proteins
PSC	Plant Secondary Compounds
PSM	Plant Secondary Metabolites
PUFA	Poly-unsaturated Fatty Acid(s)
RA	Rumenic Acid
RDP	Rumen Degradable Protein
RS	Rainy Season

Se	Selenium
SFA	Short-chain Fatty Acid(s)
SNV	Netherlands Development Organization
TEP	Total Extractable Phenols
TP	Total Phenols
TT	Total Tannins
VA	Vaccenic Acid
Zn	Zinc

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Summary

Livestock species of cattle, camels, goats and sheep play an important role as a source of animal products like meat and milk in different parts of the world. These animals differ in their digestive physiology and feeding behavior, and they are therefore grouped into different ruminant feeding types. These livestock species select or consume feeds with varying amounts of plant secondary metabolites (PSM). Understanding the transfer of PSM from the feed to milk and how these animal species cope with different contents of PSM in their feeds is of great importance. The aim of the doctoral thesis was to get a comprehensive view on the intake and transfer of PSM to milk in relation to either browsing (camel), intermediate feeders (goats) or grazing (cattle & sheep) herbivores. The research addresses questions about intake of PSM by camels and cattle in different seasons or dairy sheep and dairy goats fed under controlled conditions and the transfer of PSM from feed to milk.

The research was carried out in two different climatic regions. The first study was conducted in the East African rangelands of Laikipia, Kenya to assess the diet selection, performance, intake of PSM, transfer of PSM to milk and effect of PSM on milk FAs of camels (*Camelus dromedarius*), crossbred (*Bos taurus* × *Bos indicus*) and local cattle (*Bos indicus*) types (n=12 per animal type) in rainy season (RS) and transition period (TP) for 36 days/season. It was further evaluated whether supplementation with rumen degradable protein (RDP) (*n*=6 per treatment) affected plant selection and performance. There were clear differences in the diet selection pattern of camel and cattle kept on semi-arid rangelands, with no competition in their diet selection while the cattle types had similar diet selection. Both cattle types selected grass as their main diet while the camels selected herbs, shrubs and trees with higher contributions of woody plants in TP than RS. The supplemented camels had overall higher browsing time in the TP than the un-supplemented camels. Supplementation had no effect on milk yield and gross composition. Camels and local cattle are better adapted to seasonal changes in terms of milk yield than the crossbred cattle in the drier TP but not in RS and extra RDP can be used to increase the intake of woody species and, therefore, can be used to manage the encroachment of woody species in the rangelands. The phenols in the feed of cattle and camels were positively transferred to their milk. The results further show that seasonal variations in forage quantity/quality, plant species selected by the animals and animal type affected milk FA profile and phenol concentration in milk. The phenol excretion with milk were correlated with phenol intake in cattle types but not camels indicating that camels have a different phenol and lipid metabolism than cattle. The research also illustrates the

importance of camels and local cattle in utilizing the semi-arid rangelands of East Africa especially in the dry periods than improved cattle breeds that require supplementary feeding.

The second study was conducted in Switzerland with dairy sheep (grazer) and dairy goats (intermediate feeder) to investigate a possible transfer of total phenols from grapeseed extract to blood, milk and excreta and side-effects on performance and milk quality in a controlled feeding experiment (n=11 sheep; n=9 goats). This study indicated that part of the phenolic compounds in grapeseed extract could be transferred to blood, milk and urine. Goats saliva had overall higher tannin binding affinity than sheep saliva at the end of the experiment with no species differences at the start of the experiment. There was no effect of the phenolic diet on the tannin binding capacity of the saliva. An inclusion level of 4 % of grapeseed extract in dietary dry matter in the feed of dairy goats and sheep did not influence performance, blood antioxidant capacity, concentration of phenols in feces and total protein in saliva. The outcomes of this study show that agro-industrial by products like grapeseed extract rich in polyphenols can be used in animal feeding and do not have negative effects on performance.

In conclusion, the two studies investigated in this project revealed that polyphenols in the diets of cattle and camels in free range environments and polyphenols in grapeseed extract fed to goats and sheep in a controlled feeding experiment can be transferred to milk. Browsers (camels) and intermediate feeders (goats) seem to have a better coping mechanism to limit harmful effects and transfer of phenols to milk in response to intake of phenols than are grazers (cattle and sheep).

Zusammenfassung

Rinder, Kamele, Ziegen und Schafe zählen zu den wichtigsten Nutztieren, da sie eine grosse Rolle als Lieferanten von Fleisch und Milch in verschiedenen Regionen der Welt spielen. Diese Tierarten unterscheiden sich in ihrem Fressverhalten und ihrer Verdauungsphysiologie, dementsprechend differenziert man zwischen Browser (Kamel), Intermediate Feeder (Ziegen) and Grazer (Rinder, Schafe). Sie wählen oder konsumieren Pflanzenarten mit unterschiedlichen Mengen an sekundären PSM. Es ist wichtig, zu verstehen, wie die Pflanzeninhaltsstoffe aus dem Futter in die Milch übertragen werden, und, wie die genannten Tierarten mit unterschiedlichen Gehalten an Pflanzeninhaltsstoffen in ihrem Futter umgehen. Ziel der Dissertation war es, einen umfassenden Überblick über die Aufnahme und den Transfer von PSM in die Milch in Bezug auf verschiedene pflanzenfressende Tierarten zu erhalten, die sich hinsichtlich ihres Fressverhaltens unterscheiden: browsing (Kamel), intermediate feeding (Ziegen) oder grazing (Rinder und Schafe). Die Arbeit befasst sich mit Fragen der Futteraufnahme von Pflanzeninhaltsstoffen durch Kamele und Rinder in verschiedenen Jahreszeiten. Ausserdem wurde der Transfer von Pflanzeninhaltsstoffen aus dem Futter in die Milch von Milchschafen und Milchziegen, die unter kontrollierten Bedingungen gehalten und gefüttert wurden untersucht.

Die Forschung wurde in zwei verschiedenen Klimaregionen durchgeführt. Die erste Studie wurde im ostafrikanischen Weideland von Laikipia in Kenia durchgeführt, um die Futterauswahl, die Leistung, die Aufnahme von PSM und die Auswirkungen auf das Milchfettsäuremuster von Kamelen (*Camelus dromedarius*), Kreuzungsrindern (*Bos taurus* × *Bos indicus*) und lokalen Rinderrassen (*Bos indicus*) (n = 12 pro Tierart) in der Regenzeit (RS) und Übergangszeiten (TP) für 36 Tage/Saison zu beurteilen. Es wurde weiter evaluiert, ob die Ergänzung mit im Pansen abbaubarem Protein (RDP) (n = 6 pro Behandlung) die Pflanzenauswahl und Tierleistung beeinflusste. Es gab deutliche Unterschiede im Muster der Futterauswahl von Kamelen und Rindern, die auf semi-aridem Weideland gehalten wurden, wobei keine Überschneidungen in der Pflanzenauswahl auftraten. Beide Rindertypen wählten Gras als Hauptfutter, während die Kamele Kräuter, Sträucher und Bäume, mit einem höheren Anteilen der letzten beiden funktionelle Gruppen in der TP als in der RS, auswählten. Die mit RDP zugefütterten Kamele hatten insgesamt eine längere Zeit die mit Fressen von Bäumen und Sträuchern verbracht wurde in der TP als die nicht mit RDP ergänzten Kamele. Die Supplementierung hatte keinen Einfluss auf die Milchleistung und die Zusammensetzung der Milchinhaltsstoffe. In Bezug auf Milchleistung sind sowohl Kamele und lokale Rindertypen besser an die saisonalen Änderungen angepasst als

Kreuzungsrinder, allerdings nur in der trockeneren TP und nicht RS. Die Ergänzung mit zusätzlichem RDP kann dazu verwendet werden/Eine Ergänzung, um die Aufnahme und Verwertung an holzigen Pflanzenarten in den Weidegebieten zu erhöhen. Die Phenole aus dem Futter der Kamele und der Rinder wurden positiv in die Milch transferiert. Die Ergebnisse zeigten im Weiteren, dass saisonale Schwankungen in der Futtermenge und Qualität und der Tierart das Muster an Fettsäuren (FA) und die Phenolkonzentration in der Milch beeinflussten. Die Exkretion der Phenole korrelierte mit der Aufnahme der Phenole, allerdings nur bei den Rindertypen aber nicht bei Kamelen. Das weist auf einen unterschiedlichen Effekt von Phenolen auf den Fettstoffwechsel der Kamele im Vergleich zu den Rindern hin. Die Forschung illustriert im weiteren die Bedeutung der Kamele und der lokalen Rindertypen hinsichtlich der Nutzung des semi-ariden Weidelands Ostafrikas, wobei im Besonderen während der Trockenperioden Kreuzungsrinder zusätzliches Futter benötigen.

Die zweite Studie wurde in der Schweiz mit Milchschafen und Milchziegen durchgeführt, um den Transfer an Gesamtphenolen aus Traubenkernextrakt ins Blut, die Milch und die Exkremente, sowie die Nebenwirkungen auf die Leistung und Milchqualität in einem kontrollierten Fütterungsversuch (n = 11 Schafe; n = 9 Ziegen) zu untersuchen. Diese Studie deutete darauf hin, dass ein Teil der Gesamtphenole aus dem Traubenkernextrakt ins Blut, die Milch und den Urin transferiert werden können. Die Futterration hatte keinen Effekt auf die Tannin-Bindungsaffinität des Speichels. Die Ergebnisse im Speichel der beiden Tierarten zeigten am Ende des Experiments eine insgesamt höhere Tannin-Bindungsaffinität des Ziegen-Speichels gegenüber dem Speichel von Schafen. Zu Beginn des Experiments zeigten die beiden Spezies keine Unterschiede in der Tannin-Bindungsaffinität des Speichels. Bei einem Anteil von 4% Trockensubstanz Traubenkernextrakt im Futter von Schafen und Milchziegen wurden die Leistung, antioxidative Blutkapazität, die Konzentration an Phenolen im Kot und das Gesamtprotein im Speichel nicht beeinflusst. Die Ergebnisse dieser Studie zeigen, dass agroindustrielle Nebenprodukte wie polyphenol-reiche Traubenkernextrakte in der Tierernährung verwendet werden können und keine negativen Auswirkungen auf die Leistung haben. Spezifische agro-industrielle Nebenprodukte wie Traubenkernextrakt, welche reich an Polyphenolen sind, sollten auf ihre Wirkung und Verwendung in der Tierernährung im Hinblick auf Nebenwirkungen, Leistungen und Produktqialität evaluiert werden.

Zusammenfassend zeigen die beiden Studien dieser Doktorarbeit, dass Phenole in der Nahrung von Rindern und Kamelen in Freilandumgebungen und Polyphenole in Traubenkernextrakt, die Ziegen und Schafen in kontrollierten Fütterungsexperimenten gefüttert werden, in die Milch überführt werden

können. Browser (Kamele) und Intermediate Feeder (Ziegen) scheinen die schädliche Wirkungen von Phenolen und deren Transfer in die Milch besser regulieren als Grazer (Rinder und Schafe)

Chapter 1

General introduction

1.1 Agriculture and livestock production in developing countries with focus on Kenya

Livestock and livestock products contribute to the livelihood of many people around the world and the trend in livestock production is changing due to urbanization and increases in income especially in the developing countries and as a result, the demand for livestock products will continue to increase (Herrero et al. 2009). About 50% of the world's land area is rangeland (Friedel et al. 2000) and they are an important resource as they support a large population that derive their livelihood from them. Rangelands are important feed resources for livestock in the rural areas where majority of the people depend on livestock in different parts of the world and it is estimated that rangelands provide about 70% of the feed for domestic ruminants (Nicholson 2000).

In Kenya, agriculture is one of the most important sectors, contributing approximately 26% of the gross domestic product (GDP), and employs more than 40% of the national labor force (Kenya Bureau of Statistic, KNBS, 2009). A big population of Kenyans live in the rural areas and they depend directly or indirectly on agriculture. Livestock production, a sub sector of agriculture, is an important component of the Kenyan economy, with 21 million head of cattle, 27 million goats, 19 million sheep and 3 million camels in 2016 (FAO). Livestock production contributes to almost 90% of the livelihood of the rural population and accounts for nearly 95% of family incomes in the arid and semi-arid areas (ASALs) of Kenya and more than 70% of all Kenya's livestock is found in the ASAL (Kenya Ministry of Agriculture, 2008). According to Netherlands Development Organization (2008) they estimated that the Kenyan livestock sector contributes about 12% to the Kenya's national GDP and 30% to the agricultural GDP.

Arid and semi-arid rangelands cover about 80% of the total Kenya surface area which is home to over 25% of the total estimated 40 million inhabitants (KNBS, 2009). Kenyan rangelands play an important role in livestock production as they support a wide range of livestock production systems. The main livestock production systems in Kenya are the high potential areas in central and western Kenya that receive high rainfall with smallholder dairy farmers, the medium potential rangelands with commercial ranches and small mixed farming and the low potential arid rangelands inhabited by the pastoralists (Kahi et al. 2006). In high potential areas livestock production is threatened by population pressure while in ASAL land degradation, droughts and soil erosion are the main threats (Kahi et al. 2006).

The main cattle types kept in Kenya comprise of the local small East African shorthorn Zebu (*Bos indicus*), European cattle breeds (*Bos taurus*) mainly the Holstein, Friesian, Ayrshire, Jersey, Guernsey and their crosses with local Zebu (Bebe et al. 2002). In Kenya, all indigenous cattle are of the shorthorn,

thoracic humped zebu type and are found throughout the country and are the majority of cattle in Kenya (Bebe et al. 2003). Among the local cattle are the Pokot cattle from the Pokot ethnic group and the Boran cattle that originate from southern Ethiopia. The European cattle and their crosses are not popular in the ASALs compared with local Zebu cattle because they are more susceptible to tsetse flies and tick-borne diseases (Maloo et al. 2001). Crossbreeding between the high yielding European cattle breeds and the local cattle like "Pokot cattle" has also been promoted in the high potential areas to improve performance of live weight and milk yield. The dromedary camel (*Camelus dromedarius*) is an important livestock species that is well adapted to ASALs in Kenya. Camels and local cattle have the ability to live and perform better than the exotic cattle under the harsh climate, low feed and other management conditions that is prevalent in the ASALs, and hence their acceptance among the pastoralist (Yagil, 1994). The purebreds of European breeds are not adapted to the conditions in the dry areas and are only kept in the high potential areas of Kenya.

1.2 Diet selection by ruminants

In general, rangelands comprise of different habitat types ranging from open grasslands, shrub dominated areas to woody or bush vegetation with different amounts and composition of plant cover and plant species (Holeck et al., 1998). The composition of plant species and plant cover on the rangelands depend on environmental conditions like rainfall and seasonal variations in temperature (Tieszen et al. 1979). Ruminant species are found in different rangelands throughout the world and they feed on plants differing in physical and chemical compositions (e.g., grasses and woody species). Hofmann and Stewart (1972) (Figure 1) classified ruminants as: 1) "bulk and roughage" feeders or grazers that select diets containing < 25% browse; 2) "concentrate selectors or browsers" that select diets containing at least 75% fruits, dicot foliage, and tree and shrub stems and foliage; or 3) intermediate or mixed feeders that select both grasses and browse. They used this scheme to classify 65 ruminants on four continents. Hofmann (1989) classified these ruminant species: 25% were grazers, 40% were browser, and 35% were mixed feeders. Hofmann (1973) observed the foraging behavior of ruminants according to their digestive anatomy, and found that the digestive system adaptations correspond to the ecological role of the animal. The grazers are better adapted to consuming slowly digested plant fiber (typical of grasses) while browsers are adapted to consuming highly digestible plant material. Grazers have a large rumen with a complex omasum, a structure suitable for selective retention and prolonged fermentation of cellulose particles while the browser rumens are densely

papillated, have a rapid absorption capacity, and they have large salivary glands that produce saliva to help buffer the digestible plant material and aid in the passage of feed from the rumen (Hofmann 1989). Intermediate feeders have a digestive system that is in between those of browsers and grazers and their resemblance of the digestive tract to those of grazers or browsers depends on the amounts of grass or browse in their diet, and varies according to season (Hofmann 1989). The highly digestible diets are digested more rapidly and more completely than the low digestible diets like grasses. The diets consumed by the different animal types have differences in plant chemical composition especially between grasses (monocot) consumed by grazers, herbs and browse (dicot) that is consumed by browsers (Coppock et al. 1987). Grasses are characterized by thick cell wall with less digestible fiber while browse which consist of thin cell wall with the cell content containing highly digestible plant compounds (Demment and Van Soest 1985).

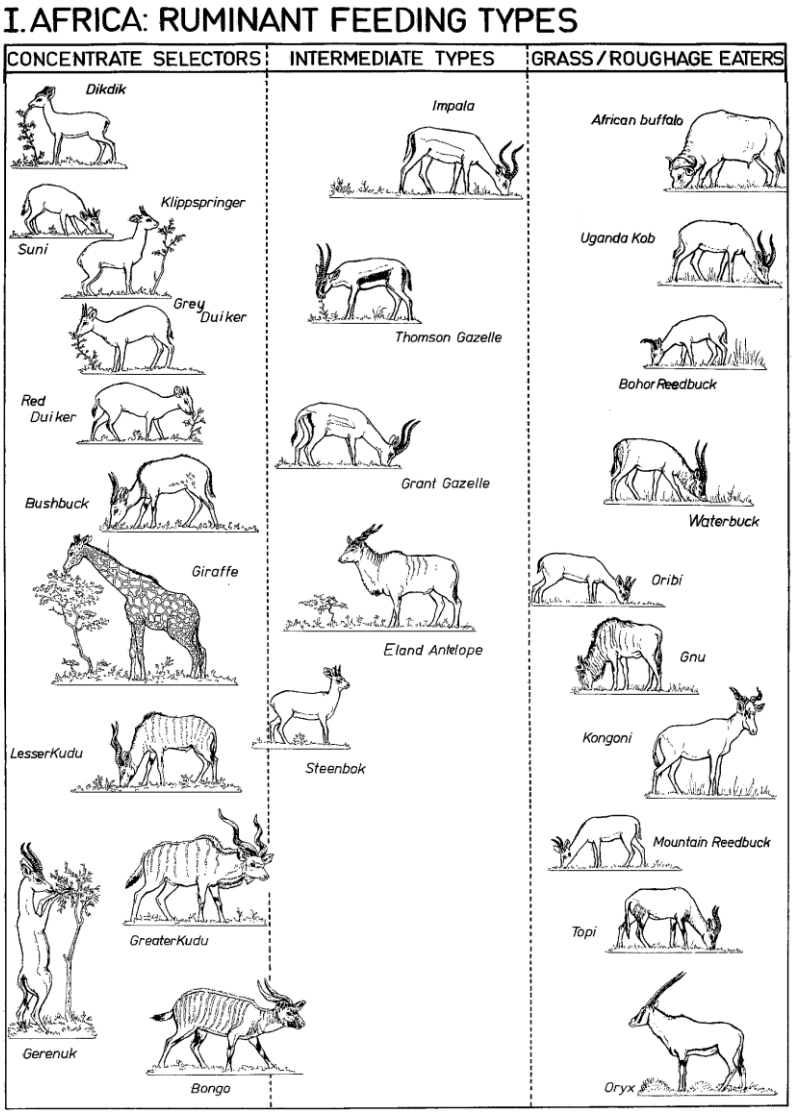

Fig. 1. African ruminants classified according to feeding type (Hofmann, 1973)

1.3 Secondary metabolites in plants

Plant secondary metabolites are a group of bioactive compounds that are products of plants/plant metabolism which are not directly involved in the growth, development, and reproduction of the plants (Irchhaiya et al. 2015). Plant secondary metabolites (PSM) exist in different classes in plants, and their concentration in plants is affected by several factors that include genetics and environmental factors (Estell 2010). Plant secondary metabolites are prevalent in dicotyledons, but almost absent in monocotyledons (Duncan and Poppi 2008) (Figure 2). For instance, the African browse species contain higher levels of PSM especially polyphenols than plants from other regions (Makkar and Becker 1998). Plant secondary metabolites act as defense mechanisms against pathogens, pest, and grazing herbivores

(Swain 1979). Plant secondary metabolites can be grouped based on their chemical structure (e.g., terpenoid, phenols, alkaloid), modes of action, or their concentration in plants (Dearing et al. 2005). A common group of PSM are the phenolic compounds that are present in plants and plant derived foods and beverages. Phenolic compounds, are a group of chemical compounds consisting of an aromatic ring structure with one or more hydroxyl groups, which are important in a variety of plant physiological functions ranging from plant growth and reproduction and protects against herbivory and insects (Bravo, 1998). They comprise a large diversity of structures, including simple molecules (e.g., phenolic acids) to large molecules such as tannins (Bravo 1998). Polyphenols can be categorized into extractable polyphenols which are of low to medium molecular mass that can be extracted using different solvents and non-extractable polyphenols of high-molecular-weight that remain insoluble in the solvent (Bravo et al. 1994).

Tannins are complex phenolic compounds found in plants (Makkar 2003) and are divided into condensed tannins and hydrolysable tannins. Tannins can have negative and positive effects on the livestock depending on their concentration in plants, animal species and physiological state of the animal (Makkar 2003). These effects on animals include improved performance of milk and body weight to interference with feed intake, digestion or metabolism of energy or nutrients and acute toxicity and death (Janzen 1979). Ruminants can cope with negative effects of tannins using several strategies that include behavioral responses, physiological mechanisms, microbial adaptations and detoxification strategies (Estell 2010).

Production of salivary rich proline proteins and liver detoxification are physiological strategies used by animals to cope with tannins (Estell 2010). Salivary rich proline proteins have the ability to bind with tannins and form complexes that inhibit the negative effects of tannins like reduce feed intake (Estell 2010). These complexes formed are excreted in feces (Shimada 2006). Browsers like deer have been shown to produce these proteins (Robbins et al. 1991). Studies on goat and sheep saliva show contrasting results regarding the role of saliva as a defense mechanism against tannin ingestion with some reporting that goats and sheep do not produce salivary rich proline proteins as a way to neutralize tannins (Austin et al. 1989; Robbins et al. 1987) while others studies report the presence of tannin binding salivary proteins in goats and sheep (Alonso-Díaz et al. 2010). Hanovice-Ziony et al. (2010) did not detect tannin binding salivary proteins in goats while Lamy et al. (2009) found differences in amounts of salivary protein between goats and sheep. The saliva of cattle fed tannin rich oak leaves are not rich in proline (Makkar and Becker 1998). Robbins et al. (1987) suggested a possibility of salivary

proteins in sheep being induced by consumption of plants rich in tannins. Lamy et al. (2011a) reported increased saliva total protein concentration in goats and sheep after consumption of quebracho tannin enriched diets.

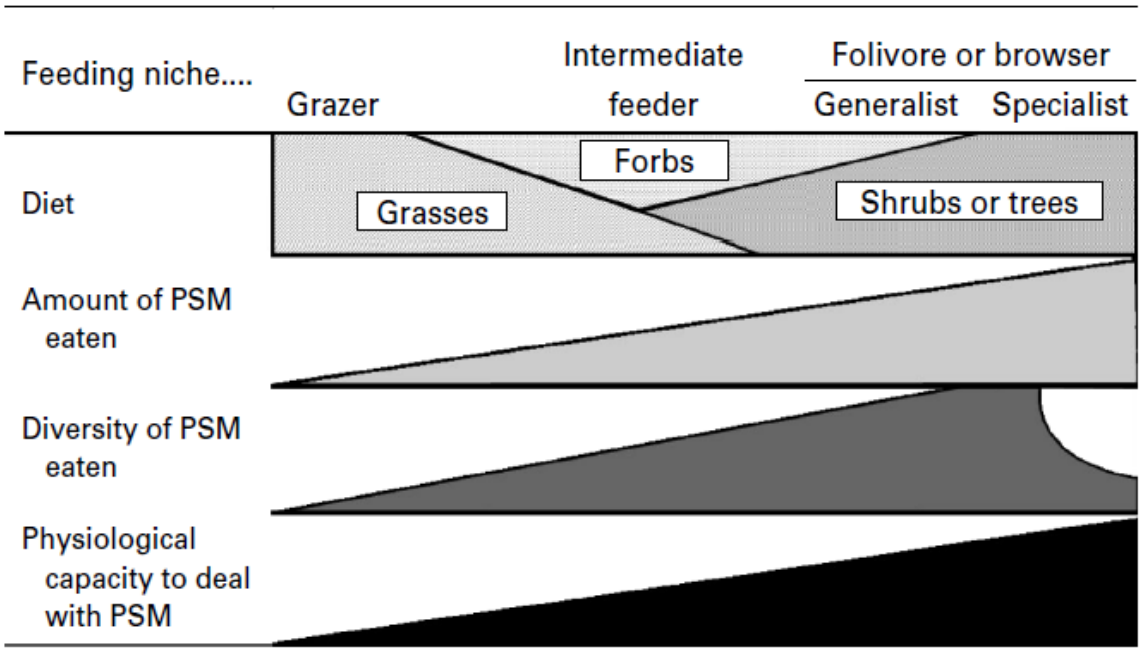

Fig. 2. Types of foraging behavior in relation to the consumption of plant secondary metabolites (PSM) (Iason 2005).

1.4 Transfer of plant secondary metabolites to animal products

Transfer of substances from feed to animal products has been investigated in several small ruminant studies (Jordán et al. 2010; Moñino et al. 2008), mainly for the purpose of assessing the risk to consumers of animal-source products posed by these substances in animal feed (Leeman et al. 2007). The inclusion of compounds like polyphenols in animal feed products has become an important issue for the producers, to satisfy consumer's needs for quality of animal products. From several studies it is evident that transfer of PSM to animal products vary greatly depending on the type of PSM, differences in animal metabolism, concentration in feed and exposure periods for each livestock species and breed (Leeman et al. 2007). The main pathways through which these compounds are transferred to livestock products is through the gastro-intestinal tract and into the bloodstream, mammary gland and urinary excretion. Majority of plant phenols are metabolized into conjugates in the liver which are then excreted via urine (Scalbert and Williamson 2000; Singleton 1981). Excretion in the urine is the primary route

for elimination of most water-soluble substances and the indigestible complexes that are formed between condensed tannins and protein that pass through the intestine are excreted in feces (Bravo et al. 1994). The differences in the excretion rate of polyphenols either in urine and feces is because of their chemical differences mainly related to their size, nature of the phenolic compounds and degree of solubility (Bravo 1998). O'connell and Fox (2001) reviewed the importance of phenolic compounds to improve the quality of milk and dairy products. Dietary polyphenols, or their metabolites, can be transferred to the animal products and can influence the quality and the characteristics of products, mainly due to their antioxidant properties (Moñino et al. 2008; O'grady et al. 2008). For instance, the inclusion of 10% and 20% levels of rosemary leaves, a plant rich in polyphenols in the sheep diet did not affect the animal performance or milk quality (Jordán et al. 2010) and showed a positive transfer of polyphenols like flavonoids, and terpenes to milk and in blood plasma of the suckling goat kids. López-Andrés et al. (2013) showed that the plasma and liver of lambs that are fed a diet rich in quebracho tannins exhibited greater antioxidant capacities than do the same tissues of lambs that are fed a control diet.

1.5. The use of agro industrial by-products in ruminant nutrition

Plant secondary compounds are also found in agro-industrial by-products arising from the food processing industries which have the potential to be used as animal feed. The use of agro industrial by-products in ruminant feeding is of interest due to their importance in nutrient supply like protein and their bioactive compounds like polyphenols (Schieber et al. 2001). Including these by products in moderate amounts in the diet of livestock like sheep and goats have revealed positive effects on milk quality and milk yield (Jordán et al. 2010). The transfer of the antioxidant activity in these compounds to animal tissues can improve the quality of livestock products and this is linked to their ability to increase oxidative stability for instance in lamb meat (Moñino et al. 2008). Bravo (1998) explained that the antioxidant efficiency of polyphenols depends on the extent of their absorption and metabolism of these compounds in the animal body. Grapeseed extract which is a residue of the food industry is rich in polyphenols (Torres et al. 2002) and can be used as alternative livestock feed (Makris et al. 2007). Santos et al. (2014) observed high antioxidant capacity of milk of Holstein dairy cows fed grape residue silage without influencing their milk yield. Gladine et al. (2007) likewise reported significant increase in plasma total antioxidant capacity and appearance of several phenolic compounds like rosmarinic acid and terpenes in blood plasma in sheep following a direct ruminal administration of grape extracts.

1.6. Effect of PSM on milk fatty acid composition in ruminants

There are several factors that affect the milk composition in ruminants that include animal type, feed and environmental factors (Palmquist et al. 1993). The type of forage the animal feeds on greatly affects the milk FA composition and this varies between the different plant species that are eaten by the animals, the quality, and quantity of the plants ingested, growth stage of the plants and seasonal effects on the plant quality (Palmquist et al. 1993). Different dietary forages with high or low amounts of PSM affect differently the composition of the milk FAs. Changing the composition of the animal's diet is the major factor that modifies milk FA composition. The major effect of PSM for instance tannins in the feed of ruminant is the reduction of biohydrogenation of polyunsaturated FA (PUFA), and their intermediates in rumen enhancing the extent of rumen escape of these FAs, increasing the amount of these FA reaching mammary gland and, therefore, secreted in milk (Buccioni et al. 2015; Correddu et al. 2016). Vasta et al. (2009) found that supplementing tannin rich carob pulp (*Ceratonia siliqua*) to lambs decreased ruminal biohydrogenation leading to higher accumulation of PUFA like C18: 2 *n*-6 and lowering the level of C18:0 in the meat of lambs. The α-linolenic acid (ALA) transfer rate from feed to milk was found to be influenced by the different flowering catch crops with different contents of polyphenols in the diet of lactating dairy cows (Kälber et al. 2011). Turner (2005) showed increased levels of ω-3 FA and conjugated linoleic acid (CLA), in milk from cows fed *Lotus corniculatus* as source of tannins. Abarghuei et al. (2014) found that supplementing pomegranate peel extract to dairy ewes increased the concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and lower concentration of short chain FA (SFA) implying that the polyphenols in the pomegranate inhibited the growth and metabolism of bacteria that are capable of biohydrogenation.

1.7. Scope of the doctoral thesis and objectives

Cattle, sheep, goats and camel are important livestock species in semiarid and arid regions, but little scientific research has be performed on comparisons among these livestock species on plant selection and intake of PSM. Camels classified like browsers, goats are intermediate feeders while cattle and sheep are grazers (Hofmann 1989) thus relying on different diets with higher and lower amounts of browse and thus PSM, respectively. Camels and goats are thus assumed to ingest higher amounts of PSM as compared to cattle and sheep when they are allowed to freely choose their diets. Studies on the transfer of PSM into milk, blood and urine are limited. Differences in the transfer of PSM from the feed to animal products between grazers, intermediate feeders and browsers could be due to different

plant selection patterns, different amount of PSM included in the feed and thus intake of PSM or due to different abilities of these animals to inactivate PSM as a strategy of adaptation such as tannin-binding salivary proteins, among others.

The main objective of the present doctoral thesis project consists, therefore, in getting a comprehensive view on PSM in relation to either browsing (camels), intermediate feeders (goats) or grazing (cattle & sheep) herbivores. The research addresses questions about plant selection, intake of PSM and the effect of PSM on milk FA profile of camels and cattle in different seasons or dairy sheep and dairy goats fed under controlled conditions on the effect of dietary inclusion of grapeseed extract on performance, antioxidant capacity of the blood, salivary differences and the transfer of polyphenols from grapeseed extract to the milk, blood, urine and feces. For this purpose, two extensive experiments were carried out.

1.7.1 Experiment 1: Performance, plant selection, transfer of phenols into milk and milk fatty acid profile of camels and cattle in East African rangelands of Laikipia, Kenya.

Objectives:

To compare the diet selection, the intake of PSM, transfer of PSM to milk and the effect of PSM on milk fatty acid profile of camels, improved cattle and local cattle types in rainy and transition periods.

Hypotheses

1) Performance.

Hypothesis 1: Improved cattle types (between local cattle and exotic cattle) have a better performance (milk yield) than local cattle and camels during the more favorable season with good forage quality on offer, but will be outperformed by camel and local cattle during the period between rainy and dry season (transition period).

2) Plant selection and behavior.

Hypothesis 2: Camels, and to a lesser extent local cattle, do rely more on browse than crossbred cattle during the transition period.

3) Response to supplementation.

Hypothesis 3: Supplementation has an effect on diet selection by enabling the animals to include more browse (containing PSM) in their diet.

Hypothesis 4: Animals supplemented with rumen degradable protein (RDP) have a better performance compared to non-supplemented animals

Hypothesis 5: Cattle respond more to extra RDP and thus in performance compared to camels

4) Total phenols and fatty acid profile in milk.

Hypothesis 6: Camel milk, compared to cattle milk, contains more total phenols in milk due to their browsing behavior.

Hypothesis 7: The FA profile of camel and cattle milk differs between the two animal types.

Hypothesis 8: The milk of local cattle and crossbreds differs in these compositional variables as they are presumed to exert a different plant selection pattern.

Hypothesis 9: There are seasonal differences in the milk total phenols and milk FA profile of both, camels and cattle.

1.7.2 Experiment 2: Transfer of plant secondary compounds from feed to sheep and goat milk and side-effects on performance and milk quality

Objectives

The objective of the second experiment was to determine the transfer of total phenols from grapeseed extract to milk, blood and excreta and side-effects on performance and milk quality in dairy goats as compared to dairy sheep. The experiment further evaluated the effect of feeding grapeseed extract on blood antioxidant capacity and the saliva differences of goats and sheep.

With the second experiment the following hypothesis were addressed:

Hypothesis 1: The phenols present in grape seed extract can be transferred to and detected in milk, blood, urine and feces.

Hypothesis 2: In milk, blood, urine, feces of sheep and goats fed with phenols, the total phenol content and antioxidant capacity will be increased compared to the respective control animals.

Hypothesis 3: When feeding the same amount of phenols, goats as intermediate feeders are better adapted to phenol-rich extract and will excrete less phenols with the milk and blood due to established or upregulated detoxification strategies as compared to sheep, classified as grazers, but will excrete higher amounts with feces and/or urine.

Hypothesis 4: In relation to this, tannin-binding affinity and total saliva protein will be higher in goat saliva compared to sheep saliva and especially in animals fed phenol diets.

Chapter 2

Plant Selection and Performance of Two Cattle Types and Camels on Semi-arid Rangelands in Kenya

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2.1 Abstract

Plant selection pattern and performance of lactating cattle and camels were compared on semi-arid savanna rangelands in Kenya in the rainy season (RS) and a transition period (TP) between the RS and the main dry season. It was further evaluated whether supplementation with rumen-degradable protein (RDP) had an effect on these parameters. In both seasons, two cattle types (local 'Pokot' cattle and Guernsey × Boran crossbreds) and camels were used, with six females per treatment group (supplemented and non-supplemented) each (n = 72 animals in total). The experimental periods consisted of 8-10 d of adaptation and 36-40 d of data and sample collection. The diet selected by the cattle types was similar and consisted almost exclusively of grasses. The camel diet consisted mainly of herbs and shrubs with higher contributions of woody plants in the TP than in the RS. Forage from woody plants overall made up a higher proportion of the diet, which was also reflected by a longer browsing time (overall and in the TP), of the supplemented camels compared to the non-supplemented camels. This result indicates that supplementation of browsers like camels with RDP can be used to increase the intake of forage from woody plants rich in plant secondary compounds which could be an effective measure for managing rangeland to limit bush encroachment. Overall, no seasonal differences in milk yield were found for the camels and the Pokot cattle, but crossbreds had a lower yield in the TP compared to the RS. Overall, the cattle had higher milk fat content than the camels while the camels had slightly higher protein content. Supplementation had no effect on milk yield and composition. The results of diet selection and performance (milk yield) reflect the advantage of camels in arid rangelands.

2.2 Introduction

The arid and semi-arid areas of Kenya cover more than 80% of the Kenyan land mass and provide rangelands for livestock husbandry (Sombroek et al., 1982*)*. However, in recent years these areas have experienced frequent droughts that represent a particularly serious challenge for populations whose livelihood depends on livestock (Kiage, 2013; Tseyage et al., 2013). The consequences of climate change, droughts, and over-grazing have led to land degradation and changes in vegetation cover in these dry regions where encroachments of woody plant species in previously grassland dominated areas have increased over time (Archer et al., 2001; Wiegand et al., 2006). Pastoral communities in these areas have developed adaptation and coping strategies necessary to cope with the changes taking place in the rangeland conditions, such as diversification of herd composition and livestock species, where traditionally cattle-dominated ethnic groups have switched to dromedary camels, goats, and sheep (Österle, 2008; Speranza, 2010). The livestock species most widely used in the dry areas of Kenya (million heads) are goats (25), sheep (15), and cattle (12), and in lower numbers, camels (3: KNBS, 2009). These animals are an important source of livelihood for pastoral communities as the animals provide meat or milk or both (Wardeh, 2004). Camels (classified as browsers; Schwartz et al., 1983) are more suitable than grazers like cattle for utilizing woody rangelands as camels feed to a large part or exclusively on browse. In addition, compared to cattle, camels (*C. dromedarius*) are more drought-tolerant, perform better under adverse conditions, and have a lower metabolism and therefore, lower energy requirements (Maloiy et al., 2009; Dittmann et al., 2014). Additionally, camels are better adapted to high daytime temperatures, forage scarcity, and fluctuations in water supply (Yagil, 1994). Traditionally, local cattle breeds (*B. indicus*) are kept by local communities and together with camels are important livestock species for the more arid lands. Although the local zebu cattle are generally considered relatively well adapted to low-feed availability, frequent drought, and disease challenges prevalent in sub-Saharan Africa, cattle are more sensitive to drought compared to camels (Rege and Tawah, 1999). Additionally, local cattle breeds have lower growth rates and performance (Kavoi et al., 2010). However, the overall productivity of local cattle types and camels is lower than that of exotic or crossbred dairy cattle (Kimenye and Russell, 1975). Therefore, deliberate efforts were introduced by the Kenyan government to improve the milk yield of the local cattle breeds by promoting crossbreeding of local Zebu cattle and European exotic breeds; this predominantly in the more humid and productive areas (Bebe et al., 2002, 2003). In contrast, the purebred exotic high genetic merit cattle (Holstein,

Friesian, Ayrshire, and Guernsey) were found to be too susceptible to drought and a concomitant scarcity of forage of sufficient quality (Bebe et al., 2003).

Controlled studies comparing camels, improved crossbred cattle, and local cattle types simultaneously regarding performance, diet selection, and behavior on rangeland and in different seasons are lacking. Few studies on diet selection have focused on cattle, sheep, and goats, while even fewer included camels (Migongo-Bake and Hansen, 1987; Rutagwenda et al., 1990), despite their importance in semi-arid and arid rangelands. Additionally, studies on the effect of supplementation on diet selection and performance in free range comparing camels and cattle are missing. A study by Odadi et al. (2013) in semi-arid Kenyan rangelands with free-ranging cattle showed an influence of protein supplementation (here, cotton seedcake), with higher intakes of protein-rich forbs by the unsupplemented cattle during the dry season. In controlled barn-feeding experiments, sheep and goats supplemented with extra protein were able to increase their consumption of a diet rich in certain plant secondary metabolites (PSM; Villalba et al., 2002b; Utsumi et al., 2009), compounds that are largely present in browse, which might be related to an enhanced ability for detoxification and additionally, facilitated ruminal fiber digestion and rumen emptying, thus allowing a higher forage dry matter (DM) intake (Utsumi et al., 2009).

The hypotheses tested in the present study thus were the following: (i) Improved cattle types (such as crossbreds of local and exotic cattle) have a better performance (milk yield) than local cattle and camels during the more favorable season with good forage quality on offer but are outperformed by camels and local cattle during the period between the rainy and dry seasons (the transition period). (ii) Camels, and to a lesser extent local cattle, rely more on browse than improved cattle during the transition period. (iii) Animals supplemented with rumen degradable protein (RDP) have a better performance compared to non-supplemented animals. (iv) Cattle respond more to extra RDP and thus, in performance compared to camels. (v) Supplementation has an effect on diet selection by enabling the animals to include more browse (containing PSM) in the diet. These hypotheses were tested during the rainy season and the transition period with local Pokot cattle, Guernsey × Boran crossbreds, and camels in free range either unsupplemented or receiving extra RDP.

2.3 Methods

2.3.1 Study Area and Study Sites

The experiment was conducted on semi-arid savanna rangelands of the Suyian Ranch, Laikipia County, Kenya, located approximately 90 km northwest of Nanyuki town (00° 30'06"N to 36° 41' 46"E, 1800 m a.s.l.). Suyian Ranch covers an area of 17,600 ha of land. The study area is classified as semi-arid (agro-ecological zone 5, i.e., < 1100 mm rainfall; Sombroek et al., 1982). With a bimodal rainfall, precipitation ranges between 450 mm and 700 mm (Jaetzold and Smith, 1982). The rainy seasons occur in April and May (the main rainy season) and October and November (the short rainy season), and the main dry season is between January and March, while in late June and July and September and October there are smaller dry seasons, with some rainfall in between. The mean annual temperatures are between 16 °C and 26 °C (Notter, 2003).

Due to rotational grazing management, two different study sites on the farm, both located at an altitude of 1800 m a.s.l., were used for the experiment in the rainy season (RS) and the transition period (TP). Although both study sites had the same plant species composition and similar plant cover (with the exception of a higher abundance of shrubs in the study site used in the TP) and were near each other (approximately 15 km apart), the season effect cannot fully be separated from the site effect. The main habitat type of study site 1 used in the RS was grassland (mainly *Hyparrhenia papillipes* and *Themeda triandra*), interspersed with Acacia tree species (*Acacia drepanolobium* and *Acacia seyal*). The habitat of study site 2 used in the TP was mainly shrub-grassland. It consisted mainly of grasses interspersed with herbs (mostly *Barleria delamerei*), shrubs (with the dominant species *Acacia brevispica*, *Gymnosporia putterlickioides* and *Pyrostria phyllanthoidea*), and trees (mainly *Boscia angustifolia*).

2.3.2 Experimental Animals

Two groups of 36 animals per season were chosen for the experiment which comprised two cattle types and camels. The first cattle type used was a local breed called Pokot, which is of indigenous small East African shorthorn zebu origin (*Bos indicus*). A total of 12 lactating cows were used during the RS (5.7±1.0 years of age, 199±19.3 kg body weight and parity of 2.6±0.9, with 24.8±10.1 days in milk; means ± standard deviation). Twelve different lactating cows were used during the TP (224±33.1 kg body weight and 31±14 days in milk). The second cattle type consisted of lactating crossbreds of Guernsey and Boran (*B. taurus* × *Bos indicus*). Nine of the lactating experimental crossbreds used in the RS were F1 generation animals (50% Boran and 50 % Guernsey) and three were F2 generation

animals (75% Boran and 25% Guernsey). They were, on average, 6.17±2.37 years old, weighed 304±56.8 kg, were in 3.6±2.0 parities, and were 46.3±24.6 days in milk. In the TP, 12 F1 generation crossbreds were used (352±30.5 kg body weight; 42.5±29.7 days in milk). The 2 × 12 lactating camels (*Camelus dromedaries*) used were of a Somali type. Their biological data for the RS were 8.2±2.4 years of age, 523±58.6 kg body weight, 3.3±1.4 parities, and 87.4±75.5 days in milk, and for the TP, 502±53.9 kg body weight and 76±28 days in milk. For all three animal types, different animals were used in the RS and the TP, in order to have animals in both periods in the same stage of lactation.

The experimental animals were selected from larger herds based on the best possible similarities between periods and animal type in days in milk, body weight and parity. The camels and the crossbreds originated from herds of the Suyian Ranch, and the Pokots were rented from a local Pokot tribe near the farm. Before the experiment started, the camels grazed pastures consisting of *Pennisetum mezianum* and *Acacia nilotica* for a week; whereas, the pasture of the crossbreds mainly consisted of *Themeda triandra* and *Pennisteum mezianum*. The Pokots were moved to the farm on 1 May 2015 for the RS and on 15 August 2015 for the TP and were kept on grassland pastures as well for 2 w before the observation periods started.

2.3.4 Experimental Design and Treatments

The experiment was conducted during the RS from 14 May to 2 July 2015 (i.e., during the expected period of long rains), consisting of a 10 d adaptation period and a 40 d data collection period, and in the TP from 22 August to 7 October 2015 (i.e., during the expected short dry period), including an 8 d adaptation period and a 36 d data collection period). Eleven days before the measurement periods started in the RS and the TP, the experimental animals were milked to assess the milk yield and milk composition, and the body weight was measured, for the cattle with a balance (model TT40, TAL-TEC, South Africa; accuracy ±1 kg). The body weight of the camels was assessed from girth circumference using a tape and Yagil's (1994) equation as cited in Mehari et al. (2007). Animals from each animal type were randomly allocated to two subgroups of six animals per period per animal type each, and they were balanced, on average, for days in milk, milk yield, body weight, and parity. Different from one subgroup, the second subgroup received a supplement containing urea as a source of RDP (RDP+) from the adaptation period throughout the experiment. Therefore, the subgroups and animal types were kept separately during nighttime in six enclosures (Boma gates; in the RS, the three non-supplemented subgroups were kept together). The supplement was provided at ad libitum access from 1700 h to 0700

h in metal containers that were 0.5 × 0.5 m, and protected by a shelter from rain. No other feed was offered during that time. Owing to the high salt requirements and lack of familiarity with urea by the camels, the RDP supplements were designed differently for cattle and camels. The cattle supplement consisted of 25% urea, 17% lime, and 58% molasses and had a slurry-like consistency. The supplement for the camels was provided as a block consisting of 12% urea, 10% molasses, 17% lime, and 61% mineral mix. The estimated energy and protein values of the supplement fed to the two cattle groups had estimated energy and protein contents of 8.53 MJ/kg and 751 g/kg DM, respectively. The supplement for camels had an estimated energy content of 1.47 MJ/kg and a protein content of 351 g/kg DM. Estimates are based on data for energy and protein content of molasses as provided by the online database Feedipedia (Heuzé et al., 2015). The animals were adapted to the urea in the adaptation period by starting with supplements containing only 4.8% and 5% of urea, in camels and cattle, respectively. The urea proportion was gradually increased to the final levels on the first day of the respective observation periods. When the animals were confined, they additionally received a loose mineral mix in separate buckets except the supplemented camel subgroup which received the mineral mixed into the supplement. The mineral mix contained, per kg, salt, 300 g; Ca, 230 g; P, 20 g; Zn, 5 g; Mn, 3g; Cu 1.6 g; I, 0.25 g; Se, 0.02 g; vitamin E, 1 g; and vitamin B_{12}, 3.12 mg. Accidentally, the two supplemented cattle groups did not receive any mineral mix during the observation period of the TP. The daily group consumption of the mineral mix and the RDP supplements was registered by weighing the respective containers with a spring balance. Amounts were divided by the respective number of animals in the enclosures.

All experimental animals were accompanied by their offspring which were kept separately from the dams at night. After milking from 0600 to 0800 h, the animals were released to pasture. One herder each for the cattle and camels accompanied the animals, because the camels typically grazed separately from the cattle as they moved faster in search of browse. The Pokots and the crossbred cattle grazed together. The herders herded the animals to pastures in the morning and to the enclosures in the evening with no other interferences in diet selection or activity.

2.3.5 Measurement of Plant Cover

Plant cover was measured at the beginning and end of each observation period at each study site. For recording the plant species on offer, a point-intercept method was used based on Bonham (1989) as described in Marquardt et al. (2010). Different transects (three per habitat category) of 100 m length and with 100 measurement points each were made, in the beginning and the end of the respective period. Categories were grassland, shrubby grassland, and bushland in the RS (study site 1) and shrubby grassland and rocky shrubland in the TP (study site 2). Transects were selected randomly after the areas in these habitat categories where the animals most often used to graze were identified. For making a transect line, a thin string wire and a measurement tape were used. Measurement points were set every meter along the measurement tape, where all single plant individuals were recorded from the ground upward together with the functional group to which they belonged. Unknown plants were collected and later determined with the help of a botanist from the National Museums of Kenya Herbarium (Nairobi, Kenya). Proportional plant cover was calculated per transect, and one plant cover value was generated per habitat from the six transects (half in the beginning and end of each observation period).

Table 2.1 Plant cover (in % of total cover) of the plant species most frequently selected by camel and cattle in two periods in different habitats (Study sites 1 and 2).

	Rainy season (Site 1)[1]			Transition period (Site 2)[2]	
Functional group (FG)	Bushland	Grassland	Shruby grassland	Shruby grassland	Rocky shrubland
Species	(10%)[3]	(45%)[3]	(45%)[3]	(90%)[3]	(10%)[3]
Total grasses	97.8	120.2	107.3	144.8	95.0
Aristida adoensis	4.5	11.2	5.2	0.5	0.4
Bothriochloa insculpta	15.5	14.9	27.4	3.4	0.3
Brachiaria dictyoneura	-[4]	2.5	0.4	-	0.2
Cynodon nlemfuensis	1.9	1.5	2.0	7.0	3.0
Digitaria macroblephara	0.2	-	-	8.7	6.0
Eragrostis tenuifolia	2.7	2.5	1.0	0.7	0.4
Heteropogon contortus	0.5	3.7	2.4	7.5	2.7
Hyparrhenia papillipes	47.2	35.3	30.4	38.7	30.5
Pennisetum mezianum	3.0	3.5	7.3	7.2	0.7
Pennisetum stramineum	-	-	-	6.0	9.7
Setaria sphacelata	-	15.2	3.7	1.4	0.2
Themeda triandra	17.8	20.5	22.7	19.9	11.9
Other grasses[5]	4.5	9.4	4.8	43.8	29.0
Total herbs	17.8	24.5	20.3	38.5	19.0
Barleria delamerei	5.3	10.9	9.0	16.7	0.7
Barleria eranthemoides	-	-	-	-	10.0
Heliotropium zeylanicum	0.5	0.8	0.3	-	-
Other herbs[5]	12.0	12.8	11.0	21.8	8.3
Total shrubs	48.8	2.0	27.7	45.2	75.0
Acacia brevispica	-	-	-	3.5	12.2
Carissa spinarum	10.2	-	0.9	2.5	0.4
Grewia tephrodermis	2.4	-	0.5	2.0	2.0
Hibiscus flavifolius	4.5	0.2	1.7	0.3	0.5
Lantana verbunoides	0.9	0.5	0.7	0.5	0.5
Lycium europeum	0.9	-	0.2	0.4	-
Gymnosporia putterlickioides	-	-	-	1.5	1.4
Pyrostria phyllathoidea	-	-	-	1.0	1.7
Searsia natalensis	7.2	-	0.3	5.0	0.5
Scutia myrtina	1.3	-	0.2	-	-
Tephrosia emeroides	-	-	-	0.7	4.2
Terenna graveolens	0.2	-	-	0.4	1.8
Other shrubs[5]	21.2	1.3	23.2	27.4	49.8
Total trees	8.2	0.2	0.8	3.8	1.3
Acacia nilotica	4.9	0.2	-	1.3	-
Boscia angustifolia	-	-	-	0.7	0.9
Other trees[5]	3.3	-	0.8	1.8	0.4

Average of two measurements at the beginning and end of the rainy season and the transition period, n = 6 transects/habitat. Only those species are listed that are among the ten most selected plant species per animal species and season.

[1] Measurements were performed on Days 6 and 34 of the observation period in the rainy season.

[2] Measurements were performed on Days 14 and 32 of the observation period in the transition period.

[3] Estimated proportion of the study site covered by the respective habitat type.

[4] Not found in this habitat.

[5] This includes the species that were not among the most frequently selected species and the not selected species.

2.3.6 Analysis of Plant Chemical Composition

In the beginning and at the end of the respective observation period, the most frequently selected plant species per animal species (i.e., separately for cattle and camels) were collected, air dried as soon as possible after collection in the field, oven dried at 60 °C for 72 h, and ground using a 1 mm screen. Proximate contents were analyzed following AOAC (1997). An automatic thermogravimetric analyzer (TGA-701, Leco Corporation, St. Joseph, MI, USA; AOAC No. 977.02) was used to analyze the DM and total ash. Nitrogen (N) was determined with a C/N analyzer (Typ TruMac CN, Leco Corporation, St. Joseph, MI, USA), and crude protein (CP) was calculated as 6.25 × N. Neutral detergent fibre (NDF) with the addition of α-amylase, acid detergent fibre (ADF), and acid detergent lignin (ADL) were analyzed according to Van Soest et al. (1991) and AOAC (No. 973.18) using Fibertec System M (Tecator, 1020 Hot Extraction, Foss Hillerød, Denmark), and ether extract (EE) was analyzed with the Büchi extraction system B-811, Flawil, Switzerland. The NDF and ADF values are always given exclusive of ash. Total extractable phenol (TEP) and total tannin (TT) contents were measured following Makkar (2003) using the Folin-Ciocalteu method with gallic acid as standard. Condensed tannins (CT) were analyzed according to the butanol-HCL-iron method, and the values are given as leucocyanidin equivalents (Makkar, 2003).

2.3.7 Measurement of Diet Selection

Direct observations were performed on one animal per day in an alternate order regarding animal type and supplementation group until all animals were observed once during the whole observation period. Observations took place at a distance of 1 to 2 m and from the time the animals were released for grazing/browsing at 0800 h until 1600 h when they were moved back to the enclosures, using scan sampling (1-min non-stop recordings and 5 min in between the observations used for collecting the respective plant samples for later determination in the herbarium), resulting in 91–97 observation min per animal and period. In each of the 1-min observations, the number of bites per plant species, its functional group, the bite category, the bite size, and the plant part consumed were recorded. For the estimation of the biomass intake, the number of bites per plant species was multiplied by the air DM weight of the respective bite category according to the method developed by Agreil and Meuret (2004). For that purpose, the diet selection behavior of the animals was mimicked at the beginning and the end of each observation period. This was done together for both cattle types, which had almost the same body size and bite sizes, and separately for the camels. The bite simulations were made separately for the most frequently consumed plant species; whereas, mixed bite categories were created for less

frequently consumed species considering the functional group and the morphological similarity (Appendices Table S2.1). Bite sizes were distinguished per category as full bite (high proportion of leaves and or leaves and stems/bite), half bite (some leaves and stems/bite), or small bite (a set of leaves or portion of a leaf/bite), adjusted from Agreil and Meuret (2004). In the RS, for the ten plant species most frequently selected by cattle and camels, two bites each were mimicked in the beginning and at the end of the observation period, resulting in $n = 4$ per category and bite size. No mixed-bite categories were sampled; thus, the mean group value of the most frequently selected single plant species fitting into the respective mixed category was calculated and used. In the TP, ten single bites per bite category and bite size for single and mixed categories were collected in a paper bag in the beginning and the end of the TP, respectively, and divided by ten, resulting in $n = 2$. Air DM content was measured by weighing the samples before and after drying for 72 h at 60 °C.

2.3.8 Measurement of Activity Pattern

The same observation minute used for diet selection recording was used to record the activity pattern of the animals. The following activities were distinguished: eating, further separated into grazing (here, feeding on grasses and herbs), browsing (feeding on shrubs and trees), and grazing and browsing (when the focus animal was doing both during the observation minute), resting (separated into lying and standing), and pica behavior (further separated into chewing bone and licking the ground). When the focus animal was moving during eating, the activity was allocated to eating. The category others included walking without eating, sand bathing, calf suckling, and drinking.

2.3.9 Measurement of Milk Yield and Milk Composition

Milking started on the first animal available. The calves were allowed to approach their dams in order to stimulate milk let-down. Afterwards, milking started until the animals were milked out. On day 11 before the start and during the entire observation period, the amount of milk per animal was registered using a 1-l measuring jug with an accuracy of ± 100 ml. Milk yield in l (MY_l) was converted to MY_{kg} by $MY_{kg} = MY_l \times 1.03$. At every milking, the milk was analyzed using a portable ultrasonic milk analyzer (Lactoscan SA-L, Milkotronic Limited, Nova Zagora, Bulgaria). The measurements were done with the setting for ‘cow’. In order to calibrate the data measured with the Lactoscan, a regression was calculated using milk samples from 16 Swiss cows analyzed in addition with a MilkoScan FT6000 (Foss, Hillerød, Denmark) at Swisslab AG (Zollikofen, Switzerland), using the following equations: fat adjusted (%) = 0.9787 × fat (%) – 0.0653, $R^2 = 0.99$; protein adjusted (%) = 0.2003 × protein (%) +

2.3036, $R^2 = 0.80$. Fat and protein corrected milk (FPCM, kg) was calculated as uncorrected milk (kg) × (0.337 + 0.116 × fat content (%) + 0.06 × protein content (%)) (Gerber et al., 2012). Values were averaged per cow per period (RS: $n = 40$ days/animal; TP: $n = 36$ days/animal). For some animals, on single days of the TP it was not possible to get milk for measuring the milk amount or milk composition data, which slightly reduced the number of days for single animals.

2.3.10 Statistical Analysis

Data were subjected to analysis of variance applying the mixed procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Model 1, used to analyze milk yield and milk compositional data, considered the period, animal type, and supplementation with all interactions as fixed effects. For that purpose, the data measured 11 days before the observations started were used as covariables. One camel of the supplemented group was not included in the milk sampling. In the table, LS means (adjusted by covariance analysis), standard error of the mean, *P* values, and in brackets, the arithmetic means (data as measured) are presented.

The proportion of the total biomass of the botanical functional groups selected by the animals and the activity data were analyzed with Model 2, where the period, animal type, supplementation and all possible interactions were considered fixed effects. In case of non-normal distribution of the residuals, which were checked graphically, log-, or arcsine transformations were performed on some parameters (information given in table footnotes). If in the transformations no normal distribution of the residuals could be achieved (because some plant items were selected by one animal species only and not the other), the data were split and analyzed separately for the respective animal species (information is given in table footnotes). If not otherwise indicated, data represent LS means. Comparisons among the means were performed with Tukey's method. The significance level was set to $P < 0.05$.

2.4 Results

2.4.1 Composition of Plant Cover

Across all plant cover measurements, plant species comprised 47 grasses, 78 herbs, 60 shrubs, and six tree species. Grasses were the most dominant functional group across all habitats (Table 2.1). Herbs were present in all habitats, having mostly between 20% and 40% of the plant cover. Shrubs made up most of the plant cover in the rocky shrubland of study site 2, while they were least abundant in the grassland of study site 1. Trees never exceeded 10% of the plant cover. *H. papillipes* and *T. triandra* were among the most abundant grass species in the diet of the cattle and were found in all habitat types of both study sites. *B. delamerei*, the herb most frequently selected by the camels, was present in all five habitats at, on average, 9% of the plant cover.

2.4.2 Chemical Composition of the Most Frequently Consumed Plants

The most frequently consumed plant species analyzed (Appendices Table S2.2) had NDF contents between 40% and 80% of DM. The leaves from the woody plant species (trees and shrubs), overall, were slightly lower in NDF content and richer in ADL content compared to the herbaceous plants, especially the grasses. Grasses were low in CP across both periods compared to herbs, shrubs, and trees. The highest CP values in the RS were found in the shrubs *A. brevispica, G. tephrodermis, L. europeum,* and the herb *H. zeylanicum* and in the TP, in the tree *B. angustifolia*. Total phenols were most prevalent in the woody plants *A. nilotica,* followed by *C. spinarum, S. myrtina, G. putterlickioides,* and *T. graveolens* and never exceeded 3.5% of the DM in case of the herbaceous plants (Appendices Table S2.2).

2.4.3 Intake of RDP and Mineral Supplements

Consumption of the RDP-based supplement (g/day and head during the data collection period for the supplemented animals) was 140±200 and 170±180 in the camels, 260±390 and 360±240 in the crossbreds, and 130±140 and 210±160 in the Pokots in the RS and the TP, respectively (data not shown in table). The intake of the mineral mix (g/day and head) in RS, was 10±40 and 60±120 in the supplemented crossbreds and Pokots (the supplemented camels received the mineral mix mixed in the RDP supplement), and on average, 60±80 in the three non-supplemented subgroups kept together, respectively. The corresponding values for the mineral mix intake of the unsupplemented subgroups in

the TP were 170±190, 100±150, and 50±70 in the camels, crossbreds, and Pokots, respectively (data not shown in table).

2.4.4 Diet Selection

Plant species selected included 41 grasses, 38 herbs, 40 shrubs, and three trees across animal species and periods. Species selected by the camels across periods were 13 herbs, 39 shrubs, and three trees, those selected by the two cattle types together were 41 grasses, 29 herbs, and seven shrubs (data not shown in table). Only four out of the 38 herb species (*Heliotropium zeylanicum*, *Indigofera volkensii*, *Justicia diclipteroides*, and *J. debilis*) and six of the 40 shrub species (*Aspilia mossambicensis*, *Grewia tephrodermis*, *Helichrysum glumaceum*, *Hibiscus flavifolius*, *Jasminum fluminense*, and *Pavonia gallaensis*) were observed to be selected by both animal species.

Herbaceous plants made up almost 100% of the diet in both cattle types and in both periods; whereas, intake of herbaceous plants overall was lower in camels in the TP compared to the RS with 58% vs 80% of the total biomass intake, respectively (period × animal type interaction, $P < 0.01$, Table 2.2). Overall, the diet of the supplemented camels consisted of 61% of herbaceous plants; whereas, this was 77% in the non-supplemented camels (animal type × supplementation interaction, $P < 0.01$). Only up to 2% of the diet of the cattle types consisted of herbs, while grasses made up the main part of the cattle diets, irrespective of season. The camels did not select grasses. Woody plants were almost totally neglected by the cattle types, while woody plants made up between 16% and 53% of the camel diet. Overall, camels selected more ($P < 0.01$) woody plants (from shrubs and trees) in the TP compared to the RS. In addition, supplementation had an effect ($P < 0.05$) on the diet contribution of woody plants to the camel diet, with a higher share in the diet of the supplemented camels. The same pattern was found for the contribution of shrubs, and there was a trend ($P < 0.1$) of a higher contribution of trees to the diet of the supplemented camels, although their proportion in the diet never exceeded 3% (Table 2.2). Camel supplemented with RDP selected more of the woody species *Barleria trispinosa* and *Terenna graveolens* in the TP (plants not selected in the RS) and more of *Carissa spinarum* in the RS compared to the non-supplemented camels (period × supplementation interaction, $P < 0.05$; data not shown in table).

Table 2.2 Proportion of biomass (% of total air dry matter (air DM) per animal) selected by camels, crossbred, and Pokot cattle in two periods per plant functional group.

FG	Rainy season						Transition period						SEM	*P*-values						
	Camel		Crossbred		Pokot		Camel		Crossbred		Pokot			P	S	A	A×P	P×S	S×A	A×S×P
	+	-	+	-	+	-	+	-	+	-	+	-								
n	6	6	6	6	6	6	6	6	6	6	6	6								
Proportion of biomass (% of total)[1]																				
Herbaceous	76^{b}	84^{ab}	100^{a}	100^{a}	100^{a}	100^{a}	46^{c}	70^{b}	100^{a}	100^{a}	100^{a}	100^{a}	3.5	<0.01	0.01	<0.01	<0.01	0.18	<0.01	0.17
Grasses[2]	-	-	99	99	99	100	-	-	100	99	99	98	0.7	0.28	0.51	0.87	0.11	0.13	0.81	0.31
Herbs[3]	76^{a}	84^{a}	1^{b}	1^{b}	1^{b}	>0^{b}	46^{a}	70^{a}	>0^{b}	1^{b}	1^{b}	2^{b}	3.5	0.49	0.08	<0.01	0.02	0.07	0.71	0.46
Woody[4]	24^{b}	16^{b}	-	-	-	-	53^{a}	30^{ab}	-	-	-	-	6.1	<0.01	0.02			0.19		
Shrubs[4]	23^{b}	15^{b}	-	-	-	-	50^{a}	29^{ab}	-	-	-	-	5.8	<0.01	0.02			0.25		
Trees[4]	1^{b}	1^{b}	-	-	-	-	3^{a}	1^{b}	-	-	-	-	0.5	0.02	0.09			0.01		

[a–c] Within a row, least square means without a common superscript differ ($P < 0.05$).

[1] Rounded values are displayed.

[2] Only cattle data were used for analysis. *P* values were generated from the arcsine-transformed data.

[3] *P* values and superscripts were generated from the log-transformed data.

[4] Only the camel data used for analysis as not or almost not selected by the cattle.

2.4.5 Activity Pattern

Most of the time (83–93%) between the turnout to and the return from pastures was spent with feeding activities (Table 2.3). Camels, overall, spent more time eating ($P < 0.01$) than both cattle types. Browsing (feeding on shrubs and/or trees) was not or rarely practiced by the two cattle types, while overall, the camels spent more time browsing ($P < 0.05$) in the TP compared to the RS (period × animal type interaction, $P < 0.01$). The supplemented camels spent, across seasons and in the TP, more time browsing ($P < 0.05$) than the non-supplemented camels (48% and 33% of observation time across seasons, respectively, animal type × supplementation interaction, $P < 0.01$). Both cattle types spent more time ($P < 0.05$) grazing (feeding on grasses and/or herbs) compared to the camels in both periods, while the camels, overall, spent more time grazing ($P < 0.05$) in the RS compared to the TP. There was no effect ($P \geq 0.10$) of the period, animal type, supplementation, or of any of the interactions regarding resting activities. Pica behavior (mainly chewing bones) was absent or almost absent in the camels and Pokot cattle but was observed in the crossbreds cattle (Table 2.3).

2.4.6 Milk Yield and Composition

Period, animal type, and their interaction, but not supplementation, had an effect ($P < 0.05$) on the milk yield and FPCM (Table 2.4). Overall, different from camels and Pokot, the milk yield of the crossbreds decreased ($P < 0.05$) from the RS to the TP. The milk fat content was affected ($P < 0.01$) by period and animal type, and there was a trend ($P = 0.09$) for a supplementation effect. Overall, the cattle types had higher ($P < 0.05$) milk fat content compared to the camels, and it was overall higher ($P < 0.05$) in the RS compared to the TP. The milk protein content of the camels was slightly higher ($P < 0.05$) compared to the two cattle types. Similar to the milk yield, there was a period × animal type interaction for the fat and protein yields. This resulted from the lack of seasonal differences in the camels and Pokots and lower ($P < 0.05$) yields in the crossbreds in the TP compared to the RS.

Table 2.3 Amount of time (in %) spent by camels, crossbreds, and Pokot cattle in two periods for different activities between 0800 and 1600 h as measured every 5 min; P: Period, A: Animal type, S: Supplementation (with (+) or without (-) supplementation (S)), (*n* = 91-97min/animal, *n* = 6 animals/subgroup), LSMeans and SEM are displayed.

Parameter[1]	Rainy season						Transition period						SEM	*P*-values						
	Camel		Crossbred		Pokot		Camel		Crossbred		Pokot			P	S	A	A×P	S×A	P×S	A×S×P
	+	-	+	-	+	-	+	-	+	-	+	-								
Eating[2]	90.7	88.1	88.6	86.1	83.9	84.5	92.6	91.4	85.9	82.8	87.5	85.4	2.22	0.63	0.16	<0.01	0.15	0.81	0.81	0.81
Browsing[3,4]	32.6^{bc}	22.3^{c}	0^{d}	0^{d}	0^{d}	0^{d}	63.7^{a}	43.1^{b}	0.3^{d}	0.2^{d}	0^{d}	0^{d}	3.86	<0.01	0.02	<0.01	<0.01	<0.01	0.44	0.69
Grazing[5]	46.4^{b}	51.2^{b}	88.2^{a}	86.1^{a}	83.9^{a}	84.5^{a}	22.9^{c}	37.3^{bc}	85.5^{a}	82.6^{a}	86.6^{a}	85.2^{a}	3.31	<0.01	0.25	<0.01	<0.01	0.03	0.55	0.40
Pica behavior[6]	0.0	0.0	1.4	1.4	0.5	0.0	0.0	0.0	1.7	3.8	0.2	0.3	0.97	0.42	0.61	<0.01	0.51	0.64	0.42	0.75
Resting	3.3	5.5	4.7	5.8	6.0	5.0	0.9	2.1	5.2	5.2	5.9	5.7	1.88	0.40	0.62	0.10	0.42	0.67	0.82	0.92
Lying	1.9	0.5	1.0	3.2	0.2	0.3	0.0	0.0	1.6	0.0	1.6	0.0	1.02	0.26	0.53	0.36	0.34	0.71	0.23	0.20
Standing	1.4	5.0	3.7	2.6	5.8	4.7	0.9	2.1	3.6	5.2	4.3	5.7	1.68	0.80	0.36	0.07	0.46	0.56	0.63	0.48

[a–d] Within a row, least square means without a common superscript differ ($P<0.05$).

[1] Further activities, such as walking without eating, letting the calf suckle, drinking, and sand bathing, are not displayed in the table.

[2] Some observation minutes included both grazing and browsing; these are included in the category 'eating' but were not shown separately.

[3] 'Browsing' includes only observation minutes with feeding on shrubs and/or trees.

[4] *P* values and superscripts were generated from the arcsine-transformed data.

[5] 'Grazing' includes only observation minutes with feeding on grasses and/or herbs.

[6] 'Pica behavior' includes chewing bones and licking the ground.

Table 2.4 Milk yield (from morning milking per day) and milk composition of camels, crossbred, and Pokot cattle in two periods; P: Period, A: Animal type, S: Supplementation (with (+) or without (-) supplementation). LSMeans, adjusted by values measured before the supplementation started were used as covariables, and in brackets, uncorrected arithmetic means and SEM are displayed.

	Rainy season						Transition period						SEM	*P*-values						
	Camel		Crossbred		Pokot		Camel		Crossbred		Pokot			P	S	A	A×P	P×S	S×A	A×S×P
	+	-	+	-	+	-	+	-	+	-	+	-								
n	6	6	6	6	6	6	6	5	5	6	6	6								
Milk yield per day (kg/day)																				
absolute	2.98^{ab}	2.71^{abcd}	3.23^{a}	3.11^{ab}	1.97^{cde}	2.17^{bcde}	2.86^{abc}	2.63^{abcde}	2.33^{abcde}	2.10^{cde}	1.76^{e}	1.83^{de}	0.207	<0.01	0.37	<0.01	<0.01	0.73	0.28	0.94
	(2.98)	(2.84)	(4.12)	(4.10)	(1.25)	(1.75)	(2.90)	(2.53)	(3.07)	(2.67)	(0.72)	(0.87)								
FPCM[1]	2.97^{abc}	2.81^{abc}	3.65^{a}	3.51^{ab}	1.73^{def}	2.21^{cde}	2.63^{bcd}	2.55^{bcd}	2.56^{bcd}	2.37^{cde}	1.27^{f}	1.42^{ef}	0.223	<0.01	0.94	<0.01	0.02	0.67	0.16	0.75
	(2.84)	(2.78)	(4.29)	(4.45)	(1.24)	(1.84)	(2.53)	(2.29)	(3.12)	(2.76)	(0.71)	(0.90)								
Fat	115.1^{ab}	112.5^{bc}	157.2^{a}	158.4^{a}	81.7^{bc}	98.4^{bc}	94.8^{bc}	99.8^{bc}	97.2^{bc}	87.1^{bc}	69.2^{c}	71.4^{c}	10.07	<0.01	0.67	<0.01	<0.01	0.54	0.51	0.62
(g/day)	(112.2)	(114.0)	(188.1)	(202.2)	(52.0)	(81.7)	(89.3)	(85.2)	(133.5)	(120.3)	(29.5)	(39.4)								
Protein	87.5^{ab}	79.8^{abcd}	94.5^{a}	91.3^{ab}	57.6^{de}	63.7^{bcde}	84.0^{abc}	77.2^{abcde}	68.5^{abcde}	61.7^{cde}	51.4^{e}	53.6^{de}	6.03	<0.01	0.38	<0.01	<0.01	0.73	0.27	0.94
(g/day)	(88.6)	(84.0)	(120.3)	(119.7)	(36.6)	(51.1)	(85.5)	(74.4)	(89.8)	(78.1)	(21.2)	(25.4)								
Milk composition (%)																				
Fat	3.84^{ab}	4.07^{ab}	4.55^{a}	4.70^{a}	4.30^{ab}	4.75^{a}	3.39^{b}	3.95^{ab}	4.07^{ab}	3.99^{ab}	4.30^{ab}	4.30^{ab}	0.245	<0.01	0.09	<0.01	0.44	0.65	0.52	0.43
	(3.69)	(4.04)	(4.57)	(4.97)	(4.04)	(4.74)	(3.09)	(3.59)	(4.59)	(4.47)	(3.99)	(4.48)								
Protein	2.94	2.94	2.93	2.93	2.93	2.93	2.94	2.94	2.93	2.93	2.93	2.94	0.006	0.35	0.58	0.02	0.85	0.63	0.58	0.97
	(2.97)	(2.96)	(2.92)	(2.92)	(2.93)	(2.92)	(2.95)	(2.95)	(2.92)	(2.92)	(2.93)	(2.93)								

[a–f] Within a row, least square means without a common superscript differ ($P < 0.05$).

[1] FPCM (fat and protein corrected milk)

2.5 Discussion

2.5.1 General Differences Between Cattle Types and Camels

In the present study, there were clear differences between the two animal species in terms of diet selection. In line with the classification of cattle as grazers (Hofmann, 1989) and camels as browsers (Schwartz et al., 1983), both cattle types mainly selected grasses and almost totally neglected woody forages, while the camels totally avoided grasses and relied on herbs, shrubs, and trees. Coppock et al. (1986), in a study comparing the diet selection of camel, cattle, and small ruminants in free range in the arid region of Ngisonyoka, a northwestern region in Kenya, reported that ≥ 95% of the diet of the cattle consisted of grasses and herbs while camels relied on woody vegetation (95% of the diet), and grasses and herbs accounted for 5% of their diets. Other diet selection studies in free range in similar rangelands in Kenya or Somalia found grass and herb proportions of 6% (Elmi, 1989) and 10% (Migongo and Hansen, 1987) in camel diets in the dry season and of 5% (Migongo and Hansen, 1987) and 14–19% (Lusigi et al., 1984) in the rainy season. The study sites in the present study had sufficient offer of woody vegetation that remained green throughout both seasons. Although grasses are the most important components of cattle diets (Coppock et al., 1986, Marquardt et al., 2010), forage from woody plants was found to be included in the diet of local cattle types kept in free range in Bolivian mountain forests during the dry season, with the concomitant decline in quantity and quality of herbaceous forage resources (Marquardt et al., 2010). In general, the grazing behavior of cattle and camels differed. The cattle moved together in a group while grazing, taking several successive bites at one spot before slowly moving to the next. The camels, however, did not browse together and spent less time on a single plant, only taking a few bites of each plant before quickly moving to the next. This behavior by the camels is assumed to be a strategy to avoid ingesting high amounts of plant secondary metabolites (PSM) in certain plants (Dereje and Uden, 2005a).

Milk yields of the cattle types were lower than those reported for crossbred cattle and local Boran cattle in Ethiopia; that were, however, kept and fed indoors (Jenet et al., 2004). In addition, the milk yields of the camels were comparably lower than reported elsewhere for camels in free range (Dereje and Uden, 2005b); however, in that study the camels were milked twice a day and supplemented with concentrate. Overall, as expected, the crossbreds outperformed the local Pokot cattle in milk yield in the more favorable RS with higher forage quality and availability. The small body size, poor feed conversion efficiency, and low frequency of genes for high production of local cattle compared to improved breeds with high genetic potential (Rege and Tawah, 1999) might explain the overall low

milk yield of the Pokot cattle. The local cattle and camels are adapted to environmental stress, can survive on poor-quality pastures, and are able maintain milk production in extreme conditions compared to exotic cattle (Hansen, 2004; Wilson, 1998). The higher fat content in the milk of local cattle compared to camel milk was also found by Yagil (2000); however, in that study no differences were found in milk protein content.

2.5.2 Differences in the Response to the Period by Cattle and Camels

Different from previous studies in Kenya by Ego et al. (2003), who found that browse components in cattle diets were twice as high in the dry season compared to the rainy season (on average, 8% and 4% of the diet, respectively), in the semi-arid vegetation in south-central Kenya, the proportion of browse in the cattle diets during the TP never exceeded 0.1% of the diet. Although the TP was more unfavorable in terms of availability of forage resources and forage quality than the RS, the quantity and quality were considerably higher compared to the situation of a dry season; thus, the cattle were not forced to switch to forage resources other than grasses. The relative browsing time of the camels was higher in the TP compared to the RS. This difference might be related to the more pronounced decrease in the quality and quantity of the herbs in comparison to trees and shrubs from the RS to the TP but also site-specific aspects; for instance, the higher shrub cover in study site 2, which was used for TP observations, cannot be totally excluded. Camels are able to select high-quality forage throughout the seasons (Wardeh, 2004) and thus, are able to maintain their milk production (Dereje and Uden, 2005b). Different from the RS, the crossbreds did not have a better performance than the Pokot cattle for milk yield in the TP. During the drier TP, the quality of the forage on offer declined, and this might have led to the observed decline in the milk yield of the crossbred cattle with higher nutritional requirements compared to the local cattle types (Jenet et al., 2004). These authors showed that there was no response in milk yield by local cattle (pure Boran) compared to crossbreds (Boran × Holstein) that significantly responded in milk yield to additional feed levels (Jenet et al., 2004).

2.5.3 Differences in the Response to Supplementation by Cattle and Camels

No effects of supplementation with an RDP-based supplement were found in the cattle diets regarding the proportions of the different functional group. Although, Judkins et al. (1985) also found no effect of protein supplementation on plant selectivity of cattle based on chemical and botanical composition, Odadi et al. (2013) found in a study conducted in semi-arid Kenyan rangelands close to the present study area that the cattle supplemented with extra-protein (cotton seedcake) had a lower intake (measured as % of bites) of forbs during the dry season. These authors related the higher intake of forbs (which overall ranged between 1% and 5% of total bites) of the non-supplemented cattle to higher CP demands, as the forbs were, in comparison to the grasses, relatively rich in CP. These significant differences described above, however, were found only for the dry season and not for the rainy season (Odadi et al., 2013). Although in the present study low levels of crude protein (3–11% DM) were found in the most frequently selected grass species, they were analyzed in samples collected during the RS and the TP, but not during a dry season. In the TP, the grass was already dry but of a better quality compared to the conditions during a dry season. This might be an explanation for the absence of a supplementation effect on cattle diet selection.

Different from the cattle, the camels that were supplemented with RDP had proportionally higher intakes of woody plants, especially shrubs, compared to the non-supplemented camel. In line with these results, the camels receiving supplement spent proportionally more time browsing (i.e., ingesting trees and shrubs) compared to the non-supplemented camels, especially in the TP. Woody plants are often rich , compared to grasses (reviewed by Duncan and Poppi, 2008). Supplements high in energy and protein content can help ruminants increase their diet of PSM rich forages and detoxify PSM, such as terpenes or tannins (Villalba and Provenza, 2005; Dziba et al., 2006; Mkhize et al., 2016). Sheep supplemented with extra-protein and energy spent more of the observation time feeding on a woody plant (*Artemisia tridentata*) rich in terpenes compared to control sheep (Dziba et al., 2007). In a study by Mkhize et al. (2016), supplementation with extra-protein or energy (here, soybean meal or maize grain) increased intake of woody plants (and concomitantly, of CT) by local goats in a semi-arid savanna rangeland of South Africa compared to non-supplemented goats. Supplementation in this study helped to counterbalance the effects of CT, which have the ability to form complexes with proteins and other nutrients. In the present study, the woody plant species that were preferred by the camels supplemented with RDP had comparably high amounts of total extractable phenols (*C. spinarum* 18% and *T. graveolens* 11%) and CT content of 6.5% and 0.2%, respectively. Thus, the extra-protein

provided by the supplementation might have enabled the camels to ingest more of the browse rich in phenols.

Supplementation had, other than expected, no influence on milk yield or milk composition in the camels and cattle. Dereje and Uden (2005b) found that protein- and energy-rich concentrate feeding (4 kg/day per camel) had a significant effect on milk yield and fat content, but not protein content, in lactating dromedary camels in a grazing study conducted in eastern Ethiopia. In a similar study, Moges et al. (2016) reported a considerable increase in milk yield and quality in response to different levels of concentrate (made from a mixture of wheat bran, sorghum grain, noug seedcake, and mineral vitamin premix) supplementation (amounts of 250, 500, and 750 g/kg milk) for the browsing camels. The low levels of protein supplement in this study (on average, 155 and 240 g/day in camels and cattle, respectively) might have led to the lack of effect on milk yield and milk composition compared to similar studies with higher amounts of protein supplement.

2.6 Implications

The results of the present study showed clear differences in the diet selection pattern of camel and cattle kept on semi-arid rangelands, with almost no dietary overlap. Because of this, cattle and camels do not compete for forage resources and therefore, are able to co-graze/browse in the same rangelands without affecting each other's performance. Extra rumen-degradable protein increased the use of forage from woody species by camels and thus, could be considered as rangeland management practice for control of bush encroachment. The lack of an effect of supplementation in cattle does not exclude that this might be different during the dry season, a period which was not included in the present study. If improved cattle types are introduced, special attention to either calving season (best in proximity to the rainy season) or supplementation (the transition and dry periods) has to be given to avoid a major depression in milk yield and milk constituents. Regarding supplementation, the best type (energy, degradable or undegradable protein, etc.) has yet to be determined. The present results indicate that camels are best adapted to the environmental conditions of the East African savannas, which are increasingly affected by climate change and concurrent droughts. Unexpectedly, the local cattle type did not differ much in diet selection behavior from improved cattle breeds. The improved cattle were superior only to the local Pokot cattle in milk yield in the rainy season, but not in the more harsh transition period.

Chapter 3

Free ranging camels and cattle respond differently in phenol intake, phenol excretion with milk and milk fatty acid profile

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3.1 Abstract

Camels as browsers select different forage resources in savannah rangelands than cattle as grazers what might underlie seasonal changes. These effects of season and diet selection might affect phenol transfer into milk and milk fatty acid profiles, which are particularly relevant for human health. Therefore, differences between dromedary camels and cattle were tested on semi-arid Kenyan rangelands in the rainy season (RS) and in the transition period (TP) to the dry season. Two cattle types (Guernsey × Boran crossbred and local 'Pokot' Zebu cattle) were employed. Seventy-two lactating animals were used, 12 each per animal type and season. Half of each group of 12 were supplemented with a urea-molasses mix at night when the animals were confined. The intakes of nutrients and total extractable phenols (TEP) were estimated through scan sampling by following target animals. Milk samples collected on d 39 and 35 in RS and TP, respectively, were analyzed for total phenol content and fatty acid (FA) profile. In addition, milk samples before and at the end of RS were analyzed for microbial counts. Average TEP intakes varied between seasons in cattle but despite large differences between individual animals not in camels. Season, but not animal type, had an overall effect on milk phenol concentration. Concomitantly, total phenol excretion with milk was correlated with TEP intake only in the cattle. Counts of mesophilic germs, enterococci and staphylococci in milk of all animal types were higher at the end of the RS than at the start of RS. Their proliferation was not suppressed by the supplementation. Season, animal type and especially their interaction were significant in the proportion of a large number of FA in milk fat. Proportions of mono- and polyunsaturated FA were generally higher in camel milk fat, and the first was higher in RS than TP in cattle but not in camel milk. Cattle milk fat contained more conjugated linoleic acids than that of camels in RS but not in TP. Proportions of oleic, linoleic, α-linoleic, eicosapentaenoic and docosahexaenoic acid (only in RS) were higher and the n-6-to-n-3 ratio was lower in camel milk than cattle milk. The effects of supplementation were mostly small. The results indicate that camels and cattle may have a fundamentally different lipid metabolism and that camels may have developed control mechanisms limiting adverse effects and transfer of phenols to milk in response to the typically higher intake of TEP-rich browse.

3.2 Introduction

Camels (*C. dromedarius*) and cattle (*Bos indicus*) are important livestock species in the arid and semi-arid areas of Eastern Africa. Indigenous camel and cattle types are well adapted to arid environments (Yagil, 1994; Hansen, 2004) and produce milk under adverse conditions (Degen, 2007). Milk is an important source of macro- and micronutrients for people living in these areas (Farah, 2004), is a source of income and assists in fulfilling socio-cultural practices.

The milk composition of camels and cattle kept on arid and semi-arid rangelands is mostly influenced by the vegetation they graze or browse on. Camels, classified as browsers, mainly utilize forage resources from woody vegetation (Schwartz et al., 1983) and herbs, while cattle, classified as grazers, rely mostly on herbaceous vegetation, especially grasses (Hofmann, 1989). Dicotyledons, and especially woody plants, contain more plant secondary compounds (PSC) than grasses (Mariaca et al., 1997). However, there are large, often seasonal differences in occurrence and level of PSC in plants (Abilleira et al., 2011). Some PSC like terpenes (Abilleira et al., 2011) or phenols (Di Trana et al., 2015; Kälber et al., 2011; Cornu et al., 2005) were found to be transferred into milk.

The FA profile of the milk fat is important for human health. Based on comparisons from different literature reports, camel milk in comparison to cattle milk seems to have smaller proportions of short- and medium-chain FA and high proportions of MUFA and PUFA including α-linolenic acid (C18:3*n*-3) (Gorba and Izzeldin, 2001; Konuspayeva et al., 2008). It is, however, unclear if this results from differences between camels and cattle in either metabolic or ingestive processes. Studies in dairy cows (e.g., Leiber et al., 2005) showed that ruminal biohydrogenation saturates the largest share of the dietary PUFA. Accordingly, PSC influencing ruminal biohydrogenation are often more important in determining the milk FA profile than are dietary PUFA. Khiaosa-ard et al. (2009) found that certain tannins increased the formation of ruminal biohydrogenation intermediates, like rumenic acid (C18:2 *c*9, *t*11), the most important conjugated linoleic acid (CLA), and its precursor (vaccenic acid, C18:1 *t*11). Dietary sources of PSC modified the milk fat accordingly (Kälber et al., 2011; Vasta and Luciano, 2011). Considering that camels prefer PSC-rich browse, a different milk fat composition between camels and cattle can be expected even when kept on the same biodiverse rangelands. To the knowledge of the authors, such direct species comparisons have not yet been performed.

The hypotheses tested were the following. i) Camel milk, compared to cattle milk, contains more phenols due to differences in diet selection, and concomitantly, ii) lower microbial counts compared to the cattle types, and iii) the FA composition of camel and cattle milk differs. iv) The milk of pure

indigenous cattle and crossbreds differs in milk phenol content and FA profile as they are presumed to exert a different nutrient intake pattern. v) There are seasonal differences in the milk phenol content and FA profile of camels, indigenous and crossbred cattle. These hypotheses were tested in an experiment carried out in Kenya on semi-arid rangelands. Specific results on plant selection and animal performance are reported in Leparmarai et al. (2018). In addition, effects of supplementation with rumen degradable protein (RDP) were tested as this could improve fiber digestion and help to detoxify PSC and, with that, possibly alter plant selection and milk composition.

3.3 Materials and Methods

3.3.1 Experimental Periods and Study Site

The experiment was performed on the Suyian Ranch (00° 30’06”N to 36° 41’ 46”E, 1800 m a.s.l.) with a size of 17,600 ha and located in the semi-arid rangelands of Laikipia County, Kenya. The field research was approved by the Kenyan National Commission for Science, Technology and Innovation (NACOSTI).The experiment was performed in 2 periods, i) from May 14 to July 2, 2015 (rainy season, RS) and ii) from August 22 to October 7, 2015 (transition period, TP, between the subsequent dry and the next RS). In both seasons, sufficient vegetation was available, which was, however, of lower quantity and quality in TP compared to RS. The experimental period consisted of 10 and 8 d of adaptation and 40 and 36 d of observation in RS and TP, respectively. The rangelands used in the 2 seasons were 15 km apart. They consisted of semi-arid savannas, mainly on vertisols, covered by grassland and shrubby grassland interspersed with trees. The woody vegetation was mainly represented by *Acacia drepanolobium* and *A. nilotica* on the RS site, and by *Boscia angustifolia* and *Gymnosporia putterlickioides* on the TP site. The herbaceous layer was dominated by perennial grasses (*Hyparrhenia papillipes* and *Themeda triandra*) and herb (*Barleria delamerei*) in RS and perennial grasses (*Pennisetum stramineum* and *Hyparrhenia papillipes*) and herb (*Barleria delamerei*) in TP.

3.3.2 Experimental Animal Types

Twenty-four multiparous lactating animals (12 per season with similar characteristics as in the respective other season, see Table 3.1) from each of camels and 2 cattle types were used. The camels (*Camelus dromedarius*) were of a ‘Somali’ type. The local cattle were ‘Pokots’, an indigenous small east African shorthorn zebu (*Bos indicus*). The other cattle type were crossbreds of East African Boran

(*B. indicus*) and Guernsey (*B. taurus*), which were F1 crossbreds, except in RS where 3 animals had 75% Boran and 25% Guernsey blood levels. Camels and crossbreds were selected from the Suyian Ranch herds, and the Pokots were obtained from the neighboring local community. The criteria used for selecting the animals were similar DIM, parity/age and milk yield as well as healthiness and tameness across all 3 animal types, and BW was similar within animal type (Table 3.1; more details in Leparmarai et al., 2018). All animals were accompanied by their offspring.

Table 3.1 Characteristics of the different experimental animals used in the rainy season (RS) and the transition period (TP) either supplemented (RDP+) or not supplemented (RDP–) with rumen-degradable protein (arithmetic means ± SD)

Season	Animal type	Supplementation	*n*	BW, kg[1]	Milk yield, kg/d	Age, yr	Parity, number	DIM[2]
RS	Camel	RDP+	6	534±38	2.10±0.65	7.2±1.5	2.5±1.2	86.7±71.5
		RDP–	6	513±76	2.19±0.77	9.2±2.9	4.0±1.1	88.2±86.1
	Crossbred	RDP+	6	307±28	3.82±1.01	6.0±2.2	3.5±1.9	47.7±25.8
		RDP–	6	302±79	3.79±1.59	6.3±2.7	3.7±2.3	44.8±25.8
	Pokot	RDP+	6	207±24	0.97±0.32	5.8±1.2	2.7±1.0	24.5±10.1
		RDP–	6	191± 9	1.38±0.42	5.5±0.8	2.5±0.8	25.2±10.9
TP	Camel	RDP+	6	489±57	2.10±0.87	-[3]	-	72.8±32.9
		RDP–	6	519±51	1.98±0.55	-	-	80.0±26.0
	Crossbred	RDP+	6	350±26	3.07±1.03	-	-	44.5±35.6
		RDP–	6	354±37	2.85±0.54	-	-	40.5±25.8
	Pokot	RDP+	6	236±41	0.54±0.36	-	-	26.3±14.1
		RDP–	6	212±20	0.65±0.49	-	-	38.5±11.8

[1]Measured 10 and 8 d before starting the supplementation in RS and TP, respectively. In TP BW is from 11 camels.
[2]Only from 10 Pokots.
[3]No information available.

3.3.3 Experimental Supplementation

Half of the animals of each type remained either unsupplemented or were supplemented (*n*=6 per treatment, animal type and season). The supplemented animals received urea as a source of RDP and a vitaminized mineral mix containing, per kg, NaCl, 300 g, Ca, 230 g, P, 20 g; Zn, 5 g; Mn, 3 g; Cu, 1.6 g; I, 0.25 g; Se, 0.02 g; vitamin E, 1 g; vitamin B_{12}, 3.12 mg. Supplementation which is described in detail in Leparmarai et al. (2018), where also the amounts of RDP supplements consumed are specified (155, 310 and 170 g/d per head on average for camels, crossbreds and Pokots, respectively).

3.3.4 Animal Management

From 0800 h to 1700 h, local herders accompanied the animals (dams and calves) on pasture. Camels grazed separately from the cattle. The animals got access to water at noon. From 0600 h to 0800 h, the animals were hand-milked in so called bomas, where they were confined at night separated from their calves, by the same herders. Calves initiated milk letdown. After milking and during the day, the calves could suckle the remaining milk.

3.3.5 Data Collection and Sampling

Once per wk and, additionally, at the start of the observation periods, BW was assessed at 0800 h before the animals left for grazing. In RS, cattle were weighed with a digital livestock balance (model TT40, TAL-TEC, South Africa; 0 to 2000 kg; accuracy ± 1 kg). In TP, only the first and the last measurements on the cattle were performed with this balance. Otherwise BW was estimated using a Rondo (Kruuse, Langeskov, Denmark) weight measuring tape to record the thoracic circumference right behind the forelegs. For that a regression was derived from tape and balance data on the first and last measurement date (BW (kg) = 0.86 circumference (cm) + 48.33; R^2 = 0.73). The BW of the camels was estimated from the linear body measurements according to Yagil (1994) as cited in Mehari et al. (2007) as BW (kg) = shoulder height (m) × chest girth (m) × abdominal girth (m) × 50. Milk yield was determined once daily using a 1-l jag with 100 ml correctness. Samples of 2 × 15 ml milk homogenized by stirring were transferred to plastic tubes on d 39 and 35 in RS and TP (ends of observation periods), respectively. Samples were immediately frozen at -20°C and later shipped to Switzerland for analysis. The plant selection behavior for estimating nutrient and phenol intakes was determined by following 1 animal per d in an alternating manner regarding animal type and supplementation group using scan sampling (1-min of recordings and 5 min of collecting of plant samples, resulting in 91 to 97 observation min/animal per season). Number of bites per plant species, its functional group, bite mass (bite category multiplied by bite size) and the plant part consumed were recorded following Agreil and Meuret (2004) with some modifications. More details are given in Leparmarai et al. (2018).

3.3.6 Laboratory Analysis

The most selected plant species, making up in total between 83 and 97% of the biomass intake, were analyzed after drying and grinding through a 1-mm screen for proximate contents by following standard procedures for nutrients (AOAC, 1997) and phenolic fractions (Makkar, 2003; see also Leparmarai et al., 2018). Total phenols in the milk were analyzed following Vazquez et al. (2015) with some modifications. A standard gallic acid was dissolved in water at 25, 50, 75, 100 and 125 µg/ml concentrations. These solutions were treated the same way as the milk samples. At first samples were vortexed, then 400 µl were transferred into a 2 ml Eppendorf tube, 500 µl of a 1:1 mixture of methanol and water were added, and vortexed. Then 30 µl of carrez I (potassium hexacyanoferrate II solution) was added, followed by 30 µl of carrez II (zinc sulphate solution) with vortexing after each step. This was followed by adding 250 µl of acetonitrile and mixing for 1 min. The mixture was left in the dark for 25 min when clot protein precipitation was complete. The resulting suspension was centrifuged (Eppendorf 5418, Eppendorf AG, Hamburg, Germany) at maximum speed for 6 min. The supernatant was obtained and transferred to new Eppendorf tubes and the analysis of total phenol contents was carried out using the Folin-Ciocalteu method. An amount of 150 µl of the supernatant was transferred into tubes and completed to 500 µl with 5:1 of Folin solution and Folin-Ciocalteu reagent (1 N; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). After being left to react for 3 min, 1 ml of sodium carbonate solution (7 g/100 ml distilled water) was added. A blank with all the reagents except milk sample but water supernatant was also prepared. All the samples were vortexed before leaving them in the dark for 40 min and then subjected to measurement with a spectrophotometer (VWR UV-6300, VWR international, Radnor, Pennsylvania) at 725 nm. Based on the standard, milk phenol concentrations were expressed as gallic acid equivalents.

To analyze the FA in milk, 0.5 ml milk were gently mixed and diluted in 5 ml n-heptane containing triundecanoin, tetradecenoic methylate and trivaleranoin as internal standards. Na-methylate was added for cold transesterification to FAME (Suter et al., 1997). The individual FAME were analyzed with a CP7421 column (200 m × 0.25 mm × 0.25 µm; Varian Inc., Darmstadt, Germany) on a gas chromatograph (model HP 6890 equipped with a FID detector (HP, Palo Alto, CA, USA). Injected volume was 1 µl, split ratio was 1:10, and hydrogen was applied as carrier gas at 1.7 ml/min. The temperature program was 60°C for 12 min, ramp 5°C/min up to 170°C; isotherm at 170°C for 60 min, ramp 5°C/min up to 250°C, isotherm for 20 min. The FA were identified based on a FAME standard

(Supelco 37 Component, Supelco Inc., Bellefonte PA, USA) and by comparing as a reference with chromatograms of milk lipids from Collomb and Bühler (2000) and Kramer et al. (2002). The proportions of individual FA were calculated by integrating peak areas. A response factor was applied obtained from pure 6:0, 13:0 and 19:0 triglyceride standards to correct for the low response of short-chain FA.

For microbial analysis, 0.1 ml of serial milk dilutions (in 1% peptone and 8.5% NaCl) were spread in duplicate on different agar media. Plate count agar (PC; Merck, Becton Dickinson AG, Allschwil, Switzerland) was used to enumerate mesophilic aerobic germs (further on called 'total germs') at 30°C after 48 h. KF *Streptococcus* agar (KFS; Becton Dickinson AG) served as medium to enumerate *Enterococcus* spp. at 43°C after 48 h and Baird Parker agar (BP; Merck) for staphylococci at 37°C after 30 h, and to identify coagulase-positive staphylococci colonies by its halo which represent most probably *Staphylococcus aureus*. The detection limit was 10 cfu/ml.

3.3.7 Calculations and Statistical Analysis

Intakes of nutrients and phenolic compounds determined in the 1-min observation bouts were extrapolated to daily biomass intake values (i.e., to 8 h by subtracting the proportion of time that was not spent eating) from these observation taking into account that no biomass was available in the enclosures. The equation used reads: $Intake_x$ (g/d per animal) = ($Intake_x$ as calculated from observation time / number of observation minutes × 480 min) × % of time spent eating/100; where $_x$ are the single nutrients or phenolic compounds. The average nutrient and phenol contents of the biomass were calculated from the composition measured in samples of individual plant species collected twice in each season and proportionate intake of these individual plant species data calculated as: DM $intake_x$ (no. of $bites_x$ × estimated bite $weight_x$ × % of DM_x/100) + … DM $intake_n$ (no. of $bites_n$ × estimated bite $weight_n$ × % of DM_n/100, with $_{x\text{-}n}$ being the single plant species). It is acknowledged that these extrapolations were associated with uncertainty. Intake data in table are presented in relation to metabolic BW ($BW^{0.75}$) to account for differences between animal types. Data were analyzed by ANOVA using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The model considered season, animal type and supplementation, and all possible interactions as fixed effects. Normal distribution of the residuals was tested graphically. Comparisons among means were performed with the help of Tukey's procedure. Regression analysis was done with the REG procedure of SAS. In the tables, LSM and SEM are displayed. $P < 0.05$ was considered statistically significant,

and $0.05 \leq P < 0.10$ as a trend. As supplementation was rarely significant in FA profile, subgroup data are not presented, but significances in relation to supplementation are presented in the footnotes.

3.4 Results

3.4.1 Estimated Intake of Nutrients and Phenols

The proportions of the most consumed plants in the diet of the animals that were used for calculation of nutrient intake ranged, with 1 exception, from 90 to 97% of all consumed plants (Table 3.2). Across all animal and supplementation types, season affected ($P < 0.05$) the average daily intakes of DM and any of the constituents presented (all related to $BW^{0.75}$). In detail, DMI was higher ($P < 0.05$) in RS compared to TP in both cattle types but not in the camels (season × animal type (**S × A**), $P < 0.05$). There were also S × A interactions in intakes of OM, CP, NDF, TEP and NTP. The camels consumed more ($P < 0.05$) TEP, TT, CT and NTP than cattle in both seasons. Intakes of DM, OM, NDF, TEP and NTP were lower ($P < 0.05$) in both cattle types in TP compared to RS, with no such difference in the camels. Within season, the intake of CP was higher in camels than cattle. Within animal types CP intake was higher in RS than TP (S × A, $P < 0.001$). Intake of ADF was overall twice as high ($P < 0.001$) in RS than TP, and the camels had a more than 2 times higher ($P < 0.001$) ADL intake than the cattle types. Intakes of TT and HT intakes slightly increased with supplementation ($P < 0.05$). In CT intake there were animal type × supplementation ($P < 0.05$) and 3-way interactions ($P < 0.05$). In RS supplemented camels ate more ($P < 0.05$) CT than non-supplemented camels.

Table 3.2 Estimated intake (LSM; g/d and kg $BW^{0.75}$)[1] of nutrients and total phenols during the 2 seasons (S), the rainy season (RS) and the transition period (TP) (*n*=6 per subgroup)[2]

S	Animal type	SU[3]	Proportion[4] (%)	DM	OM	CP[5]	NDF	ADF	ADL[5]	TEP[5]	TT[6]	CT[6]	NTP[6]	HT[6]
RS	Camel	RDP+	96.3	154.2^{a}	139.1^{a}	16.42^{a}	89.2^{abc}	64.6^{a}	22.33^{a}	7.54^{a}	2.33^{a}	1.17^{a}	5.21^{a}	1.16^{a}
		RDP–	96.3	135.6^{abcd}	121.9^{abcd}	14.58^{ab}	79.6^{abc}	57.8^{ab}	19.67^{a}	5.54^{abc}	1.40^{ab}	0.50^{b}	4.14^{ab}	0.90^{ab}
	Crossbred	RDP+	94.8	156.6^{a}	140.4^{a}	10.19^{b}	112.5^{a}	65.4^{a}	10.36^{bcd}	3.65^{bcd}	0.56^{cd}	0.10^{cd}	3.09^{b}	0.46^{bcd}
		RDP–	97.0	145.8^{abc}	130.5^{abc}	9.25^{b}	104.6^{ab}	61.3^{a}	9.67^{bcd}	3.46^{cd}	0.56^{cd}	0.10^{cd}	2.90^{b}	0.46^{bcd}
	Pokot	RDP+	92.3	148.8^{ab}	134.2^{ab}	10.44^{ab}	106.8^{ab}	61.3^{a}	10.28^{bc}	3.36^{d}	0.51^{cd}	0.08^{cd}	2.85^{b}	0.44^{bcd}
		RDP–	94.7	163.8^{a}	147.2^{a}	11.77^{ab}	117.2^{a}	67.2^{a}	11.06^{bc}	3.62^{bcd}	0.49^{cd}	0.11^{cd}	3.12^{b}	0.39^{cd}
TP	Camel	RDP+	83.0	103.2^{abcde}	94.9^{abcde}	9.68^{b}	63.2^{bc}	28.6^{c}	16.27^{ab}	5.67^{ab}	1.72^{ab}	0.41^{b}	3.95^{ab}	1.31^{a}
		RDP–	91.1	138.9^{abcd}	126.3^{abc}	12.93^{ab}	89.6^{abc}	42.6^{abc}	21.56^{a}	5.56^{abc}	1.33^{bc}	0.36^{bc}	4.23^{ab}	0.97^{abc}
	Crossbred	RDP+[7]	91.5	84.9^{bcde}	77.2^{bcde}	3.01^{c}	65.2^{bc}	31.7^{bc}	7.19^{cd}	1.71^{e}	0.34^{d}	0.01^{d}	1.38^{c}	0.33^{cd}
		RDP–	91.5	75.5^{de}	68.7^{de}	2.67^{c}	58.6^{c}	28.6^{c}	6.60^{cd}	1.45^{e}	0.25^{d}	0.01^{d}	1.19^{c}	0.25^{d}
	Pokot	RDP+	91.2	83.8^{cde}	76.1^{de}	2.93^{c}	64.4^{bc}	31.4^{c}	6.99^{cd}	1.60^{e}	0.28^{d}	0.01^{d}	1.32^{c}	0.27^{d}
		RDP–	90.1	70.5^{e}	64.4^{e}	2.43^{c}	54.8^{c}	26.8^{c}	5.98^{d}	1.32^{e}	0.22^{d}	0.01^{d}	1.09^{c}	0.22^{d}
SEM				14.70	13.09	1.242	10.03	5.94	1.531	0.505	0.225	0.096	0.348	0.145
P-values														
Season (S)				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.028
Animal type (A)				0.13	0.11	<0.001	0.67	0.88	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Supplement (SU)				0.97	0.94	0.91	0.92	0.95	0.85	0.084	0.028	0.054	0.24	0.047
S × A				0.010	0.009	<0.001	0.002	0.43	0.071	<0.001	0.94	0.69	<0.001	0.21
S × SU				0.56	0.55	0.55	0.59	0.55	0.48	0.82	0.97	0.22	0.65	0.48
A × SU				0.62	0.63	0.54	0.49	0.65	0.56	0.57	0.096	0.008	0.82	0.40
S × A × SU				0.096	0.098	0.058	0.102	0.13	0.056	0.14	0.67	0.047	0.092	0.93

CT, condensed tannins; HT, hydrolysable tannins; NTP, non-tannin phenols; TEP, total extractable phenols; TT, total tannins.
[1]Data calculated from estimated air DM intake and chemical composition based on observations made every 5 min for 1 min from 8:00 until 16:00. Intake data was extrapolated considering times spent eating and non-eating to 8 h (time spent on pasture, i.e. equivalent to total daily forage intake). Body weight is the average of 5 measurements per observation period.
[2]Means within column carrying no common superscript differ at $P < 0.05$.
[3]SU, supplement; RDP+, supplemented, RDP–, not supplemented with rumen-degradable protein.
[4]Proportion of air DM intake originating from the most selected plant species used for intake calculations.
[5]Log-transformed, but LSM and SEM are non-transformed data.
[6]Arcsine-transformed, but LSM and SEM the non-transformed data.
[7]*n*=5.

3.4.2 Total Phenols and Microbial counts in Milk

There was a decrease ($P < 0.001$) in milk total phenol concentration across all animal types from 49.4 in RS to 24.4 mg/l in TP (Table 3.3), whereas animal type and supplementation had no effect. Accordingly, also the daily excretion of total phenol with the milk was overall higher ($P < 0.001$) in RS with 137 mg/d compared to 42 mg/d in TP. The total phenol excretion with the milk was higher ($P < 0.05$) in camels and crossbreds compared to Pokot cattle as the result of the milk yield differences between animal types. Across data of both seasons and all animal types, total phenol excretion with milk increased ($P < 0.001$) with estimated TEP intake in cattle but not in camels (Fig. 3).

Milk pH value measured before ('start') and after RS ('end') was on average 6.63 and 6.21 across all 6 animal types and treatment groups, respectively (Table 3.3). There were 100 times or more times higher titers of total germs. A similar trend was visible when counting the considerable amounts of the fecal indicators Enteroccocci and at, to lower extent, Staphylococci. Presumed *Staphyloccoccus aureus* colonies were only found occasionally in camel milk samples at the start and in cattle milk of both types at the end. There was no substantial impact on the counts log-range of the 3 bacterial groups in pairwise comparisons of milk from RDP-supplemented or not-supplemented individuals.

Table 3.3 Contents and excretion (LSM) of total phenols (TP) in milk (expressed as gallic acid units), and counts of total germs and individual microbes in milk of the animals either supplemented (RDP+) or not supplemented (RDP–) with rumen-degradable protein (SU) collected in the rainy season (RS, Day 39) and the transition period (TP, Day 35). S: season; A: animal type; SU: supplementation. n.d.: values under detection limit (i.e., < log 1).

Season	Animal type		Supple-mentation	TP, mg/l[1]	TP, mg/d	pH		Total germs (log cfu/ml)[2]		Enterococci (log cfu/ml)[2]		Staphylococci (log cfu/ml)[2]		*S. aureus* (log cfu/ml)[2]	
Time				End of season[4]		Start of season[3]	End of season[4]	Start of season[3]	End of season[4]	Start of season[3]	End of season[4]	Start of season[3]	End of season[4]	Start of season[3]	End of season[4]
RS	Camel	6[5]	RDP+	45.9^{abc}	146^{abcd}	6.68	6.13	5.38	7.37	2.85 (4.27)	6.63	n.d.[5]	5.64	0.75(4.48)	n.d.
		6	RDP–	57.8^{a}	191^{a}	6.70	6.18	4.52 (5.43)	6.83	2.83 (4.25)	5.91	1.37 (2.74)	4.61 (5.53)	0.38 (2.30)	n.d.
	Crossbred	6	RDP+	46.7^{ab}	168^{ab}	6.63	6.37	4.67	6.56	0.50 (3.00)	6.19	0.45 (2.70)	3.90 (4.68)	n.d.	0.64 (3.87)
		6	RDP–	44.6^{abc}	160^{abc}	6.56	6.10	5.10	7.23	1.20 (3.59)	6.51	0.60 (3.61)	4.38	n.d.	2.17 (3.26)
	Pokot	6[6]	RDP+	42.9^{abc}	54^{cde}	6.59	6.28	3.85 (4.62)	6.41	0.71 (4.26)	5.82	0.90 (2.69)	4.79	n.d.	2.16 (4.31)
		6	RDP–	58.3^{a}	102^{abcde}	6.62	6.19	2.39 (4.78)	6.85	0.64 (3.85)	6.08	0.67 (4.01)	4.27	n.d.	1.00 (3.01)
TP	Camel	6	RDP+	21.2^{d}	52^{cde}	-[7]	-	-	-	-	-	-	-	-	-
		6	RDP–	18.5^{d}	42de	-	-	-	-	-	-	-	-	-	-
	Crossbred	6[5]	RDP+	25.3^{cd}	67^{bcde}	-	-	-	-	-	-	-	-	-	-
		6	RDP–	28.2^{bcd}	62^{bcde}	-	-	-	-	-	-	-	-	-	-
	Pokot	6[6]	RDP+	25.0^{cd}	13^{e}	-	-	-	-	-	-	-	-	-	-
		6	RDP–	28.1^{bcd}	18^{e}	-	-	-	-	-	-	-	-	-	-
	SEM			5.89	24.7	0.050	0.105	0.686	0.244	0.762	0.296	0.538	0.655	0.343	0.617
	P-values		S	<0.001	<0.001										
			A	0.22	<0.001	0.14	0.69								
			SU	0.23	0.35	0.87	0.26								
			S × A	0.10	0.19										
			S × SU	0.36	0.23										
			A × SU	0.56	0.58	0.50	0.35								
			S × A × SU	0.31	0.65										

Means within column carrying no common superscript differ at $P < 0.05$.

[1]Log-transformed; estimates and SEM taken from non-transformed data, *P* values and superscripts taken from the log-transformed data.

[2]Log-transformation was done after adding "1" to all data to account for 0 values. Values are the mean of 6 samples/subgroup with values of <log 1 set as 0 (SEM generated from these data). Values in brackets are mean values excluding samples having values of < log 1 (n.d.).

[3]Measured on Day 1 of adaptation period (RS).

[4]Measured on Day 39 (RS) and Day 35 (TP) of the respective observation period.

[5]n.d not identified.

[6]Only *n*=5 for the TEP excretion data due to lack of corresponding data on milk yield.

[7]- no data available.

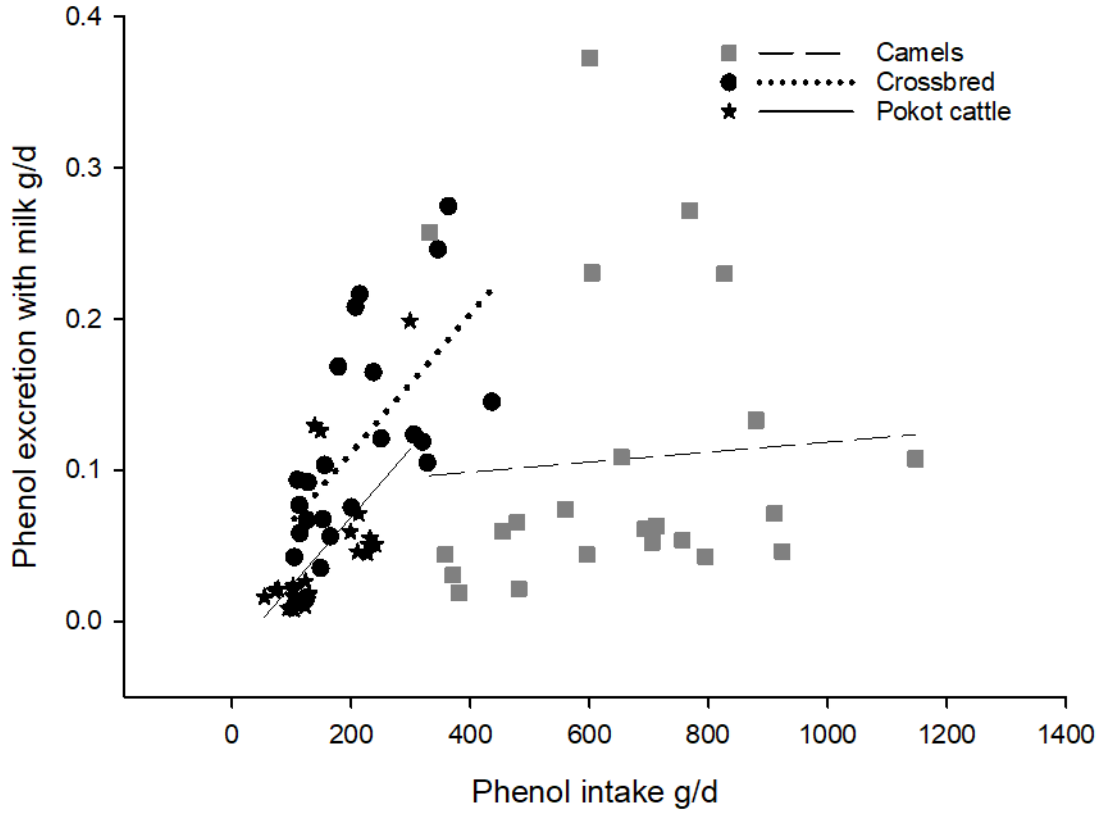

Fig. 3. Linear regression of the excretion of total phenols with the milk (y, g/d) on estimated phenol intake (x, g/d) across seasons.
Camels (*n*=23): y = 0.000 *X* + 0.086; R^2 = 0.005; RMSE = 0.098; *P* = 0.742.
Crossbreds (*n*=23): y = 0.001 *X* + 0.020; R^2 = 0.410; RMSE = 0.054; *P* = 0.001.
Pokots (*n*=22): y = 0.001 *X* – 0.022; R^2 = 0.406; RMSE = 0.038; *P* = 0.001.

3.4.3 Fatty Acid Profile of the Milk Fat

Proportions of total SFA were higher ($P < 0.05$) in cattle milk than camel milk, with no seasonal differences in camel but an increase in SFA proportions in the cattle milk from the RS to the TP (S × A, $P < 0.001$; Table 3.4). The same pattern was found for C16:0, the most abundant SFA, and almost a similar pattern occurred with C14:0. In C18:0 proportion, being similar in camel and cattle milk in RS, there was decrease ($P < 0.05$) by almost half from RS to TP in cattle milk, but not camel milk (S × A, $P < 0.001$). Camel milk fat had higher ($P < 0.05$) proportions of C14 *aiso*, C15 *iso*, C16 *iso* and C16 a*iso* than cattle milk in both seasons, while cattle milk had higher ($P < 0.05$) proportions of C4:0, C6:0, C8:0, C10:0, C12:0 and C12*iso* in both seasons. The proportions of C13:0, C15:0 and C17:0 were higher ($P < 0.05$) for camels than cattle in RS but not TP (S × A, $P < 0.001$). Proportions of C17*iso* and C22:0 were higher ($P < 0.05$) in cattle than camels in TP but not RS. Cattle milk had higher

C17*aiso* proportions than camel milk in RS but not TP, where it increased ($P < 0.05$) in all 3 animal types. The *iso/aiso* FA ratio was higher in camel than cattle milk and higher in TP than RS (both $P < 0.001$).

Table 3.4 Proportions (LSM) of saturated fatty acids (FA), including branched-chain *iso*- and *antiiso*-FA, in milk fat (% FAME) (n=6 per subgroup)[1]

	Rainy season			Transition period			SEM	*P*-values		
	Camel	Crossbred	Pokot	Camel	Crossbred	Pokot		Season (S)	Animal type (A)	S × A
C4:0[2,4]	<0.01[b]	1.58[a]	1.37[a]	<0.01[b]	1.34[a]	1.33[a]	0.135	0.172	<0.001	0.220
C6:0[2]	0.10[b]	1.69[a]	1.52[a]	0.09[b]	1.69[a]	1.69[a]	0.068	0.206	<0.001	0.075
C8:0[2]	0.09[d]	1.20[bc]	1.11[c]	0.10[d]	1.31[ab]	1.38[a]	0.053	<0.001	<0.001	0.008
C10:0[2]	0.16[c]	2.66[ab]	2.40[b]	0.15[c]	2.88[a]	2.88[a]	0.143	0.007	<0.001	0.019
C12:0[3]	0.79[c]	3.17[ab]	2.96[b]	0.78[c]	3.56[a]	3.61[a]	0.162	<0.001	<0.001	0.012
C12*iso*[2,5]	0.02[d]	0.06[c]	0.07[bc]	0.02[d]	0.08[b]	0.10[a]	0.006	<0.001	<0.001	<0.001
C13:0[6]	0.12[a]	0.07[b]	0.08[b]	0.12[a]	0.12[a]	0.13[a]	0.006	<0.001	<0.001	<0.001
C13*iso*[2]	0.34[ab]	0.28[c]	0.30[bc]	0.39[a]	0.37[a]	0.38[a]	0.024	<0.001	0.022	0.352
C14:0	10.67[de]	12.40[bc]	11.79[cd]	9.86[e]	13.67[a]	13.10[ab]	0.469	0.023	<0.001	0.001
C14*iso*[7]	0.02[a]	0.01[b]	0.02[a]	0.02[a]	0.02[a]	0.02[a]	0.002	<0.001	0.001	0.824
C14*aiso*	1.12[b]	0.65[c]	0.73[c]	1.28[a]	0.67[c]	0.73[c]	0.053	0.039	<0.001	0.076
C15:0	1.91[a]	1.23[c]	1.35[c]	1.84[ab]	1.67[b]	1.81[ab]	0.076	<0.001	<0.001	<0.001
C15*iso*[2,8]	0.12[a]	0.02[c]	0.02[c]	0.08[b]	0.01[c]	0.02[c]	0.012	<0.001	<0.001	0.076
C16:0	29.38[c]	34.60[b]	34.14[b]	28.82[c]	41.81[a]	42.12[a]	1.305	<0.001	<0.001	<0.001
C16*iso*[2]	0.61[b]	0.22[c]	0.20[c]	0.85[a]	0.23[c]	0.22[c]	0.029	<0.001	<0.001	<0.001
C16*aiso*	0.54[ab]	0.43[c]	0.46[c]	0.59[a]	0.48[bc]	0.47[bc]	0.027	0.007	<0.001	0.466
C17:0	1.11[a]	0.74[c]	0.80[c]	1.07[ab]	0.96[b]	1.00[ab]	0.041	<0.001	<0.001	<0.001
C17*iso*[9]	0.03[b]	0.02[b]	0.02[b]	0.02[b]	0.04[a]	0.04[a]	0.004	<0.001	0.089	<0.001
C17*aiso*	0.08[c]	0.10[b]	0.10[b]	0.12[ab]	0.13[a]	0.12[a]	0.007	<0.001	0.007	0.212
C18:0	10.97[a]	10.30[a]	10.14[a]	10.99[a]	5.60[b]	5.95[b]	0.616	<0.001	<0.001	<0.001
C20:0[10]	0.01	0.02	0.01	0.03	<0.01	0.01	0.011	0.809	0.009	0.375
C22:0	0.10[c]	0.11[bc]	0.13[bc]	0.10[c]	0.13[ab]	0.16[a]	0.010	0.010	<0.001	0.095
Iso/aiso	2.89[b]	1.79[d]	1.92[cd]	3.37[a]	2.03[cd]	2.10[c]	0.112	<0.001	<0.001	0.103
Total	55.42[c]	69.78[b]	67.79[b]	53.96[c]	74.75[a]	75.17[a]	1.289	<0.001	<0.001	<0.001

[1]Means within row carrying no common superscript differ at $P < 0.05$. Measured on Day 39 (RS) and Day 35 (TP) of the respective observation period. Only 5 camels and 5 Pokots in rainy season, respectively.
[2]Arcsine-transformed but LSMand SEM are non-transformed data.
[3]Log-transformed, but LSMand SEM are non-transformed data.
[4]P=0.008 for S×A×SU. SU = supplementation.
[5]P=0.041 for A×SU.
[6]P=0.030 for SU.
[7]P=0.006 for S×SU.
[8]P=0.047 for S×SU.
[9]P=0.023 for SU; P=0.045 for S×SU; P=0.035 for A×SU.
[10]P=0.016 for SU.

Proportions of total MUFA were higher in camel than cattle milk in both seasons, with a decrease in cattle milk, but not in camel milk, from RS to TP (S × A, $P < 0.001$; Table 3.5). This pattern was almost the same for C18:1 *n*-9, but increased in camel milk from RS to TP. In both seasons, C16:1 proportion was 4 to 5 times higher ($P < 0.05$) in camel than cattle milk. The proportion of C18:1 *t*11 was more than 2 times higher ($P < 0.05$) in the cattle than camel milk in RS (3.4 vs 1.5% of total FAME, respectively), but more than 2 times higher ($P < 0.05$) in camel than cattle milk in TP (2.0 vs 0.8 % of total FAME), with an increase ($P < 0.05$) in camel milk and a strong decrease ($P < 0.05$) in cattle milk from RS to TP. Camel milk fat had higher ($P < 0.05$) proportions of C12:1, C16:1, C16:1 n-7, C17:1, C18:1 *c*11, C18:1 *c*12, C18:1 *t*6+*t*7+8*t*, C18:1 *t*10, C18:1 *t*13+*t*14+*c*6+*c*7+*c*8 than cattle milk fat, whereas the opposite ($P < 0.05$) was the case with proportions of C10:1 and C20:1 *c*11.

Table 3.5 Proportions (LSM) of MUFA in milk fat (% FAME) (n=6 per subgroup)[1]

	Rainy Season			Transition Period			SEM	*P*-values		
	Camel	Crossbred	Pokot	Camel	Crossbred	Pokot		Season (S)	Animal type (A)	S × A
C10:1[2]	0.02^{d}	0.24^{c}	0.27^{bc}	0.01^{d}	0.31^{ab}	0.35^{a}	0.019	<0.001	<0.001	0.014
C12:1	0.07^{b}	0.05^{d}	0.05^{d}	0.08^{a}	0.06^{cd}	0.07^{bc}	0.005	<0.001	<0.001	0.597
C14:1	1.11^{c}	1.33^{bc}	1.57^{ab}	1.12^{c}	1.66^{ab}	1.89^{a}	0.121	0.002	<0.001	0.084
C15:1	0.57^{a}	0.39^{b}	0.43^{b}	0.57^{a}	0.58^{a}	0.63^{a}	0.032	<0.001	0.001	<0.001
C16:1[2]	6.81^{a}	1.26^{b}	1.31^{b}	6.52^{a}	1.77^{b}	1.96^{b}	0.323	0.002	<0.001	0.006
C16:1n-7	0.72^{b}	0.44^{e}	0.47^{de}	0.84^{a}	0.54^{cd}	0.57^{c}	0.029	<0.001	<0.001	0.879
C17:1	0.75^{a}	0.28^{c}	0.29^{c}	0.78^{a}	0.44^{b}	0.44^{b}	0.034	<0.001	<0.001	0.011
C18:1n-9	17.95^{b}	15.16^{c}	16.36^{bc}	20.53^{a}	12.83^{d}	11.75^{d}	0.831	0.002	<0.001	<0.001
C18:1 *c*10[2]	0.19^{a}	0.09^{bc}	0.08^{bc}	0.14^{ab}	0.06^{c}	0.06^{c}	0.030	0.026	<0.001	0.660
C18:1 *c*11	1.33^{a}	0.57^{b}	0.55^{b}	1.41^{a}	0.51^{b}	0.46^{b}	0.044	0.371	<0.001	0.010
C18:1 *c*12	0.10^{a}	0.04^{c}	0.04^{c}	0.08^{a}	0.04^{c}	0.03^{c}	0.005	0.003	<0.001	0.002
C18:1 *c*13[3]	0.12^{a}	0.10^{ab}	0.12^{a}	0.13^{a}	0.10^{ab}	0.08^{b}	0.013	0.088	0.009	0.022
C18:1 *t*6+*t*7+*t*8	0.50^{a}	0.24^{b}	0.26^{b}	0.51^{a}	0.11^{c}	0.11^{c}	0.016	<0.001	<0.001	<0.001
C18:1 *t*9[2,5]	0.19^{b}	0.19^{b}	0.21^{ab}	0.25^{a}	0.09^{c}	0.10^{c}	0.017	<0.001	<0.001	<0.001
C18:1 *t*10[2,6]	0.28^{a}	0.21^{b}	0.23^{b}	0.20^{b}	0.08^{c}	0.08^{c}	0.013	<0.001	<0.001	<0.001
C18:1 *t*11	1.46^{c}	3.37^{a}	3.38^{a}	2.04^{b}	0.77^{d}	0.76^{d}	0.136	<0.001	0.001	<0.001
C18:1 *t*12[2,7]	0.09^{a}	0.07^{ab}	0.05^{b}	0.10^{a}	0.04^{b}	0.05^{b}	0.012	0.194	<0.001	0.039
C18:1*t*13+*t*14+*c*6+*c*7+*c*8	0.32^{a}	0.19^{b}	0.22^{b}	0.20^{b}	0.13^{c}	0.14^{c}	0.017	<0.001	<0.001	0.044
C18:1 *c*14+*t*16[2,8]	0.28^{a}	0.15^{c}	0.20^{b}	0.14^{c}	0.06^{d}	0.06^{d}	0.018	<0.001	<0.001	0.059
C20:1 *c*9[2,9]	0.04^{c}	0.07^{a}	0.08^{a}	0.06^{b}	0.02^{d}	0.01^{d}	0.005	<0.001	0.001	<0.001
C20:1 *c*5[3]	0.04^{ab}	0.03^{bc}	0.04^{ab}	0.05^{a}	0.03^{bc}	0.02^{c}	0.007	0.002	<0.001	0.004
C20:1 *c*11[2]	0.08^{c}	0.19^{b}	0.24^{ab}	0.06^{c}	0.24^{ab}	0.28^{a}	0.020	0.028	<0.001	0.017
C20:1 *t*[2]	0.02^{b}	0.01^{c}	0.01^{c}	0.03^{a}	0.01^{c}	0.02^{bc}	0.003	0.019	<0.001	0.008
Total	33.02^{a}	24.66^{b}	26.45^{b}	35.86^{a}	20.47^{c}	19.92^{c}	1.123	<0.001	<0.001	<0.001

[1]Means within row carrying no common superscript differ at $P < 0.05$. Measured on Day 39 (RS) and Day 35 (TP) of the respective observation period. Only 5 camels and 5 Pokots in rainy season, respectively.
[2]Arcsine-transformed, but LSM and SEM are non-transformed data.
[3]Log-transformed, but LSM and SEM are non-transformed data.
[4]P=0.045 for S×A×SU.
[5]P=0.002 and 0.044 for SU and S×SU, respectively.
[6]P=0.019 for SU.
[7]P=0.004 for S×A×SU.
[8]P=0.023 for S×A×SU.
[9]P=0.036 for S×SU.

The PUFA proportion was more than twice as high in camel milk compared to cattle milk ($P < 0.05$), and PUFA proportion was overall higher ($P < 0.001$) in RS than TP (Table 3.6). Total n-3 and n-6 FA proportions were always higher ($P < 0.05$) in camel than cattle milk, and they decreased ($P < 0.05$) in camel milk from RS to TP. The n-6/n-3 ratio was always lower ($P < 0.05$) for camel milk than cattle milk, with an increase ($P < 0.05$) for all animal types in TP. The proportion of C18:3 *n*-3 was about 4

times higher ($P < 0.05$) in camel than cattle milk, with a decrease ($P < 0.05$) from RS to TP in all animal types. The C18:3 *n*-6 proportion was higher in cattle than camel milk in TP, and higher in Pokot milk than camel milk in RS (both $P < 0.05$). The increase ($P < 0.05$) in this FA from RS to TP was only found in cattle milk. The proportions of C20:5 *n*-3, C22:5 *n*-3 and C22:6 *n*-3 were generally low, but also higher in camel than cattle milk in each season (except in TP for C22:6 *n*-3). The proportion of C18:2 *c*9 *t*11 was higher ($P < 0.05$) in cattle than camel milk in RS, but not in TP (S × A, $P < 0.001$; Table 3.6).

There were only few effects of supplementation and interactions ($P < 0.05$) with season or animal type, and this often only in minor FA (see footnotes to Tables 3.4, 3.5 and 3.6).

Table 3.6 Proportions (LSM) of PUFA in milk fat (% FAME) (n=6 per subgroup)[1]

	Rainy Season			Transition Period				*P*-values		
	Camel	Crossbred	Pokot	Camel	Crossbred	Pokot	SEM	Season (S)	Animal type (A)	S × A
C18:2 n-6	2.72^{a}	0.71^{c}	0.64^{c}	2.10^{b}	0.76^{c}	0.67^{c}	0.088	<0.001	<0.001	<0.001
C18:2 *c*9,*c*15[2,3]	0.27^{a}	0.21^{c}	0.21^{c}	0.27^{ab}	0.22^{bc}	0.25^{abc}	0.018	0.011	<0.001	0.165
C18:2 *c*9,*c*11[2]	0.22^{a}	0.03^{c}	0.03^{c}	0.19^{b}	0.04^{c}	0.04^{c}	0.009	0.049	<0.001	<0.001
C18:2 *t*6	0.21^{a}	0.18^{b}	0.20^{ab}	0.18^{b}	0.06^{c}	0.06^{c}	0.010	<0.001	<0.001	<0.001
C18:2 *c*9,*t*13+*t*8,*c*12[2]	0.52^{a}	0.13^{c}	0.15^{c}	0.24^{b}	0.07^{d}	0.06^{d}	0.022	<0.001	<0.001	<0.001
C18:2 *c*9,*t*12[2,4]	0.13^{a}	0.04^{cd}	0.05^{c}	0.07^{b}	0.03^{de}	0.02^{e}	0.006	<0.001	<0.001	<0.001
C18:2 *t*11,*c*15+*t*9,*c*12[2,3]	0.94^{a}	0.54^{c}	0.54^{c}	0.73^{b}	0.10^{d}	0.09^{d}	0.046	<0.001	<0.001	<0.001
C18:2 *c*9,*t*11[2]	0.54^{b}	0.83^{a}	0.92^{a}	0.52^{b}	0.44^{b}	0.51^{b}	0.054	<0.001	<0.001	<0.001
C18:2 *t*9,*t*11[2]	0.07^{a}	0.03^{b}	0.03^{b}	0.03^{b}	0.01^{c}	0.01^{c}	0.007	<0.001	<0.001	0.284
C18:2 total[2]	5.62^{a}	2.69^{c}	2.77^{c}	4.33^{b}	1.71^{d}	1.72^{d}	0.162	<0.001	<0.001	0.586
C18:3n-3[2]	1.87^{a}	0.53^{c}	0.48^{cd}	1.28^{b}	0.29^{de}	0.28^{e}	0.070	<0.001	<0.001	0.229
C18:3n-6	0.13^{c}	0.16^{bc}	0.18^{b}	0.14^{bc}	0.23^{a}	0.23^{a}	0.013	<0.001	<0.001	0.001
C20:2n-6[5]	0.15^{a}	0.08^{d}	0.08^{d}	0.15^{a}	0.11^{bc}	0.12^{b}	0.009	<0.001	<0.001	0.013
C20:3n-6[2]	0.10^{b}	0.03^{c}	0.03^{c}	0.17^{a}	0.07^{bc}	0.07^{bc}	0.019	<0.001	<0.001	0.998
C20:4n-6[2]	0.19^{a}	0.05^{d}	0.05^{d}	0.20^{a}	0.09^{b}	0.09^{b}	0.013	<0.001	<0.001	0.023
C20:3n-3[2]	0.07^{a}	0.01^{b}	0.01^{b}	0.07^{a}	0.01^{b}	0.01^{b}	0.004	0.547	<0.001	0.031
C20:4n-3[2]	0.06^{a}	0.04^{b}	0.04^{b}	0.08^{a}	0.03^{b}	0.03^{b}	0.021	0.228	<0.001	0.318
C20:5n-3[6]	0.15^{a}	0.06^{c}	0.05^{c}	0.12^{b}	0.05^{c}	0.05^{c}	0.006	<0.001	<0.001	0.004
C22:4n-6	0.09^{a}	0.05^{cd}	0.06^{bc}	0.04^{d}	0.07^{ab}	0.08^{a}	0.006	0.606	0.037	<0.001
C22:5n-3[2]	0.23^{a}	0.07^{b}	0.07^{b}	0.21^{a}	0.07^{b}	0.08^{b}	0.015	0.810	<0.001	0.063
C22:6n-3	0.02^{a}	0.01^{b}	0.01^{b}	0.02^{a}	0.02^{a}	0.02^{a}	0.003	<0.001	0.176	<0.001
n-3 FA[2]	2.41^{a}	0.72^{c}	0.68^{cd}	1.78^{b}	0.47^{d}	0.49^{cd}	0.090	<0.001	<0.001	0.115
n-6 FA	3.58^{a}	1.26^{c}	1.24^{c}	2.98^{b}	1.38^{c}	1.33^{c}	0.108	0.029	<0.001	0.001
n-6/n-3	1.49^{d}	1.76^{c}	1.84^{c}	1.71^{c}	2.95^{a}	2.73^{b}	0.069	<0.001	<0.001	<0.001
Total	8.67^{a}	3.78^{c}	3.84^{c}	6.81^{b}	2.75^{d}	2.81^{d}	0.260	<0.001	<0.001	0.03

[1]Means within row carrying no common superscript differ at $P < 0.05$. Measured on Day 39 (RS) and Day 35 (TP) of the respective observation period. Only 5 camels and 5 Pokots in rainy season, respectively.
[2]Arcsine-transformed, but LSM and SEM are non-transformed data.
[3]P=0.002 for S×A×SU.
[4]P=0.044 for S×SU.
[5]P=0.030 for S×A×SU.
[6]P=0.030 for S×SU.

3.5 Discussion

3.5.1 Phenols in the Milk and Their Relationship to Dietary Phenol Intake

The camels and the cattle selected diets differing in botanical functional groups (Leparmarai et al. 2018), and concomitantly, nutrients and other constituents. Accordingly, the camels' browse was rich in CP, lignin and TEP while in RS the cattle diet was high in fiber. The camels of the present study were estimated to have ingested (in g TEP/kg $BW^{0.75}$ per d) 6.5 and 5.6 in RS and TP, whereas the values estimated for the cattle types were 1.5 and 3.5, in TP and RS, respectively.

Only occasionally contents of plant secondary compounds in the milk of ruminants were reported in literature (see review by O'Connell & Fox, 2001). The more recent investigations included the determination of contents of phenols or distinct phenolic compounds (Besle et al. 2010, Jordan et al. 2010, Di Trana et al. 2015, Santos et al. 2016), terpenes (Viallon et al. 2000, Fedele et al. 2007, Poulopoulou et al. 2012) and phytoestrogens (Höjer et al. 2012) in milk of different ruminant species. For example, Di Trana et al. (2015) found higher contents of polyphenols in goat milk (57 vs. 49 mg/l) when feeding *Sulla coronarium* (5 g polyphenols/kg DM) instead of grass hay (1 g/kg DM). This is in the range of the 43 to 58 mg total phenol/l found in the milk of the 6 subgroups of cattle and camels in RS in the present study. Even fewer studies compared phenol contents of the milk of different ruminant species (Velázquez Vázquez et al., 2015), and to our knowledge none compared transfer rates from feed to milk in different livestock species. Unexpectedly, despite the large intake differences, milk total phenols contents did not differ between the 2 livestock species in the present study and there was no clear relationship between TEP intake (varying from almost 400 to 1100 g/d) and excretion with milk (varying from almost zero to 0.4 g/d) in the camels. The estimated daily TEP intake in the camels was numerically higher in RS compared to TP (6.5 vs. 5.6 g/kg $BW^{0.75}$) but this does not sufficiently explain the large differences between seasons in milk total phenol content, either. It has to be emphasized that the intake data were based on estimations including only species contributing to 83-91 % of total air DM intake in the camels. In both cattle types, there was the expected clear relationship between estimated phenol intake and its excretion in milk.

Different from cattle, camels might have developed coping strategies to limit TEP activity in the forestomach and TEP transfer into the milk to non-harmful levels. Besides a number of behavioral adaptations to forages containing PSC (reviewed by Estell, 2010), there are also physiological adaptations of mammalian herbivores to PSC consumption. Tannin-binding salivary proteins (reviewed

by Dearing et al., 2005), were found in browsers, but are assumingly absent in grazers (Austin et al., 1989) possibly because these species are normally not exposed to high TEP contents in feed. There seems to be a link between the types of salivary tannin-binding proteins and the specific tannins found in the corresponding diets (Bennick, 2002, Alonso-Díaz et al., 2012). Studies on the presence of tannin-binding salivary proteins in camels are limited to the study of Schmidt-Witty et al. (1994) which indicate that mucin glycoproteins in camel saliva might bind to tannic acid. As further coping strategy of ruminants, adaptation of certain rumen microbes to PSC and their capability of tannin degradation were described (Nelson et al. 1995, Kumar et al. 2014).

3.5.2 Microbial Counts in Milk

Our hypothesis of microbial growth suppression including pathogens such as *S. aureus* in camel milk compared to cattle milk was rejected because, other than expected, the camel milk was not richer in phenols than cattle milk and there was also no obvious difference in the prevalence of *S. aureus*. A lower milk pH (in the order of the 0.6) and a higher abundance of total germs at the end of the RS indicate a certain impairment of milk quality, what might be related to the end of RS conditions with no more rainfall as compared to the beginning of RS with frequent rainfall. Wullschleger et al. (2013) described lower microbial counts in cattle milk in the rainy season compared to a season characterized by higher temperature and low level of rainfall.

3.5.3 Effect of Season on Milk Fatty Acid Profile

As the experimental animals were kept under free ranging conditions, the season effect was linked to the quality of the forage on offer which varies in response to soil moisture (Kassilly 2002). However, the forage quality declined from the RS to the drier TP. It has to be stated that the season effect cannot fully be separated from a possible site effect in the present study. Although the study sites were near to each other and had a similar plant species composition, there were differences in the abundance of certain plants or plant functional groups like shrub cover (higher in the TP site). Dietary forage and forage maturity are indeed important factors that affect the FA composition of the milk fat (Bauman et al., 2000). Certain nutrients and PSC present in the plants ingested by the animals influence ruminal lipid metabolism and, consequently, milk FA profile (Iussig et al., 2015). For instance, temperate grasses (*Lolium perenne, L. multiflorum* and *Phleum pratense*) in their initial growth phase led to a

higher CLA concentration in the milk compared to the temperate grasses utilized later or in their second cut (Dewhurst et al., 2001). Haddadin et al. (2008) reported that C18:1 *n*-9 proportion in milk fat of dromedary camels increased when the growth of fresh grass and herbs was stimulated by winter rains. Konuspayeva et al. (2008) and Faye et al. (2008) described that feeding autumn forage of low nutritive value increased the SFA proportion in the milk fat of camels compared to feeding forage in spring or summer. Proportions of PUFA and CLA were found to be highest in milk fat of dairy cows in spring and autumn with a decline during summer that reflected the reduced temperate climate pasture quality (Thomson and Van der Poel, 2000). In the present study, there was sufficient rainfall in RS to promote plant growth and forage quality. This triggered season effects in the proportions of the majority of all FA. However, season-only effects were not very frequent, as mostly also interactions of season × animal type occurred. One clear season effect was that in RS the proportions of total n-3 FA and C18:3 *n*-3 were higher compared to TP. This could be explained by either higher n-3 FA concentrations in the RS forages or, more likely, the higher presence of PSC in RS, reflected by the overall higher TEP contents in milk, partially inhibiting ruminal biohydrogenation of these PUFA thus preserving a part of it for incorporation into milk fat.

3.5.3 Effect of Animal Type on Milk FA Profile

To the knowledge of the authors, there are no studies directly comparing the milk FA profile of cattle and camel fed on the same diet. Thus, it is not possible to quantify the extent to which the animal type effects found were related to either lipid digestion and metabolism or different plant selection habits. Comparative literature gives an indication for higher proportions of MUFA and PUFA (Faye et al., 2008) and lower proportions of SFA (Konuspayeva et al., 2008) in camel milk compared to cattle milk. This was also the case in the present study, and this also with the most prominent MUFA and PUFA representatives C18:1 *n*-9, C18:2 *n*-6 and C18:3 *n*-3. Gorban and Izzeldin (2001) reported that camel milk does not contain C4:0 and C6:0, while Sawaya et al. (1984), Abu-Lehia (1989) and Konuspayeva et al. (2008) found these FA in very small proportions similar to the present study. Abu-Lehia (1989) also found higher C16:1, C18:2 and C18:3 in the milk of dromedary camels than milk from Holstein cows kept in another farm. Sawaya et al. (1984) found no differences in C16:0 and C18:1 proportions in milk from cattle and Najdi camels kept at different farms. In the present study, the proportions of almost every individual FA differed in the milk fat of camels and cattle, and there were almost no differences between the 2 cattle types. The latter coincides with the quite similar diet selection pattern

(Leparmarai et al. 2018) and estimated nutrient and phenol intake of the 2 cattle types. Other than expected, the possibly better adaptation of indigenous cattle to the conditions of the semi-arid rangelands was not reflected by differences in diet and estimated nutrient intakes. It has to be shown if this is true also in the dry season with extended droughts and even lower forage quality.

3.5.4 Interactions of Season and Animal Type on Fatty Acids in Milk, and in Relation to Phenols

The frequent occurrence of season × animal type interactions was likely based on differences in plant selection between the 2 animal species, but also points towards species-specific differences in lipid metabolism. The latter was most striking in those milk FA which are basically related to ruminal biohydrogenation and which were actually affected by both, season and estimated TEP intake. In cattle the most prominent response to TEP intake and its assumed effects on biohydrogenation was in the elevated excretion with milk of vaccenic acid (C18:1 *t*11), a major ruminal biohydrogenation intermediate, and the corresponding CLA (C18:2 c*9*t*11*) excretion, mostly synthesized from C18:1 *t*11 in the mammary gland, with increasing phenol intake (reflected by milk total phenol contents) in the RS. Consistent with this assumed relationship, vaccenic acid levels in cattle milk fat dropped sharply in TP, when also the estimated intake of phenols decreased in the cattle types. Still the entire biohydrogenation process was affected as amounts of C18:3 *n*-3 excreted with milk had increased to some extent with the higher estimated TEP intake, at least in the cattle types, in RS. However, the decrease of the concentration of the terminal product of ruminal biohydrogenation, C18:0, from RS to TP in the cattle milk indicates that the RS forage might have been richer in C18 FA (not analyzed). In the camels there was no relationship between phenol intake and excretion of FA involved in biohydrogenation. This again points to a possible inactivation of phenols in the camels, but this warrants further detailed investigations.

3.5.5 Effect of Supplementation of Phenols and Fatty Acids in Milk

The effect, if any, from the extra RDP (here: urea) on fiber digestion, rumen emptying and, with that, feed intake and possibly plant selection was obviously so small that it did not substantially affect phenol content and FA profile of the milk. There was a small effect of supplementation on overall estimated TT and HT intakes, and the supplemented camels had a higher estimated intake of CT than

the non-supplemented camels. These results are in line with the effects of supplementation on intake of woody plants by camels and time spent browsing of camels described in Leparmarai et al. (2018).

3.6 Conclusions

In the present study, differences observed in phenol excretion with milk and FA profile of the milk between camels and cattle could be partially related to seasonal variations in quality and quantity of forages and, consequently, nutrient and phenol intake. Differences in milk FA profile of camel and cattle suggest that there may be differences in phenol and lipid metabolism between camels and cattle possibly resulting from control mechanisms against detrimental phenol effects developed in camels which have to cope with forages rich in TEP. The complex interactions of animal species and seasonal variations in forages of rangelands have therefore to be taken into account in predicting contents of phenols and fatty acids in milk. Controlled experiments have to confirm and further quantify the species differences found in the present study.

Chapter 4

Transfer of total phenols from a grapeseed-supplemented diet to dairy sheep and goat milk, and side-effects on performance and milk quality.

Based on: P.T. Leparmarai, S. Sinz, C. Kunz, A. Liesegang, S. Ortmann, M. Kreuzer and S. Marquardt. Manuscript in final preparation for submission to *Journal of Animal Science*.

4.1 Abstract

Polyphenols are known to affect digestion of ruminants, whereas little is known on their metabolic effects. In a 2 × 2-factorial experiment the effect of supplementing a highly phenolic grapeseed extract on performance, antioxidant capacity of the blood, saliva properties and changes in total phenol concentrations in blood, milk, urine and feces was compared in 11 East Friesian dairy sheep and 9 Saanen goats. The concentrate supplemented with 6.6% grapeseed extract had contents of 3.5% DM additional phenols compared to the low phenolic control concentrate. The experiment lasted for 74 d from parturition to late lactation, with an initial adaptation phase of 7-d. Milk yield was measured daily after weaning after about 26-d in milk, and samples were repeatedly collected for analysis of gross composition, phenol concentration, and antioxidant capacity. Blood and saliva samples were collected for analysis of phenol concentration and antioxidant capacity (four times, blood) and protein content and tannin-binding capacity (twice, saliva). Urine and feces were collected two times after weaning for analysis of phenol concentration. The phenolic diet increased phenol intake and phenol concentrations in blood, milk and urine at some, but not all sampling dates. A slight relationship ($P < 0.05$) was found for phenol intake and phenol excretion with milk for sheep but not goats. The phenolic diet did not influence blood antioxidant capacity and tannin binding capacity of the saliva. The effects of the extract on milk yield were inconsistent between sheep and goats. Goats were heavier, yielded more milk and had higher feed and nutrient intakes. Accordingly, phenol intake of the goats was higher than in the sheep ($P < 0.01$). Additionally, milk protein and lactose contents were higher and milk urea content was lower in sheep than goats. The saliva of the goats had a higher tannin binding capacity than sheep saliva. There were no clear species differences in phenol concentrations in blood plasma, milk, urine and feces, but the supplemented goats had higher urinary phenol concentrations compared to the non-supplemented goats at the end of the experiment. Supplementing grapeseed extract led to partially higher phenol concentrations in milk and blood but does not seem to have negative effects on performance. Intermediate feeders (goats) seem to have developed coping mechanisms like a higher salivary tannin binding capacity, which are less pronounced in grazers (sheep).

4.2 Introduction

Agro-industrial by-products play an important role as part of the feed of small ruminants (Corredu et al., 2015). Agro-industrial by-products often contain plant secondary compounds (PSC) such as tannins (which are either hydrolysable (HT) or condensed (CT), which may have adverse effects on animal performance when present in high amounts in the diet (Makkar 2003; Min et al., 2003). In addition, high dietary tannins levels were found to reduce digestibility (Silanikove et al., 2001) and palatability; the latter due to their astringency (Provenza, 2000). As tannins may also have positive effects in the ruminant, they are among an important plant derived bioactive molecules and their contributions to the diets of ruminants is of great interest. Accordingly, the consumption of low to moderate amounts of tannins can be beneficial because they may protect dietary proteins protection against microbial degradation in the rumen by forming protein-tannin complexes (Barry et al., 1986). Tannins are present in several plant groups as well as in the agro-industrial by products used for livestock feeding and have been shown to affect several aspects of ruminants nutrition and product quality. In addition, PSC including tannins may improve ruminant-source food quality, as the PSC also partially inhibit ruminal biohydrogenation of valuable dietary polyunsaturated fatty acids (Vasta et al., 2008) and may possess antioxidant properties (Hagerman et al., 1999). Acccordingly, feeding concentrate diet containing the tannin-rich quebracho extract to lambs improved antioxidant capacity in muscle (Luciano et al 2011), liver tissue and blood plasma (Lopez-Andres et al. (2013). Grapeseed extract, a by-product from the wine industry, which is rich in polyphenols (Nudda et al., 2015, Monagas et al., 2003), is also known for its antioxidant properties and there is therefore a growing interest for the use of grapeseed extract in animal nutrition (Llobera and Cañellas, 2007). Its polyphenols are especially tannins, and the extract also contains lignified cell wall contents (Dumont et al., 1978). However, little is known about such effect of grapeseed extract in ruminants.

The degree to which PSC can be transferred from the feed via blood plasma to the milk, and are not lost with feces and urine, is an important aspect for the nutritional quality of milk and meat for humans. Several studies have shown that majority of the phenolic compounds found in milk originate from the feed (Besle et al., 2010; Petit et al., 2009). Terpenes were found in cattle milk after feeding forages rich in terpene (Viallon et al., 2000). There was also flavonoid transfer to the milk of cattle fed different grass clover silages (Steinshamn et al. 2008, Höjer et al. 2012). Polyphenols from *Sulla coronarium* were transferred to the milk of goats and could also be detected in their blood plasma (Di

Trana et al., 2015). To our knowledge there are no studies investigating the loss of polyphenols with urine and feces.

Goats, classified as intermediate feeders, prefer diets containing more browse than cattle and sheep classified as grazers (Hoffman 1989). Browse is typically richer in PSC than grass, and intermediate feeders and browsers have therefore, developed coping mechanism (Rogosic et al., 2006; Utsumi et al., 2009). Strategies to cope with PSC include behavioral ways of adaptation like avoidance, selecting specific plant parts, lower intake of plants with PSC and physiological ways of adaptation like production of salivary proteins that bind to tannins and detoxification processes (Estell, 2010). These tannin-binding proteins were for instance found in the saliva of deer, a browser, but not in sheep and cattle saliva (Austin et al., 1989). This coping strategy seems to be diet dependent, as quebracho tannin increased saliva total protein concentration in sheep and goats (Lamy et al., 2011), but this was not the case when goats and sheep received a tannin-free diet in a different experiment (Lamy et al., 2009).

In the present study the following hypotheses were tested: 1) The polyphenols present in grapeseed extract can be partially transferred to blood and milk, but will also be partially lost via feces and urine in dairy sheep and goats. 2) In blood of the animals receiving grapeseed, the antioxidant capacity will be increased compared to the respective control animals. 3) When fed the same amount of phenols, goats will excrete less total phenols with the milk and have lower blood plasma total phenol levels than sheep due to established or upregulated detoxification strategies, and thus will excrete higher amounts with either feces or urine or both. In this respect, the tannin binding affinity and total protein concentration will be higher in goat saliva compared to sheep saliva. 4) Exposure to elevated dietary TEP levels will enhance the tanning binding affinity of the saliva. These hypotheses were tested in goats and dairy sheep fed with or without grapeseed extract.

4.3 Material and methods

4.3.1 Experimental animals and housing management

The experiment was performed from February to July 2017 in the facilities of University of Zurich, Switzerland. It was approved by the Cantonal Veterinary Office of Zurich (license number ZH 267/16). Nine Saanen goats and 11 East Friesian sheep were included from parturition to late lactation (74 ± 4 and 74 ± 2 days in milk for sheep and goats respectively). The animals were grouped into two subgroups per animal species in a complete randomized design considering milk yield from previous lactation, body weight (BW), parity and age. One of the two subgroups each received a phenolic diet (five sheep

and four goats), the other two subgroups were offered a non-supplemented control diet (six sheep and five goats). The animals were housed in a barn with separate sections for each animal species. The animals were kept in individual pens of a floor size 1 × 2 m. One exception had to be made for two groups of two sheep each kept together and were tied in opposite corners of the pen during feeding. The floor was covered with wood shavings. All animals had free access to water from automatic drinkers, a plastic tray for the hay and a bucket for the concentrate. The animals were fed individually twice daily, at 0800 h in the morning and at 1700 h in the evening. At the start of the experiment, the goats weighed on average 77 ± 15 kg (mean ± standard deviation), were 3 ± 1 years of age and a milk yield of 1.9 ± 0.5 kg/day in the previous lactation. The corresponding data for the sheep were 76 ± 17 kg, 3 ± 2 years of age and 1.2 ± 0.3 kg milk/day. On average, the goats had 2.0 ± 1.0 and 1.5 ± 1.0 kids in control and phenolic group, respectively, and the sheep had 2.0 ± 0.8 and 2.0 ± 1.0 lambs in control and phenolic group. The goat kids and the lambs were weaned approximately 7 weeks after birth. Until weaning they were kept together with their mothers. One sheep on the phenolic diet was excluded from the experiment due to poor body score and declining BW.

4.3.2 Experimental diets

During the experiment the animals were fed restrictively with forage and concentrate in a ratio of 40:60. The control concentrate used (1099-60, 1099-70. UFA AG 3360 Herzogenbuchsee) consisted of (in %) yellow maize (21.97), barley (17.2), wheat (20.1), sugar beet pulp (15), mill by-products (15), molasses (3), soybean meal (2.5), wheat starch (2), soybean oil (0.5), $CaCO_3$ (1.3), NaCl (0.34) and a vitamin-mineral premix (1.1). According to the producer's statement, the concentrate contained per kg DM 80 g metabolizable protein and 6.7 MJ net energy for lactation. The vitamin-mineral premix added to the concentrate provided, per kg of concentrate, 10'500 IU vitamin A, 1,300 IU vitamin D_3, 50 mg vitamin E, 500 mg nicotinic acid, 100 mg Fe, 50 mg Zn, 50 mg Mn, 1.85 mg I, 0.5 mg Co, and 0.35 mg Se. In addition, all animals had free access to mineral licking block (Agrosal Heilbronn, Germany) containing the following nutrients per kilogram: 375 g Na, 8 g Mg, 800 mg Zn, 80 mg Mn, 100 mg I, 30 mg Se, and 30 mg Co. The phenolic concentrate was produced by mixing 6.6% grapeseed extract (70.7% total extractable phenols (TEP)) as analyzed; OmniVin 10R 6.6%, purchased from Ajinomoto OmniChem, N.V, Belgium) into the control concentrate in a pellet form. Hay from mixed swards (grass, legumes and herbs) was used as a forage. Two different hay origins were used in the two animal species. The sheep and goats were also offered 500 g sugar beet pulp/day from Days 55 and 16 of the experiment

onwards, respectively, to counteract weight loss observed. The beet pulp was soaked in water overnight at a ratio of 1 part dry beet pulp and 4 parts water before feeding the next day. The composition of the diet ingredients as analyzed is described in Table 1. The animals had always free access to fresh water *ad libitum* daily. The animals were fed according to estimated requirements for maintenance (adjusted weekly from BW measurements) and for milk yield (considering the number of offspring). The basis were the recommendations of Agroscope (2015) for lactating sheep using the following equation:

Total feed supply = 1.5 kg – 0.1 ((80 kg – BW (kg))/10) + (number of lambs –1) × 0.9.

Before the experiment started, the animals were fed 2 kg/day of mixed hay and 0.5 to 0.8 kg/day of a commercial sheep and goat concentrate (Combifloc 2921, Meliofeed AG 3360 Herzogenbuchsee, Switzerland).

4.3.3 Experiment schedule

The experiment, which had a total duration (from giving birth to the last day of the experimental period) of 75 ± 3.6 d and 74± 3 d was divided into two periods: adaptation period (length: 8 ± 2 d for the sheep and 5 ± 3 d for the goats) and experimental period. The adaptation period started 5.4 ± 1.7 and 9.2 ± 2.9 d after giving birth. During the adaptation period the animals were familiarized with the concentrate, which was gradually increased until reaching a ratio of 40:60 concentrate:hay in the diet at the first day of experimental period. The experimental period thus started on average 13 ± 3d and 14± 3 d after giving birth for the sheep and goats. The concentrate:hay ratio needed to be adjusted to 45:55 on d 21± 6 in sheep and d 20 ± 4 because of the reduced BW of the animals and was kept at this ratio until end of the experiment. Weaning took place on d 48 ± 2 and 48 ± 4 for sheep and goats. Milking started after weaning until the last day of experimental period (on average 25±4 d in sheep and 26 ±45 d in goats, respectively.)

4.3.4 Measurement protocol

Amounts of feed supplied and leftovers were recorded daily per animal during adaptation and experimental period. Weekly, the animals were weighed after morning feeding. Feed items were sampled five times in approximately monthly intervals and ground to pass through a 1 mm sieve. Starting with the post-weaning period, the animals were milked in the morning (from 0730 h) and in the afternoon (from 1630 h) in a milking parlor and yields were recorded. Milk samples for phenol analysis were first collected in the pre-weaning period (sheep: Day 31; goats: Day 35) and then in the

post-weaning period on Day 55 and Day 56 as well as on Day 76 and Day 75 for sheep and goats, respectively. The milk samples were stored on ice until being frozen at -20°C. Milk samples for gross composition analysis were collected once during the experiment (Days 58 ± 4 and 46 ± 7 in sheep and goats, respectively). These samples were collected in a plastic vessel which containing Bronopol® and were analyzed by a Fourier transform infrared spectrophotometer (Milkoscan FT 6000, Foss, Hillerod, Denmark) at the Suisselab (Zollikofen, Switzerland). Four blood samples were collected from the jugular vein, two each in EDTA and lithium heparin tubes 4 times, on Day 5 ± 2 (baseline, 1 day before starting adaptation), on Day 32 ± 3 (pre-weaning), and on Day 54 ± 3 and Day 74 ± 3 (post-weaning). Plasma was obtained by centrifuging at 2000 × g for 20 min. The plasma was then stored at -80°C. Urine and feces samples were collected within 2 h after morning feeding from all animals two times on Day 30 ± 3 (sheep) and Day 55 ± 3 (goats) as well as Day 77 ± 4 (sheep) and Day 74 ± 3 (goats) (post-weaning). Urine was collected by spot sampling, feces were obtained by grab sampling, stored at ice and frozen at -20°C. Feces were later dried at 60°C for 48 h. Saliva samples were taken on Day 3 ± 1 and Day 7 ± 3 (baseline) and on Day 75 ± 4 and Day 74 ± 3 (end of experiment) in sheep and goats, respectively. The saliva was collected by inserting a synthetic sponge (Sarstedt Salivetten) into the mouth of the animals which then were allowed to chew for a few minutes in order to soak the sponge with saliva. The sponge was then centrifuged for 10 min at 3500 × g at 4°C and transferred to Eppendorf tubes and stored at -80°C.

4.3.5 Laboratory analyses

Feed items were analyzed for dry matter (DM) and total ash using an automatic thermo-gravimetric device (TGA-701, Leco Corporation, St. Joseph, MI, USA). Crude protein (CP) was calculated as 6.25×N, with N content determined with a C/N analyzer (type TruMac CN, Leco Corporation, St. Joseph, MI, USA). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as described by Van Soest et al. (1991) using Fibertec System M (Tecator, 1020 Hot Extraction, Foss Hillerød, Denmark). Values were corrected for ash content, and α-amylase was added in NDF analysis. Ether extract was analyzed by Büchi extraction system B-811 (Flawil, Switzerland).

Total tannins (TT) and TEP were determined by the Folin-Ciocalteu method (Makkar, 2003). The same method was used to analyze TEP in feces. The butanol-HCl-iron method was used to analyze CT. In blood plasma and urine, TEP were analyzed by the method developed by Serafini et al. (1998) and

the Folin Ciocalteu method (ISO, 2005). Briefly, the thawed samples were vortexed and subjected extraction and hydrolysis. They were centrifuged at 2500 rpm for 1.5 min. 100 µl sample was transferred into a tube and for hydrolyzing the conjugated forms of polyphenols, 200 µl of 1.0 mol/L HCl was added, vigorously vortexed for 1 min and incubated at 37ºC for 30 min. After, 200 µl of 2.0 mol/L NaOH in 75% methanol was added, vortexed for 1 min and incubated at 37ºC for 30 min. This step breaks the links of polyphenols with lipids and provides a first extraction of polyphenols. For precipitating protein 300 µl of 0.75 mol/L MPA (meta-phosphoric acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was added. vortexed for 1 min and the samples were centrifuged at 16,000 *g* for 15 min. 700 µl of the supernatant was transferred into a tube and precipitating protein again by adding 100 µl of 0.75 mol/L MPA, vortexed and centrifuged for 15 min at 16,000 *g*. 100 µl of the supernatant was transferred into a tube; 500 µl of Folin-Ciocalteu reagent (1 N; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in the ratio of 10 parts water and 1 part Folin-Ciocalteu reagent was added and left to react for 3 min; 400 µl of sodium carbonate solution (7.5 %, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was added. A blank with all the reagents and water instead of blood or urine sample was also prepared. All samples were thoroughly vortexed before leaving them in the dark for 1 h. A spectrophotometer (VWR UV-6300, VWR international, Radnor, Pennsylvania) was used to determine absorbance at 765 nm. In milk, TEP were analyzed according to Vazquez et al. (2015) with some modifications. A standard gallic acid was dissolved in water at 25, 50, 75, 100 and 125 µg/ml concentrations. These solutions were treated the same way as the milk samples. At first samples were vortexed, then 400 µl were transferred into a 2 ml Eppendorf tube, 500 µl of a 1:1 mixture of methanol and water were added, and vortexed. Then 30 µl of carrez I (potassium hexacyanoferrate II solution) was added, followed by 30 µl of carrez II (zinc sulphate solution) with vortexing after each step. This was followed by adding 250 µl of acetonitrile and mixing for 1 min. The mixture was left in the dark for 25 min when clot protein precipitation was complete. The resulting suspension was centrifuged (Eppendorf 5418, Eppendorf AG, Hamburg, Germany) at maximum speed for 6 min. The supernatant was obtained and transferred to new Eppendorf tubes and the analysis of total phenol contents was carried out using the Folin-Ciocalteu method. An amount of 150 µl of the supernatant was transferred into tubes and completed to 500 µl with 5:1 of Folin solution and Folin-Ciocalteu reagent (1 N; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). After being left to react for 3 min, 1 ml of sodium carbonate solution (7 g/100 ml distilled water) was added. A blank with all the reagents except milk sample but water supernatant was also prepared. All the samples were vortexed before leaving them in

the dark for 40 min and then subjected to measurement with a spectrophotometer (VWR UV-6300, VWR international, Radnor, Pennsylvania) at 725 nm. Based on the standard, milk phenol concentrations were expressed as gallic acid equivalents. All TEP and TT contents were expressed as gallic acid equivalents whereas CT were presented as leucocyanidin equivalents.

The antioxidant capacity of blood plasma was measured using the ferric reducing ability of plasma (FRAP) assay according to Benzie and Strain (1996). The FRAP reagent was prepared by mixing 50 mL acetate buffer, pH 3.6 (1.349 g Na acetate trihydrate, 8 ml glacial acetic acid mixed with 500 ml millipore H_2O), 5 mL of 2,4,6-tripyridyl-s-triazine (TPTZ, Sigma Aldrich, USA) solution in 40 mM HCl, and 5 mL ferric chloride hexahydrate ($FeCl_3 \times 6\ H_2O$) solution in a ratio of 10:1:1. The trivalent complex Fe compound ferric-tripyridyl triazine (Fe^{3+}-TPTZ) was reduced to the blue colored ferrous (Fe^{2+}) by mixing 0.01 mL of sample with 0.3 mL FRAP reagent. Absorbance was measured with a spectrophotometer at 620 nm after 15 min of incubation at 37ºC while shaking (700 rpm). An 1 mM stock solution of L-ascorbic acid was used for calibration.

Before analysis of salivary protein contents, the samples were centrifuged for 10 min at 35000 rpm. Salivary protein concentrations were determined according to Bradford (1976). The tannin binding capacity of the saliva was measured by the tannin binding assay of Fickel et al. (1999). Two tannin sources were used, quebracho tannins as a representative of the CT and tannic acid for the HT.

4.3.6 Calculations and statistical analysis

All intake data were related to BW. The HT were calculated as TT – CT and the non-tannin phenols (NTP) as TEP – TT. Energy corrected milk (ECM) was calculated as outlined by Agroscope (2015) as

ECM (kg) = milk (kg) × [0.38 × fat (%) + 0.24 × protein (%) + 0.17 × lactose (%)]/3.14.

Data were statistically analyzed using the SAS software 9 (SAS Inst. Inc., Cary, NC) using the mixed procedure of ANOVA. The experimental units were the single animals. The model considered animal species (goat or sheep), diet (control or phenolic) and the interaction of animal species × diet as fixed effects. Animal was set as random factor. For the statistical analysis of the saliva data, two outliers (defined as ± 2 standard deviations) each were excluded for saliva protein content, tannin binding capacity using quebracho tannins and tannin binding capacity using tannic acid. The tables give the arithmetic means across all data in brackets for these variables. The relationship between phenol intake and phenol excretion with milk was analyzed with PROC REG of SAS and Sigmaplot 13 (Systat software, Inc.) was used to make the graph. Normal distribution of the residuals was tested graphically.

Comparison of means was performed by Tukey's test. The significance level was set as $P < 0.05$ and $0.05 < P < 0.10$ as a trend. If not stated differently, the tables give Least Square means and standard errors of the mean.

4.4 Results

The two hay types fed to sheep and goats were quite similar in their proximate composition (Table 4.1). The grapeseed extract was calculated to contribute 4.7% additional TEP to the phenolic diet. The recovered difference between the two concentrates was 3.5% TEP in DM. The control concentrate contained slightly less CP but clearly more NDF than the phenolic concentrate.

Table 4.1 Chemical composition (% of DM) of the experimental feeds and complete diets (means ± standard deviations.

	Single feed components					Diets (55% hay, 45% concentrate)[1]			
	Hay		Concentrate		Sugar	Control		Phenolic	
	Sheep	Goats	Control	Phenolic	beet pulp	Sheep	Goats	Sheep	Goats
n	2	3	4	4	1				
Nutrients									
DM	90.8 ± 0.9	91.3 ± 0.4	88.4 ± 0.4	88.9 ± 0.0	90.6	90.6	90.9	90.8	91.1
OM	92.2 ± 0.9	91.6 ± 0.8	93.5 ± 0.2	94.4 ± 0.1	92.4	93.7	93.4	94.1	93.8
CP	10.5 ± 0.9	11.9 ± 0.3	13.8 ± 0.1	12.9 ± 0.0	8.6	12.1	12.8	11.6	12.4
NDF	51.1 ± 0.3	54.5 ± 5.7	25.6 ± 1.6	15.9 ± 0.9	44.4	39.6	41.4	35.2	37.0
ADF	34.6 ± 0.0	34.9 ± 0.9	9.8 ± 0.2	5.2 ± 0.3	24.3	24.3	23.4	22.2	21.3
ADL	6.2 ± 0.0	6.0 ± 0.6	2.3 ± 0.4	2.5 ± 0.9	12.5	4.9	4.6	5.0	4.7
Ash	2.9 ± 0.1	4.6 ± 0.5	2.9 ± 0.1	2.5 ± 0.0	7.6	7.3	7.6	6.9	7.2
Phenols									
TEP[a]	1.38 ± 0.08	1.62 ± 0.23	0.54 ± 0.02	4.04 ± 0.02	0.46	0.98	1.11	2.59	2.72
NTP	1.30 ± 0.02	1.41 ± 0.16	0.45 ± 0.02	0.63 ± 0.03	0.38	0.90	0.95	0.98	1.03
TT[1]	0.08 ± 0.06	0.22 ± 0.07	0.09 ± 0.01	3.41 ± 0.04	0.08	0.08	0.15	1.61	1.68
CT[2]	0.02 ± 0.00	0.06 ± 0.05	>0.00 ± 0.01	1.56 ± 0.06	0.00	0.01	0.03	0.73	0.75
HT	0.06 ± 0.06	0.16 ± 0.02	0.08 ± 0.01	1.85 ± 0.09	0.08	0.07	0.12	0.88	0.94

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; CT: condensed tannins; DM: dry matter; NDF: neutral detergent fiber; OM: organic matter; TP: total extractable phenols; TT: total tannins.
[1]Not including beet pulp added to the diet (500 g per animal and day).
[1]Given as leucocyanidin equivalents.
[2]Given as tannic acid equivalents.

The goats fed the control diet had a higher BW compared to the sheep fed the same diet, but no such difference was found when goats and sheep received the phenolic diet (animal species × interaction, $P < 0.05$; Table 4.2). This pattern was, however, already found on d 19 and was maintained until d 68 of the experiment. There was a species effect on BW change ($P < 0.001$) and a trend in the animal species × diet interaction. The goats overall lost weight between d 19 and 68, while the sheep gained weight. Numerically, the weight gain in the sheep was larger in the control group, while in the goats the weight loss was numerically larger in the control group (animal species × diet, $P = 0.05$). Consistent with the higher feed allowance, the goats had overall higher ($P < 0.01$) intakes of total DM and hay per kg of BW and day compared to the sheep. Intakes of concentrate and beet pulp did not differ between groups. Along with the differences in overall DM intake, intakes of nutrients and phenols (TEP, TT, CT and HT) were higher ($P < 0.05$) in goats than sheep. The intakes of the phenol fractions were substantially higher ($P < 0.001$) with the phenolic diet than the control diet in both species (Table 4.2).

Table 4.2 Effects of animal species (S) and diet (D) on BW and intake of sheep and goats receiving a control diet or a diet enriched with a phenolic extract ('phenolic).

Animal species	Sheep		Goats			*P*-values		
Diet	Control	Phenolic	Control	Phenolic	SEM	Species	Diet	S×D
n	6	5	5	4				
BW (kg)								
d 19	58.8^{b}	74.3^{ab}	84.2^{a}	72.5^{ab}	5.77	0.037	0.722	0.019
d 68	62.8^{b}	75.1^{ab}	77.3^{a}	68.7^{ab}	5.07	0.388	0.683	0.036
Average	60.0^{b}	73.8^{ab}	80.4^{a}	70.2^{ab}	5.32	0.099	0.716	0.024
BW change (g/day)[2]	81^{a}	17^{ab}	-142^{c}	-77^{bc}	33.9	<0.001	0.992	0.052
Dry matter intake (g/kg BW and day)								
Total	46.3	43.9	49.7	54.7	3.18	0.024	0.657	0.214
Hay	21.5^{b}	20.7^{b}	28.8^{a}	31.5^{a}	1.53	<0.001	0.526	0.223
Concentrate	24.1	22.7	19.7	21.8	1.78	0.123	0.848	0.281
Beet pulp	1.72	1.55	1.35	1.62	0.114	0.174	0.647	0.046
Nutrient intake (g/kg BW and day)								
OM	44.7^{ab}	42.4^{b}	49.2^{ab}	54.6^{a}	3.12	0.009	0.579	0.186
CP	5.81^{ab}	5.27^{b}	6.57^{ab}	7.05^{a}	0.408	0.003	0.928	0.183
NDF	18.2^{b}	15.1^{b}	23.5^{a}	23.1^{a}	1.27	<0.001	0.147	0.238
ADF	10.4^{b}	8.8^{b}	13.1^{a}	13.5^{a}	0.72	<0.001	0.370	0.154
ADL	2.20^{b}	2.12^{b}	2.77^{a}	3.16^{a}	0.164	<0.001	0.304	0.142
Phenol intake (g/kg BW and day)								
TEP[3]	0.439^{b}	1.211^{a}	0.595^{b}	1.418^{a}	0.0653	0.004	<0.001	0.959
TT[4]	0.039^{c}	0.791^{a}	0.084^{b}	0.818^{a}	0.0427	0.032	<0.001	0.121
CT[4]	0.006^{c}	0.358^{a}	0.018^{b}	0.360^{a}	0.0194	0.052	<0.001	0.068
HT	0.032^{c}	0.431^{a}	0.062^{b}	0.454^{a}	0.0234	0.019	<0.001	0.142

ADF: acid detergent fiber; ADL: acid detergent lignin; BW: body weight; CP: crude protein; CT: condensed tannins; DM: dry matter; NDF: neutral detergent fiber; OM: organic matter; SEM: standard error of mean; TP: total extractable phenols; TT: total tannins.

[a-c]Least square means within rows are different at P < 0.05.

[2]BW change between d 19 and d 68, divided by 49 days.

[3]Data was log-transformed. Least square means and SEM displayed are from the non-transformed data.

[4]Data was arcsin-transformed. Least square means and SEM displayed are from the non-transformed data.

(Table 4.3). Yield of milk and ECM was overall almost 3.4 times higher ($P < 0.001$) in goats than sheep. There was an animal species × diet interaction ($P < 0.05$ for milk yield, $P = 0.05$ for ECM yield) which indicated an increase in yield with the phenolic diet in sheep and the opposite in goats (not significant in the multiple comparisons among means). Milk protein and lactose contents were higher ($P < 0.001$) in sheep than goats. The goats had higher ($P < 0.01$) milk urea contents than the sheep (22.7 vs 17.0), and feeding the phenolic diet overall reduced ($P < 0.01$) milk urea content. Along with the higher milk yields, goats had overall higher ($P < 0.00$) yields of fat, protein and lactose and excreted

more ($P < 0.001$) urea. In the excretion of protein, lactose and urea, there were animal species × diet interactions ($P < 0.05$) in a way that levels seemed to increase (protein, lactose) or remain constant (urea) when providing the phenolic diet to sheep, whereas this was opposite in the goats ($P < 0.05$ in urea, Table 4.3).

Table 4.3 Effects of animal species (S) and diet (D) on milk performance of sheep and goats receiving a control diet or a diet enriched with a phenolic extract ('phenolic').

Animal species	Sheep		Goats			*P*-values		
Diet	Control	Phenolic	Control	Phenolic	SEM	Species	Diet	S×D
n	6	4	5	4				
Milk yield (kg/d)								
Absolute[1, 3]	1.04[b]	1.39[b]	4.69[a]	3.56[a]	0.316	<0.001	0.675	0.027
ECM[2]	1.08[b]	1.45[b]	4.18[a]	3.30[a]	0.308	<0.001	0.382	0.044
Milk composition (%)[2]								
Fat	3.89	4.00	4.89	5.04	0.703	0.140	0.851	0.976
Protein	4.87[a]	4.67[a]	2.46[b]	2.56[b]	0.170	<0.001	0.751	0.356
Lactose	4.89[a]	4.81[a]	4.12[c]	4.23[b]	0.121	<0.001	0.878	0.397
Urea (mg/dL)	19.6[ab]	14.3[b]	26.1[a]	19.4[ab]	1.93	0.006	0.005	0.702
Constituent yield (g/d)[2]								
Fat[3]	39.6[b]	56.3[b]	213.1[a]	167.7[a]	21.05	<0.001	0.658	0.153
Protein	47.3[c]	62.5[bc]	110.9[a]	88.4[ab]	9.41	<0.001	0.684	0.048
Lactose	49.2[b]	65.5[b]	185.9[a]	147.4[a]	13.52	<0.001	0.390	0.046
Urea	0.20[c]	0.20[c]	1.18[a]	0.67[b]	0.095	<0.001	0.012	0.011

ECM: energy corrected milk; SEM: standard error of mean.
[a-c] Least square means within rows are different at $P < 0.05$.
[1]Average of 25±4 d and 26±5 d in sheep and goats, respectively.
[2]Milk samples collected on d 74±5 and d 67±7 in sheep and goats, respectively.
[3]The data was log-transformed. LSM and SEM displayed are from the non-transformed data, *P*-values and superscripts from the transformed data.

The concentration of total phenols in blood was higher ($P < 0.05$) in animals fed the phenolic diet than animals receiving the control diet, both on Day 32 (+9%) and Day 74 (+15%), but not prior to the experiment or on Day 54 (Table 4.4). Additionally, there was a trend ($P = 0.07$) for higher total phenol concentrations in the blood of the sheep compared to the goats on Day 62. Feeding the phenolic diet increased the TEP concentration in the milk by 24% on Day 33 ($P < 0.05$) and on Day 56 ($P = 0.05$). The effect was no longer present on Day 74. The total phenol excretion with the milk increased ($P < 0.05$) with phenol intake in sheep but not goats (Figure 5). There was a trend ($P = 0.07$) for a higher total phenol concentrations of the urine on Day 55 in the goats fed the phenolic diet than the control diet (no values for sheep). There was an animal species × diet interaction ($P < 0.001$) in total phenol concentration in urine on Day 74, where the two control groups did not differ between species, but

urinary total phenol was higher ($P < 0.05$) in the goats fed the phenolic diet than the control diet. The total phenol concentration in urine on D74 was higher ($P < 0.05$) in animal's fed phenolic diet than the animals fed control diets. Treatments had no effect on TEP concentration in the feces.

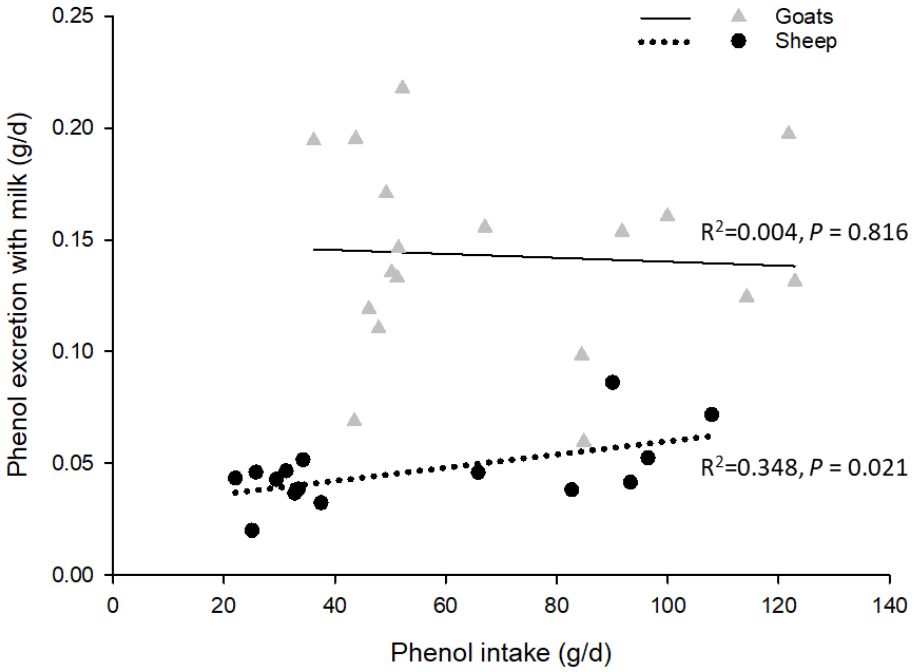

Fig. 4. Linear regression of the excretion of total phenols with the milk (y, g/d) on phenol intake on day 56 and day 74 (x, g/d) sheep and goats. The values are from the two days of both animals. Not all data was available for sheep.

There were no treatment effects on the antioxidant capacity of the blood plasma in any of the days and the animals in the four groups did not differ prior to the experiment, either (Table 4.4).

Table 4.4 Effects of animal species (S) and diet (D) on the antioxidant activity (ferric reducing ability of plasma; μmol Fe^{2+}/L) of blood plasma of sheep and goats receiving a control diet or a diet enriched with a phenolic extract ('phenolic').

	Sheep		Goats			P-values		
	Control	Phenolic	Control	Phenolic	SEM	Species	Diet	S×D
n	6	5	5	4				
Prexperimental	184	191	211	204	13.9	0.12	1.00	0.57
d 32	242[1]	240	233	220	19.2	0.42	0.67	0.77
d 54	237[3]	204[2]	195	220	18.7	0.47	0.83	0.12
d 74	227[1]	227	198	211	14.3	0.11	0.61	0.62

SEM: standard error of mean.
[1-3] One[1], two[2] or three[3] animals less than the indicated *n*-number.

Table 4.5 Effects of animal species (S) and diet (D) on the concentration of total extractable phenols in blood, milk, urine and feces and on the excretion with the milk of sheep and goats receiving control diet or a diet enriched with a phenolic extract ('phenolic').

Animal species	Sheep		Goats			*P*-values		
Diet	Control	Phenolic	Control	Phenolic	SEM	Species	Diet	S×D
n	6	5	5	4				
Blood (mg/L)								
Preexperimental	188	205	199	197	9.4	0.86	0.39	0.30
d 32	196	213	196	217	9.8	0.85	0.048	0.77
d 54	232[3]	225[2]	208	227	16.7	0.48	0.70	0.41
d 74	198[ab]	224[a]	166[b]	201[ab]	15.6	0.066	0.046	0.740
Milk (mg/L)								
d 33	32.2[2]	38.2	26.8[1]	39.5	3.25	0.530	0.012	0.317
d 56	26.3[3]	37.9[3]	28.8	34.8	4.96	0.934	0.052	0.494
d 74	.2	37.9	33.4	40.2	6.84	0.713	0.718	0.473
Urine (mg/L)								
d 30	883[1]	1858[1]	–	–	521.1	–	0.206	–
d 55	–	–	1940	3517	547.4	–	0.069	–
d 74	2483[1,ab]	1991[b]	1329[b]	3292[a]	319.2	0.805	0.025	<0.001
Feces (g/kg DM)	8.24	9.11	–	–	1.200	–	0.603	–
d 30	8.24	9.11	–	–	1.200	–	0.603	–
d 55	–	–	10.09	12.37[1]	2.155	–	0.435	–
d 74	11.54	12.17[2]	13.18[2]	11.57[1]	2.271	0.811	0.823	0.608

SEM: standard error of mean.
–: not sampled.
[a-b] Least square means within rows are different at $P < 0.05$.
[1-3] One[1], two[2] or three[3] animals less than indicated *n*-number.

Total protein concentration in saliva of goats and sheep later fed the two diets did not differ prior to the experiment and the dietary treatments had no effect when measured on Day 74 (Table 4.6). Goat saliva had overall a higher tannin binding capacity than sheep saliva as measured on Day 74 (quebracho tannins, $P < 0.05$; tannic acid, $P < 0.01$). This species difference was not obvious before the start of the treatment feeding. There was no effect of the phenolic diet on the tannin binding capacity of the saliva.

Table 4.6 Effects of animal species (S) and diet (D) on total protein content and tannin-binding capacity (% of total quebracho tannins or tannic acid) of saliva of sheep and goats receiving a control diet or a diet enriched with a phenolic extract ('phenolic').

Animal species	Sheep		Goats			*P*-values		
Diet	Control	Phenolic	Control	Phenolic	SEM	Species	Diet	S×D
n	6	5	5	4				
Total protein content (g/L)								
Preexperimental	0.98[1] (0.98)	1.77[1] (1.77)	1.20 (1.20)	1.00 (1.00)	0.285	0.32	0.31	0.089
d 74	1.85[1] (2.39)	1.70[1] (1.70)	1.78[1] (1.78)	1.18[1] (1.86)	0.396	0.41	0.30	0.53
Quebracho (%)								
Preexperimenteal	73.3[1] (73.3)	65.8 (65.8)	69.4 (69.4)	67.7 (67.7)	2.72	0.70	0.088	0.26
d 74	59.6 (59.6)	69.4 (69.4)	75.4[1] (66.0)	77.2[1] (61.0)	5.99	0.035	0.27	0.45
Tannic acid (%)								
Preexperimental	57.1[1] (57.1)	50.1 (50.1)	57.2 (57.2)	56.1 (56.1)	3.25	0.32	0.20	0.35
d 74	47.3[b] (47.3)	49.1[ab] (49.1)	61.1[ab,1] (53.6)	64.7[a,1] (53.3)	4.36	0.001	0.47	0.80

Values displayed in brackets are the arithmetic means calculated across all animals inclusive of outliers.
SEM: standard error of mean.
[a-b]Least square means within rows are different at $P < 0.05$.
[1]One animal less than the indicated *n*-number.

4.5 Discussion

Transfer of PSC from feed to animal-source foods has been the focus of many studies as a tool for improving animal product quality and potential benefits to human health (Vasta et al., 2011). The transfer of these compounds varies greatly depending on concentrations in the feed and their bioavailability and absorption in the gut (Scalbert et al., 2002). The present experiment was conducted to investigate the antioxidant capacity and changes caused by feeding a supplement rich in phenols on concentrations of phenols in milk, blood, urine and feces. As grazers and intermediate feeders may have developed different coping strategies with respect to PSC, the response to such a supplement of dairy sheep and dairy goats was compared. Including phenols via grapeseed extract at a level of 4% in

concentrate dry matter in the diets of sheep and goats of the phenolic groups corresponds to levels fed in other studies with lambs where PSC were supplemented to the diets, such as 4.7% DM terpenes (Dziba and Provenza, 2008), 6.5% DM polyphenols in total diet (Villalba et al., 2006) and 6.4% DM tannins on the diet (Lopez-Andrés et al., 2013).

4.5.1 Effects of supplementation with grapeseed extract

On average of both animal species, the grapeseed extract in the diet did not affect milk yield and composition. This is in agreement with findings of Nudda et al., (2015) where grapeseed alone or grapeseed in combination with linseed supplied to dairy sheep had no effect on milk yield and composition. As anticipated, grapeseed extract reduced the milk urea content likely by binding of part of the dietary protein by the polyphenols in the rumen preventing its microbial degradation. This phenomenon is well known for tannins, which were prevalent in the extract as well (Santos et al., 2014).

According to our results, grapeseed polyphenols are digestible to some extent as their consumption elevated TEP concentrations in blood plasma and milk, and the elevated total phenols concentration in urine indicates the same. Indeed, the mammary gland was described as one of the excretory pathways for polyphenols like flavonoids (De Feo et al., 2006). The effects were, however, not fully consistent throughout the experiment. Also Poulopoulou et al. (2012) observed such a variation between days in blood and milk phenol contents when they administered a mixture of terpenes, α-pinene, limonene and β-caryophyllene to sheep. McLean et al. (2008) and Vialloninsta et al. (2000) also reported a high variability in terpene concentration in milk on different days, regardless of their intake by the cattle being the same during the experiment period. These differences were related to microbial degradation in the rumen after a familiarization process with the compounds or because of varying modes and sites through which PSC are excreted after they are metabolized (Poulopoulou et al., 2012; Vialloninsta et al. 2000). Certain rumen microbes are able to degrade HT. In the present study, higher phenol concentrations were found in blood and milk of the animals fed the phenolic diet, although not at all the sampling dates. Other studies also reported increased levels of polyphenols in lamb meat (Monino et al., 2008), in blood plasma of sheep infused with rosemary extract (Gladine et al., 2007) as well as in milk of dairy goats fed rosemary leaves and blood plasma of their suckling offspring (Jordan et al., 2010) compared to control animals. In the present study, the milk phenol contents were in the range of 35 to 40 mg/l in the animals fed grapeseed extract. This level was higher than the 14 mg/l reported by Besle et al. (2010) for the milk of cows grazing mountain pastures containing 35 g phenols/kg DM. On

the other hand, goat milk was found to contain 57 mg milk free polyphenols/l at a high total polyphenol intake of 9 g/day from *Sulla coronarium* (Di Trana et al., 2015). De Feo et al., (2006) reported comparably low levels of quercetin and flavonoids in goat milk of 12.2 mg/l and 20 mg/l, respectively.

The pathway for metabolic detoxification and removal of dietary polyphenols absorbed through either rumen or intestinal mucosa is its renal excretion after transformation in the liver to sulfates and glucuronides (Scheline, 1991). Obviously, this pathway has been used on some days for partial removal of absorbed grapeseed polyphenols in the present study as well. The content of total tannins (TT) and condensed tannins (CT) in feces was 0.19% and 0.02%, respectively, for the animals of the present study fed phenol diets. These values are lower compared to 0.81% and 0.06% for TT and CT, respectively, measured in Holstein Friesian cattle fed tannin rich oak leaves (Makkar and Becker 1998). However, the concentration in the feces also depends on water content and digestibility of the other dietary constituents.

Several studies reported positive relationships between polyphenol intake and the occurrence of antioxidant compounds or elevated antioxidant activity in blood plasma or milk. De Feo et al. (2006) described occurrence of rutin and quercetin in goat milk. Tannins from quebracho were found to increase the antioxidant status in muscles (Luciano et al., 2011), plasma and liver (Lopez-Andres et al., 2013). Feeding rosemary distilled leaves at a rate of 10% or 20% in the ewes diet led to improved lamb meat antioxidant status (Monino et al., 2008). By contrast, the absence of an effect of the grapeseed extract on the antioxidant capacity of the blood plasma in the present study indicates that the phenols active in this respect were either not absorbed or excreted with the urine. Similarly, Goni et al. (2007) and Santos et al. (2016) did not find a distinct antioxidant capacity of the serum of grape pomace-fed birds and of the milk of cows fed propolis.

Several authors suggest that the physiological adaptation to PSC is an induced response to their presence in feed (Iason, 2005; Clauss et al., 2005; Costa et al., 2008). Alonso-Diaz et al. (2010) explained that the expression of a corresponding salivary protective system could be influenced by diet. We therefore, hypothesized that feeding the tannin-rich grapeseed extract would trigger a higher tannin binding affinity of the saliva via salivary proteins and thus also lead to an elevated salivary total protein because the specificity between salivary proteins and tannins is an adaptive response of mammalian species. This was, however, not the case any of the animal species investigated in the present study.

4.5.2 Effects of animal species and interactions with the supplementation with grapeseed extract

The greater feed (hay) intake by the goats compared to the sheep was expected as the goats had to cover requirements for a more than three times higher milk yield. The gross nutrient composition of the milk in the present study was within the ranges reported for dairy sheep and goats (Raynal-Ljutovac et al., 2008). The higher milk urea content in goat than sheep milk was likely the result of the high CP content of their diet.

The main focus of the species comparison made in the present study was the species-specific differences in phenols coping mechanisms in general and in the response to the extra phenols from the grapeseed extract. Animals regularly consuming tannin-rich feeds appear to develop defensive mechanisms against tannins and protein-rich saliva is probably the first line of defense developed by mammalian species against tannins (Skopec et al., 2004; Mueller-Harvey, 2006; Shimada, 2006). Defense mechanisms may include preventing their transfer into the metabolism and their intensive removal from metabolism via urine. Differences between sheep (grazers) and goats (intermediate feeders) (Hofmann, 1989) were, therefore, expected as the latter are likely more exposed to and, if allowed to, consume, diets richer in phenols than the sheep (Gilboa et al., 1995; Silanikove et al., 1996).. In case there would also be a different response of to the grapeseed extract (i.e., interactions) the two species would also differ directly in triggering of the coping mechanisms against the grapeseed phenols. Such an interaction was found in urinary concentration of phenols which tended to increase in goats but not in sheep with the phenolic diet. Apart from that, only milk yield showed a relevant animal species × diet interaction (more milk with extra TEP in sheep, less in goats). There were also general species differences. In the present study, goat saliva had a clearly higher tannin binding capacity in both CT (quebracho tannins) and HT (tannic acid) than sheep saliva, but there was no response to the extra phenols. Havonice-Ziony et al., (2010) also found that the affinity of salivary proteins did not respond to presence or absence of a tanniferous diet in goats. Goats have been reported to produce more protein-rich saliva during eating than sheep (Dominigue et al., 1991), but Distel and Provenza (1991) did not detect proline-rich protein in the saliva of goats fed a tannin-rich diet (blackbrush twigs). Also in the present study, there was no species difference in total protein concentration of the saliva. Longer exposure times, higher levels of dietary tannin, or different tannin sources still might stimulate saliva protein synthesis in these animals.

4.6 Conclusion

The present study showed that supplementing 4% grapeseed extract rich in polyphenols to the diet of dairy sheep and goats increased concentrations of total phenols in blood plasma and milk and thus part of the polyphenols could be absorbed. However, supplementing the grapeseed extract did not lead to an increase in the antioxidant capacity of blood. Finally, there were some, however, limited interactions indicating a different response of sheep (grazers) and goats (intermediate feeders) to the grapeseed extract. Although this did not include different tannin-binding properties of the saliva in the presence and absence of the extract, the principally higher activity of goats supported the hypothesis that tannin binding affinity will be higher in goat saliva than sheep saliva. The study also demonstrated that agro-industrial by-products like grapeseed extract can be successfully included in ruminant diets without impairing performance.

Chapter 5

General Discussion and Conclusion

The purpose of the present research was to compare animals (camels, cattle, sheep and goats) of different feeding type according to Hofmann (1989) in order to understand their intake of PSM and the transfer of PSM from their diet to milk. In chapter two, camels and two cattle types supplemented with/without RDP in the East African rangelands were compared in two seasons to assess their performance and plant selection. It was further evaluated in chapter 3 whether the nutrient intake by these animals affected excretion of phenols with milk phenol and milk FA concentration in milk. The fourth chapter examined the feeding of PSM rich grapeseed extract to dairy goats and dairy sheep to assess the transfer of polyphenols to blood, milk, urine and faeces and their side-effects on performance and milk quality.

5.1. Differences between grazing and browsing herbivores

Ruminants like cattle, sheep, goats and camels rely on a diet composed of different plant species. Browsing (feeding on woody species) and grazing (feeding on grasses and herbaceous legumes) are particularly different types of feeding behavior among ruminants (Duncan and Poppi 2008). Cattle, sheep, goats and camels have different plant selection pattern on feeding on either grasses or browse. Cattle and sheep classified as grazers (Hofmann 1989) and camels classified as browsers (Schwartz et al. 1983) have different diet selection behavior. In the present study, cattle mainly selected a diet that is composed of grasses with a small proportion of browse while the camel's diet was composed of woody species of trees, shrubs and herbaceous vegetation. Goats being intermediate feeders have rumen structures that are able to switch from grasses to browse with the ability to adapt to different fiber types (Hofmann 1989; Mkhize et al. 2016). Grasses are monocotyledonous plants while herbaceous plants and woody plants which comprise of shrubs and trees are dicotyledonous plants. Browse has higher levels of lignin than grasses but lower levels of NDF than grasses (chapter 2). Due to the high lignin content of the browse (chapter 2) the digestibility is lower than that of grasses (White and Trudell 1980). Grasses have been shown to tolerate the grazing by herbivores as their low growth form minimizes the impact of grazing and facilitates regrowth (McNaughton 1983) while browse plants have physical and chemical defenses that reduce the effect of browsing (Bryant et al. 1991).

Grazers like cattle and sheep while grazing choose plants according to how they appear on the rangeland, rather than individual plants or plant parts (Duncan and Poppi 2008). Browsers like camels are more selective and they normally select different individual plants within rangeland (Duncan et al. 1994; Hartley and Jones 1997) and also within individual plants than are cattle, which tend to be less

selective. Comparing grasses and browse, the nutritional quality (chapter 2) varies greatly in different plants, and browsers like camels select specific parts of the plant like leaves over stems (Shipley et al. 1998). Camels, therefore, may make decisions on how to maximize nutrient intake more frequently than do cattle due to their selective feeding behavior (Coppock et al. 1987).

Cattle and camels have a different use of plant resources in the rangeland as they select different plant species, distribute themselves and move around differently while grazing or browsing (chapter 2). The implication of this behavior is that cattle and camels do not compete for plant resources and this has a better use of the habitat and plant selection. In a comparative study of the feeding behavior of ruminants in free range, Coppock et al. (1986) demonstrated that goats and camels tended to move faster and spend less time feeding at a feeding station than do the cattle or sheep.

5.2 Effect of season and supplementation on grazing and browsing herbivores

Browse and grasses play an important role in the nutrition of ruminant livestock in tropical regions, however, during the dry periods there is scarcity of pastures in these areas. The changes in forage quality and quantity in different seasons is a major factor that affects animal productivity in the free ranging conditions (Selemani et al. 2013). The type of forage consumed by cattle, sheep, goats and camels differ in terms of their physical and chemical composition which depends on seasonal variations and hence the amount of nutrient ingested (Coppock et al. 1986). Grasses contain lignified material and are poor quality forage during the dry periods as it is high in total fibers (Rubanza et al. 2005). When there is little or no rainfall the nutritive value of the forage declines due to highly lignified material and a reduction of leaves and stems (Van Soest 1994). During periods of reduced quantity of forage especially in the dry season the animals may switch to less preferred plant species to save the time and energy of looking for high quality plant species (Baumont et al. 2000). The chemical composition of the most eaten plants species showed that the plant species eaten by cattle were affected by season being of lower quality in the TP (3.5 % CP) than RS (6 % CP) unlike for camels that was not affected by seasonal differences (9.2 % and 10 % CP in TP and RS respectively) (chapter 2).

The plant selection behavior of animals changes from season to season according to the available resources and the amount of plant material in the diet varies seasonally. The availability of grasses and browse declines drastically during the dry periods as the grasses dry off and some of the woody species shed off their leaves in the arid regions thereby reducing the amount of forage for herbivores (Selemani et al. 2013). Some of the woody species browsed by camels are able to remain green for long periods

of time even during the dry periods due to their deeper root system and their nutrient content does not fluctuate as do grasses and herbs (Boutton et al. 1988). Comparing cattle and camels, season was a major factor that affected diet quality especially for cattle (chapter 2). The diet of cattle consisted mainly of green leaves of grasses during the RS and grass material having lower quality during the TP while the browse for camels was consistently green across the two seasons. The two cattle types had similar plant species in their diets in the RS and TP therefore, the cattle types did not switch to browse during the drier TP because of availability of grasses. Grazing conditions are complex due to variations with time in available quantity, quality and physical structure of the vegetation and environmental conditions (Hodgson 1986). Dumont and Gordon (2003) indicated that sheep and cattle initially select grasses that allow them to meet both diet quality and quantity requirements, before using behavioral adjustments such as increasing daily grazing time to maintain diet quality for as long as possible and when total intake decreases below the animal's needs they finally switch to the poor quality alternative. In the grazing study in Kenya (chapter 2) the chemical composition of the cattle diets were variable in RS and TP while the camel diets were not (average values of crude protein contents of 6% and 11% in RS and 3.5% and 9.2%in TP for cattle and camels, respectively). Camels were always found to attain above maintenance concentrations of dietary crude proteins even during the dry periods of the year in the semi-arid areas of northern Kenya while the cattle, sheep and goats were not able to meet maintenance needs for energy and protein during the dry periods (Coppock et al. 1987). Forage quality and quantity are important factors that determine the animal performance during dry periods that normally last several months in the dry areas. In the grazing experiment in Kenya (chapter 2), the crossbreds cattle had a lower milk yield in the TP than RS and the camels and local cattle were not affected by seasonal changes indicating that camels and local cattle are better adapted to seasonal variations in feed quantity and quality than the crossbreds with high feed requirements. However, for the case of camels and local cattle it could also be that their inherently low performance allowed them to cover requirements with feed of lower quality.

Plant secondary metabolites have been extensively studied because of the adverse effects that they may have when ingested by animals especially on intake and production (Lamy et al. 2011b). However, the beneficial effects of PSM in animal nutrition and humans as antioxidants have also been investigated (Koleckar et al. 2008). The PSM ingested by the animal depends on their concentration in the diet, the amount consumed, the action within the gastrointestinal tract, absorption, transformation and excretion from the animal (Scalbert and Williamson 2000). Plant secondary metabolites have been reported to be

in low amounts in grass species but are abundant in a number of herb and especially woody species (chapter 2) (Harborne 2014). Camel and goats are adapted to feeding on forage with PSM due to their nature of feeding type. It is understood that as the concentration of PSM in the forage of ruminants increases in their diets the intake of these forage decreases (Iason 2005). The camels and goats as a defensive mechanism are able to choose mixed diets so as to increase intake of feed (Provenza et al. 2003) or switch to several plants while browsing and grazing so as to reduce the intake and consumption of PSM (Iason 2005; Mkhize et al. 2016), therefore avoiding the nutritional limitation as a result of PSM unlike the cattle and sheep whose diets contain considerably low amounts of PSM (Harborne 2014). These behavior by animals like camel and goats is a strategy to reduce toxicity associated with intake of PSM. Comparing the grazing experiment (chapter 2&3) and the controlled feeding experiment (chapter 4), the camels and cattle had the opportunity to choose diverse plant species and because of this are able to regulate the PSM in their diets better than when the animals are restricted to feeding on a specific diet with low or high PSM like sheep and goats in the second experiment of this thesis (chapter 4).

Saliva is one of the ways through which herbivores defend themselves against the effect of PSM and it's believed that this defense mechanism is gained in the process of evolution by animals that graze or browse on plants that contain PSM (Shimada 2006). The animals adapted to feeding on PSM rich diets like browsers are able to produce tannin binding proteins in their saliva (Hofmann 1985). Several mammalian species were examined for the presence of tannin binding salivary proteins (Shimada 2006) and in these studies the saliva of sheep, goats and cattle did not contain proline rich proteins that bind to tannins. Concerning the saliva of goats and sheep (chapter 4), it was hypothesized that the saliva of dairy goats have higher tannin binding affinity and total protein than saliva of sheep especially for the animals that are fed tannin rich grapeseed extract. Our results support the evidence that saliva of goats had overall higher tannin binding affinity to bind to quebracho tannin and tannic acid than saliva of sheep. However, the total protein in saliva between the two animal species did not differ. Total protein concentration of the saliva of goats and sheep was not affected by ingestion of 50 g/kg quebracho tannins in a study by Salem et al. (2014). Alonso-Díaz et al. (2012) found that salivary proteins from goats had a higher capacity to precipitate tannins from *A. pennatula* than sheep while the salivary proteins of sheep had a higher capacity to precipitate to tannins from *P. piscipula* than goats. From this study it shows that there are differences in between goats and sheep in the capacity of their saliva to interact with tannins in different plants. Goats have been reported to produce more nitrogen in saliva

than sheep during eating (Dellow and Barry 1991), Distel and Provenza (1991) did not detect proline-rich protein in the saliva of goats fed blackbrush twigs, a tannin-rich diet. It is possible that salivary proteins of each animal are adapted to the particular tannin-containing foods consumed by that animal. Tannins are more prevalent in browse than grasses. Grazers like sheep and cattle normally consume grasses with little concentrations of PSM and therefore do not need tannin binding proteins as a means of protecting themselves from negative effects of PSM unlike camels and goats that feed on browse. Supplementation with protein and energy have been found to increase the ability of animals to ingest PSM (Provenza 1996). There is a relationship between energy and protein requirements and the ability to detoxify forages rich in PSM such as tannins (Mkhize et al. 2016; Villalba et al. 2002). During the RS in our study (chapter 2), there was plenty of pasture for the animals and therefore the animals had enough nutrients derived from the pastures without requiring additional protein and energy supplementation. The supplemented camels had a proportionally higher intake of woody plants than un-supplemented camels indicating that the RDP enabled the supplemented camels to ingest more of these woody plant species with PSM. Other studies have also reported improved intake of diets containing PSM by lambs when supplemented with energy and protein feeds (Villalba et al. 2002). It has been observed that the ability of animals to tolerate PSM is related to nutrient intake because the process of detoxification depletes the protein and energy in the body (Villalba et al. 2002). Shaw et al. (2006) found that sheep fed a high quality diet (both protein and energy) consumed more PSM from three diets with different PSM vs. sheep fed a low quality diet. Utsumi et al. (2009) reported that protein sources of different degradability could potentially influence juniper intake in goats and sheep in response to seasonal variation of PSM concentration. The ability of the camels and goats to utilize browse especially shrubs and trees in the semi-arid rangelands where woody species encroachment has increasingly been observed can be used as a means to reduce the invasion of these woody species in open grasslands which are the main grazing areas for cattle and sheep.

5.3 Effect of season and animal type on milk fatty acid profile

Plant species growing in their natural environments are important feed for livestock. However, they contain moderate to high levels of PSM that can affect the concentration of milk FAs. In most of these areas, livestock production is based on free range grazing and studying the effects of the different plant species consumed by animals on the milk FA is of great importance. Different plant species consumed by the animals can affect or alter the different proportions of milk FAs due to their differences in

quality. Several feed factors like the type of feed consumed by the animals and animal factors like species and breed have been reported to affect the milk fat composition (Palmquist et al. 1993). Especially, in the first study that was conducted in Kenya (chapter 2&3) cattle diets had lower quality in the transition period than in the rainy season and this had major effect on most of the milk FAs as the changes in quality of the forage has been reported to affect the amounts of the different milk FA (Chilliard et al. 1991). Cabiddu et al. (2005) reported that different forage species like *C. coronarium* and *M. polymorpha* increased the levels of PUFA and CLA in sheep milk while different phenological stages of the forage like fresh grass in spring increased milk FA (Addis et al. 2005) and species composition (Cabiddu et al. 2005) can influence the milk FA composition.

The intake of PSM especially TEP by the cattle and camels differed as a result of cattle relying on grasses and camels browse and PSM have been found to alter rumen biohydrogenation by feeding different plants in different seasons (Cabiddu et al. 2009; Khiaosa-Ard et al. 2011). Cabiddu et al. (2009) demonstrated that C18:3 *n*-3 proportions in rumen fluid and milk lipids are positively affected by plant phenols in sheep grazing *H. coronarium*, likely through an inhibition of the first step of biohydrogenation. Camels had higher intake of phenols than cattle in both seasons and with the inhibiting effect of PSM on ruminal biohydrogenation (Vasta et al. 2009) the camel milk had overall higher proportions of PUFA in milk fat than milk fat from cattle. In the RS the proportions of FAs like *n*-3 in milk fat of camels were also higher than in the TP. CLA and RA originates from ruminal biohydrogenation of dietary linoleic and linolenic acids (Chilliard et al. 2007). An accumulation of vaccenic acid (VA) has been observed also in the ruminal fluid of alpine-grazing cattle compared to lowland-grazing cows and this result was probably due to the high PSM concentration in these pastures (Khiaosa-Ard et al. 2011). Considering the season effect on milk FA in the grazing experiment with cattle and camels (chapter 3) VA decreased from the RS to TP in cattle types indicating that there was a reduction of ruminal biohydrogenation with decrease in phenol intake.

The milk of different ruminants differ in their proportions of FAs (short, medium chain, saturated, branched, mono and polyunsaturated, cis, trans and conjugated FAs). PUFA are associated with positive effects like prevention of certain forms of cancer while the short chain FAS are associated with negative effects that are related to cardiovascular diseases (Williams 2000). For instance in the grazing experiment (chapter 3), the camel milk fat is rich in the desired FAs of PUFA and MUFA compared to the cattle milk fat that had higher SFA. Several studies have also reported that the main characteristic

of the sheep and goat milk fat is made up of short and medium chain FAs which are mainly caproic, caprylic and capric FAs (Cabiddu et al. 2005; Chilliard et al. 2006).

5.4 Transfer of PSM to milk

Studies on the transfer of PSM from feed to animal products and excreta of dairy goats, sheep or from feed to milk of cattle and camels are limited. Especially phenolic compounds are increasingly studied for their impact on animal source products (Cabiddu et al. 2009). In the second experiment, the effect of grapeseed extract in adult dairy goats and sheep and intake of TEP by grazing camels and cattle in their natural grazing areas were investigated with respect to the transfer of total phenols from feed to milk.

In the grazing experiment with cattle and camels (chapter 2&3), the camel diet was more rich in polyphenols than cattle diet and these differences are because of the plants selected by these animals. The camels ingested more plant functional groups with PSC in their diet than cattle in both seasons while the cattle diet had lower amount of PSC and especially in the TP when the forage quality was lower (chapter 2). The estimated TEP intake in camels was 6.5 and 5.6 g/kg metabolic BW in RS and TP and for the cattle was 3.5 and 1.5 g/kg metabolic bodyweight in RS and TP indicating the type of the plants species selected by these animals are affected by seasonal changes. The differences in intake of PSM between camel and cattle could be explained by the differences in their diet selection. As presumed, there was a relationship between phenol intake and its excretion in milk in both cattle types, but not the camels. This indicates that camels have a different metabolism than cattle that limits the excretion of TEP with milk. In the second experiment with sheep and goats, the animals that were fed grapeseed with phenols had similar concentration of total phenols at the end of experiment in their milk being 39.2 and 36.8 mg/l in sheep and goats respectively, compared to that of camel and cattle that was 49.4 and 24.4 mg/l in RS and TP. Comparing the four ruminant species the concentration of phenols in milk is within the range reported in other studies like Di Trana et al. (2015) who found values of between 49-57 mg/l total phenols in milk of goats.

5.5 Effect of supplementation of grapeseed extract as a source of plant secondary metabolites on grazer and intermediate feeder

The aim of the experiment was to evaluate the inclusion of grapeseed extract with phenols on performance and concentration of phenol in milk, blood, urine and faeces in dairy goats and sheep in a controlled feeding experiment (chapter 4). The experimental feeds in the study varied in chemical composition and no differences in performance were found between the goats and sheep. It was hypothesized that the polyphenols in the grapeseed extract can be transferred to the blood, milk and urine through the gastrointestinal tract in to the blood stream, mammary gland and urinary pathways (De Feo et al. 2006). The results of the controlled feeding experiment show that there was a positive transfer of total phenols from the feed to milk, blood plasma and urine of dairy sheep and goats fed grapeseed extract. Considering that the goats and sheep responded to polyphenols in blood plasma and milk in a different way it could be a different case if these animals were in a free grazing environment or on a free choice diet where differences in their feeding behavior might influence the intake of polyphenols. It has been stated that free ranging ruminants are able to select a diet which has high amounts of nutrients and lower concentration of PSM and previous experience can influence the dietary intake by animals (Duncan and Gordon 1999). In the sheep and goat experiment (chapter 4) the two animal species did not have a variety of feed to choose from like in the grazing study in Kenya (chapter 2&3) where the cattle and camels were free to choose the different plant species with varying amount of phenols available in the rangeland.

The grazing experiment and controlled feeding experiment both show that there is relationship between dietary TEP intake and transfer of polyphenols to milk of cattle and sheep. In the second experiment with sheep and goats, the control diets contained low amounts of polyphenols compared to the grapeseed extract supplemented diets which suggest that an increase in intake of TEP will lead to an increase in concentrations of phenols in milk, blood and urine as was found in the cattle types but not in the camels in the first experiment of this thesis. The transfer of polyphenols to animal products like meat and milk has been reported to improve performance and enhance quality of these products (Gladine et al. 2007; Vasta and Luciano 2011) and it’s therefore, important to promote the use of agro industrial by products that contain polyphenols. Other studies have also reported increased levels of polyphenols in lamb meat (Moñino et al. 2008), blood plasma of sheep infused with rosemary extract (Gladine et al. 2007) and blood plasma of suckling kids from dairy goats fed rosemary leaves. These

studies did not compare goats and sheep in the same experiment as in the second experiment of this thesis (chapter 4).

To assess the effectiveness of the dietary feeding of grape seed extract, the antioxidant status of blood plasma was determined. We found that there was no effect of feeding grapeseed extract on the total blood antioxidant capacity in sheep and goats. Similarly, some other studies have reported similar findings regarding relationships between dietary forage intake as sources of antioxidant compounds in cows (Santos et al. 2016) and chicken (Goni et al. 2007). However, some other studies reported that polyphenols can be important sources of antioxidants which can be transferred from feed to meat of lambs (Jordán et al. 2010; López-Andrés et al. 2013; Moñino et al. 2008).

5.6 General conclusions

In the present doctoral thesis grazers (cattle, sheep) and browsers (camels) respectively intermediate feeders (goats) were compared with regard to performance and intake of PSM either in free grazing in the east African rangeland or controlled feeding experiment.

In the grazing experiment, the camels and local cattle were found to be more adapted to the conditions of the semi-arid areas of Laikipia in terms of performance than are crossbred cattle which were affected by seasonal changes of forage quantity and quality. Supplementing crossbred cattle with protein and energy supplements like molasses and urea during dry and transition periods will help them improve on their performance and possibly increase the intake of poor quality pastures. On a similar note supplementing camels with rumen degradable protein could help them increase the intake of browse and therefore could be considered as an important rangeland management option to control bush encroachment in these rangelands.

Considering that camels and cattle differ in their feeding behavior and comparative diet selection they can utilize the rangelands in different ways and grazing studies like of the present project are important in deriving appropriate management strategies for these animals. For instance, cattle and camels in the present study showed a low level of competition for the feed resources when grazing or browsing in the same rangeland since cattle selected mainly grass species and camels selected browse. In comparing different animal species like cattle, sheep, camels and goats grazing in the same or similar rangelands emphasis must be made on defining the environment on which these animals perform satisfactorily and understanding the effect of grazing by one species on the performance of the other.

Animal species differences in their plant selection behavior and seasonal changes in forage quality and quantity are major factors that affected the milk FA profile and phenol concentration in milk of camels and cattle. From the grazing experiment (chapter 3), there was a positive relationship between estimated phenol intake and phenol excretion with milk in cattle but not in camels indicating that camels might have a different lipid metabolism compared to cattle. Camels might have developed better coping mechanism to limit the adverse effects of PSM and transfer of phenol to milk as they are more likely exposed to PSM given their feeding behavior. These response could help to explain why camels are able to thrive well in the semi-arid rangelands with high PSM rich browse.

Dietary feeding of grapeseed extract to dairy sheep and goats could be used in livestock feeding as the polyphenols in the grapeseed extract are positively transferred to milk and blood which contribute to their potential health benefits and did not affect performance. Sheep and goats responded to intake of polyphenols differently (chapter 4) in terms of their concentration of phenol in blood and milk when offered the same amount of phenol in the diet and further research might be interesting to test the same animal species when they are offered a free choice feeding or when the animals are on a free grazing environment where they are free to choose their diets without any restriction.

Appendices

Appendices 2.1 of chapter 2

Bite characteristics (small, half, and full bite) and bite weights (in grams of air dry matter ± standard deviation) of the most frequently selected plants/mixed categories of the different functional groups (FG: grasses, herbs, shrubs, and trees) by cattle (crossbred and Pokot) and camels during the rainy season and the transition period.

FG	Species		Rainy season (*n*=4)			Transition period (*n*=2)		
		Bite size	Small	Half	Full	Small	Half	Full
Cattle		Plant family						
Single grasses								
	Aristida adoensis Hochst. ex A. Rich.	Poaceae	0.36±0.13	0.78±0.18	1.11±0.33	-[2]	-[2]	-[1]
	Bothriochloa insculpta (A.Rich.) A.Camus	Poaceae	0.27±0.18	0.71±0.13	1.73±0.92	-[2]	-[2]	-[1]
	Brachiaria dictyoneura (Fig. & De Not.) Stapf	Poaceae	0.24±0.13	0.79±0.14	0.96±0.10	-[2]	-[1]	-[1]
	Cynodon nlemfuensis Vanderyst	Poaceae	0.20±0.19	0.76±0.20	0.94±0.26	0.29±0.01	1.22±0.08	2.92±0.78
	Digitaria macroblephara (Hack.) Paoli	Poaceae	-[1]	-[2]	-[1]	0.37±0.08	0.67±0.65	3.51±0.81
	Eragrostis tenuifolia (A.Rich.) Hochst. ex Steud.	Poaceae	0.61±0.20	1.13±0.24	2.81±0.63	-[2]	-[2]	-[2]
	Heteropogon contortus (L.) Roem. & Schult.	Poaceae	0.45±0.15	0.65±0.13	0.93±0.16	-[2]	-[2]	-[2]
	Hyparrhenia papillipes (Hochst. ex A.Rich.) Andersson ex Stapf	Poaceae	0.26±0.10	0.65±0.06	1.02±0.29	0.36±0.06	1.08±0.40	2.76±0.62
	Pennisetum mezianum Leeke	Poaceae	0.20±0.16	0.60±0.08	1.13±0.29	-[2]	-[2]	-[1]
	Pennisetum stramineum Peter	Poaceae	-[1]	-[1]	-[1]	0.29±0.07	0.80±0.08	2.24±0.07
	Setaria sphacelata (Schumach.) Stapf & C.E. Hubb. Ex Moss	Poaceae	0.41±0.28	1.17±0.49	2.63±0.85	-[1]	-[2]	-[1]
	Themeda triandra Forssk.	Poaceae	0.21±0.21	0.80±0.08	1.44±0.66	0.30±0.06	0.91±0.37	2.65±1.35
Mixed grass categories								
	Cattle bunch grasses	Poaceae	-[1]	0.69±0.13[4]	-[1]	0.20±0.15	0.48±0.09	1.09±0.07
	Cattle creeping grass	Poaceae	-[1]	0.76±0.20[4]	0.94±0.26[4]	-[1]	-[1]	-[1]
	Cattle small grasses	Poaceae	0.48±0.20[4]	0.95±0.27[4]	1.96±1.02[4]	0.09±0.01	0.30±0.14	1.00±0.53
	Cattle tall grasses	Poaceae	0.30±0.19[4]	0.87±0.36[4]	1.54±0.93[4]	0.23±0.04	0.58±0.00	1.55±0.01
Mixed dicot. categories								
	Broad leaved herbs		0.19±0.11[3]	0.57±0.24[3]	1.12±0.54[3]	0.19±0.11	0.57±0.24	1.12±0.54
	Broad leaved shrub		-[1]	0.65±0.66	3.37±0.95	0.24±0.27	0.65±0.66	-[1]
	Small leaved herbs		-[1]	-[1]	-[1]	0.11±0.04	0.29±0.20	0.96±0.70
Camel								
Herbs								
	Barleria delamerei S.Moore	Acanthaceae	0.17±0.04	1.06±0.24	4.10±1.98	0.54±0.21	2.86±0.63	7.74±0.99
	Barleria eranthemoides R.Br.	Acanthaceae	-[1]	-[1]	-[1]	0.53±0.04	2.14±0.23	6.58±0.52
	Heliotropium zeylanicum (Burm.f.) Lam.	Boraginaceae	-[1]	0.72±0.22	4.14±1.92	-[2]	-[2]	-[2]
Shrubs								
	Acacia brevispica Harms	Fabaceae	-[1]	-[1]	-[1]	0.50±0.28	1.66±0.61	4.20±0.03
	Carissa spinarum L.	Apocynaceae	-[1]	2.63±1.28	3.73±1.31	-[2]	-[2]	-[2]
	Grewia tephrodermis S.Mortiz	Tiliaceae	-[1]	0.52±0.28	1.41±0.47	-[2]	-[2]	-[2]
	Hibiscus flavifolius Ulbr.	Malvaceae	-[1]	1.03±0.20	1.93±0.34	-[2]	-[2]	-[1]
	Lantana verbunoides (Forssk.) Vahl	Verbanaceae	-[1]	-[1]	3.06±0.77	-[1]	-[1]	-[2]

Appendices 2.1 of chapter 2 Continued

FG Species	Bite size	Rainy season (*n*=4)			Transition period (*n*=2)		
		Small	Half	Full	Small	Half	Full
Camel	Plant family						
Shrubs							
Lycium europaeum L.	Solanaceae	0.14±0.13	0.35±0.14	1.15±0.59	-[2]	-[1]	-[1]
Gymnosporia putterlickioides Loes.	Celastraceae	-[1]	-[1]	-[1]	0.25±0.03	0.69±0.07	1.35±0.61
Pyrostria phyllathoidea (Baill.) Bridson	Rubiaceae	-[1]	-[2]	-[2]	0.34±0.19	1.09±0.21	2.51±0.35
Searsia natalensis (Bernh. ex Krauss) F.A. Barkley	Anacardiaceae	-[1]	1.48±1.12	2.60±1.30	-[1]	-[2]	-[2]
Scutia myrtina (Burm.f.) Kurz	Rhamnaceae	0.20±0.06	0.73±0.33	2.30±0.58	-[1]	-[1]	-[1]
Tephrosia emeroides A. Rich	Fabaceae	-[1]	-[1]	-[1]	0.39±0.01	0.86±0.28	2.98±0.20
Tarenna graveolens (S.Moore) Bremek.	Rubiaceae	-[1]	-[1]	-[1]	0.75±0.19	2.86±0.25	8.57±0.69
Trees							
Acacia nilotica (L.) Delile	Fabaceae	0.24±0.07	0.98±0.53	2.13±0.40	-[2]	-[1]	-[1]
Boscia angustifolia A.Rich.	Capparaceae	-[1]	-[1]	-[1]	0.33±0.38	0.70±0.79	1.49±1.29
Mixed dicot. categories							
Small leaved herbs		0.17±0.04[4]	-[1]	-[1]	0.42±0.01	0.70±0.51	2.71±0.28
Broad leaved shrubs		0.43±0.33[4]	1.50±1.07[4]	2.92±1.09[4]	0.70±0.36	1.85±0.59	4.09±1.74
Small leaved shrubs		0.20±0.14[4]	0.66±0.32[4]	2.16±1.53[4]	0.43±0.08	0.88±0.01	2.03±0.16
Small leaved trees		0.24±0.07[4]	-[1]	-[1]	0.24±0.06	-[1]	-[1]

[1] Not eaten by the animals during the respective period.

[2] Eaten but not sampled as separate category because of low frequency in the diet; therefore, it was included in the respective mixed category.

[3] The category 'cattle broad leaved herb' was not collected in the rainy season; therefore, the values measured in the transition period were used for data analysis instead.

[4]In the rainy season, the mixed-bite categories were not collected; thus, the mean group values of the most frequently selected single plant species that fit into the respective mixed category were used.

Appendices 2.2 of chapter 2

Chemical composition (% of DM) of the most frequently selected forage plants selected by cattle (crossbred and Pokot) and camels in the rainy season (R) and the transition period (T) from different functional groups; *n* = 2 per period (P)[1].

Functional group, Species	P	DM	OM	NDF	ADF	ADL	CP	EE	TEP	TT	CT
Grasses											
Aristida adoensis	R	91.7±0.5	89.0±0.1	74.6±0.1	43.7[2]	6.9[2]	5.9±0.7	1.3±0.0	1.5[2]	0.1[2]	>0.0[2]
Bothriochloa insculpta	R	91.6±1.1	89.3±0.8	71.2±2.9	40.0±3.4	4.6±0.6	6.6±1.6	1.4±0.1	2.4[2]	0.2[2]	0.1[2]
Brachiaria dictyoneura	R	91.5±0.6	89.7±2.4	74.3±4.5	42.6±5.8	5.7±1.0	6.3±2.8	1.6±0.2	1.9±0.0	0.1±0.0	>0.0±0.0
Cynodon nlemfuensis	R	91.2±0.3	91.5±0.2	69.7±5.6	36.4±4.8	7.7±3.3	9.9±3.4	1.5±0.2	1.6±0.0	0.1±0.0	>0.0±0.0
	T	95.1±0.1	91.9±1.2	80.6±0.1	39.9±2.0	8.5±0.9	2.8±0.6	1.0±0.0	1.5±0.1	0.1±0.0	>0.0±0.0
Digitaria macroblephara	T	94.9±0.0	91.1±3.7	72.9±3.6	34.4±3.6	6.1±1.0	4.4±0.6	1.5±0.9	1.4±0.0	0.1±0.1	>0.0±0.0
Eragrostis tenuifolia	R	91.2±0.3	92.1±2.1	71.5±4.2	38.1±3.2	8.0±3.5	10.8±0.6	2.1±0.3	1.7±0.1	0.1±0.1	0.1±0.0
Heteropogon contortus	R	91.8±0.7	91.8±1.9	76.4±4.5	44.4±6.2	7.6±2.4	4.3±1.2	1.3±0.0	2.5±0.4	0.4±0.1	0.1±0.1
Hyparrhenia papillipes	R	91.7±0.5	90.8±0.9	73.6±5.4	45.4±5.0	8.2±2.7	4.5±1.3	2.0±0.4	2.8±0.5	0.8±0.4	0.0±0.0
	T	94.5±0.2	87.0±2.0	68.6±2.3	33.2±1.9	6.7±0.2	3.8±0.9	2.0±0.0	2.2±0.2	0.4±0.3	>0.0±0.0
Pennisetum mezianum	R	91.8±0.0	90.8±0.9	73.9±3.0	41.6[2]	7.2[2]	6.8±1.0	1.5±0.2	2.0±0.1	0.1±0.1	>0.0±0.0
Pennisetum stramineum	T	95.1±0.4	89.1±5.0	80.9±0.8	39.8±0.4	11.9±3.1	4.5±0.1	1.5±0.2	1.6±0.1	0.1±0.0	>0.0±0.0
Setaria sphacelata	R	92.0±0.5	86.5±0.5	68.9±3.1	41.2±3.9	6.5±2.3	7.4±2.9	1.4±0.1	2.2±0.1	0.3±0.0	0.2±0.1
Themeda triandra	R	91.7±0.5	90.5±2.6	73.4±4.3	44.3±2.2	7.3±0.2	5.1±1.0	1.4±0.1	2.7±0.3	0.6±0.0	0.0±0.0
	T	94.8±0.1	92.7±2.9	77.5±2.4	37.1±1.8	9.2±1.9	3.9±1.3	1.1±0.4	2.6±0.2	0.8±0.3	0.0±0.0
Herbs											
Barleria delamerei	R	89.5±0.6	89.7±1.3	60.3±5.7	43.7±3.1	14.9±1.6	10.5±2.9	0.9±0.4	3.2±0.2	0.3±0.1	0.1±0.0
	T	94.2±0.5	90.2±1.2	68.7±1.2	32.5±1.1	17.4±0.4	9.3±1.1	0.8±0.1	2.5±0.2	0.1±0.1	>0.0±0.0
Barleria eranthemoides	R	88.6±0.3	87.0±0.8	50.6±1.5	39.1±1.5	9.0±0.6	11.7±1.2	1.1±0.1	2.1±0.1	0.2±0.0	>0.0±0.0
	T	94.2±0.5	89.1±1.9	61.7±0.4	34.1±1.1	10.7±0.1	8.4±2.8	0.7±0.3	2.7±0.2	0.1±0.0	>0.0±0.0
Heliotropium zeylanicum	R	89.2±0.2	86.8±2.1	49.1±6.7	40.5±0.2	13.2±2.8	13.7±3.0	1.8±0.1	2.9±0.2	0.3±0.2	0.2±0.1
Shrubs and trees											
Acacia brevispica	R	89.6±0.8	94.9±0.3	55.9±7.8	34.3±0.0	16.9±0.0	17.7±3.9	1.7±0.5	8.4±0.1	3.9±0.3	1.8±0.1
	T	94.8±0.2	94.3±0.3	59.5±10.3	21.9±0.3	17.5±1.4	11.8±0.2	1.7±0.2	10.0±0.0	5.1±0.2	1.5±0.2
Carissa spinarum	R	90.0±0.3	94.3±1.2	44.3±0.6	31.3±2.5	13.7±0.4	8.6±0.8	3.8±1.2	17.8±1.3	8.8±0.1	6.5±0.6
Grewia tephrodermis	R	90.2±0.0	90.6±0.7	50.5±6.2	38.2±3.0	7.7±1.0	14.1±2.7	1.3±0.2	3.0±0.4	0.7±0.1	0.5±0.1
Hibiscus flavifolius	R	90.5±0.1	89.5±0.8	54.6±0.9	42.9±1.2	8.6±1.1	12.0±0.2	2.2±0.6	2.6±0.4	0.1±0.1	>0.0±0.0

Appendices 2.2 of chapter 2 Continued

Functional group, Species	P	DM	OM	NDF	ADF	ADL	CP	EE	TEP	TT	CT
Shrubs and trees											
Lantana verbunoides	R	90.1±0.5	91.0±1.5	49.4±2.0	40.0±0.3	11.4±0.3	11.4±0.1	1.7±0.1	4.8±0.1	0.7±0.3	0.2±0.1
Lycium europaeum	R	90.1±0.7	91.2±1.9	64.1±1.3	44.4±4.8	17.1±0.8	13.7±3.0	1.7±0.5	2.7±0.3	0.5±0.1	0.3±0.0
Gymnosporia putterlickioides	T	94.3±0.0	92.3±0.2	63.1±3.3	26.7±1.2	17.5±2.5	5.5±0.1	1.8±0.2	11.7±1.0	9.2±1.4	3.3±0.2
Pyrostria phyllathoidea	T	95.3±0.4	94.5±0.6	52.7±0.3	22.1±9.2	12.0±1.0	7.3±0.3	1.9±0.0	6.8±0.5	1.9±0.3	0.6±0.1
Searsia natalensis	R	90.9±0.4	92.6±0.3	57.2±4.3	33.1±2.8	15.4±0.7	11.9±4.2	1.2±0.1	6.2±0.9	2.9±1.1	1.3±0.3
Scutia myrtina	R	88.7±0.2	94.0±0.7	53.4±6.7	37.7±3.1	17.5±0.8	9.2±1.8	0.8±0.0	13.7±0.8	9.2±0.3	7.0±0.1
Tephrosia emeroides	T	95.1±0.2	96.5±0.5	69.7±6.3	33.9±3.1	14.2±2.6	11.3±0.7	1.8±0.4	3.0±0.8	0.2±0.1	>0.0±0.0
Tarenna graveolens	T	96.5±0.3	95.8±1.1	37.0±3.2	16.0±2.7	11.4±0.2	6.8±1.4	1.5±0.4	11.1±2.1	2.8±0.5	0.2±0.2
Acacia nilotica	R	90.8±0.4	96.0±0.4	37.5±3.3	28.2±2.0	11.1±0.4	11.1±1.0	1.0±0.1	32.9±1.3	30.0±1.3	0.5±0.0
Boscia angustifolia	T	94.6±0.1	93.0±1.5	68.9±0.5	27.4±1.1	17.9±0.7	13.5±1.6	1.1±0.9	1.9±0.1	0.2±0.1	>0.0±0.0

ADF, acid detergent fiber, ADL, acid detergent lignin; CP, crude protein; CT, condensed tannins; DM, dry matter; EE, ether extract; NDF, neutral detergent fiber; OM, organic matter; TEP, total extractable phenols; TT, total tannins.

[1] Plant collection dates were 29.5.15 and 30.6.15 in R and 28.8.15 and 30.9.2015 in T.

[2] $n = 1$

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