Effects of mild water restriction on digestion and nitrogen metabolism in a desert adapted goat breed

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Dedication

To my parents for their will to make me fly

Declaration

I declare that this dissertation was written independently and without non permissible help and that I used no sources other than those specified in the dissertation. All quotations that have been extracted from published or unpublished sources have been marked as such. No part of this work has been used in other Ph.D. processes.

(Hiermit versichere ich, dass ich die vorligende Dissertation selbstuendig und ohne unerlaubte Hilfe angefertigt und keine anderen als die in der Dissertation angegebenen Hilfsmittel benutzt habe. Alle Stellen, die aus veroeffentlichten oder unver oeffentlichten Schriften entnommen sund, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotionsverfahren verwendet worden.)

Witzenhausen, …….

(Mwanaima Rajab Ramadhan)

Preface

This Ph.D. research was conducted under the framework of the Research Training Group 1397 “Regulation of soil organic matter and nutrient turnover in organic agriculture” funded by the German Research Foundation (DFG; subproject D2). The current research focused on possibilities to improve nutrient utilization and reduce nitrogen losses in animal production. The first chapter introduces the thesis and gives the research objectives addressed in the study. Chapters 2, 3 and 4 contain manuscripts prepared for publication in peer-reviewed journals.

Chapter 2:

Ramadhan, M. R., Joergensen, R. G., Mahgoub, O. and Schlecht, E. Feed digestibility, digesta passage rate and faecal microbial biomass in a desert adapted goat breed exposed to mild water restriction.

Chapter 3:

Ramadhan, M. R., Mahgoub, O., Schlecht, E. and Dickhoefer, U. Urinary excretion of purine derivatives, microbial protein synthesis and ruminal fermentation in a desert adapted goat breed exposed to mild water restriction.

Chapter 4:

Ramadhan, M. R., Dickhoefer, U., Appenburg, S., Buerkert, A., and Schlecht, E. Effect of mild water restriction on nitrogen balance, faecal nitrogen forms and nitrogen partitioning between urine and faeces in desert adapted goats.

Chapter 5 contains the general discussion and conclusions.

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List of abbreviations

λ	Passage rate of fibre-bound marker through the rumen
ADFom	Acid detergent fibre - ash free
AES	Animal experimental station
ANOVA	Analysis of variance
BEDN	Bacterial and endogenous debris nitrogen
BUN	Bacterial and undigested nitrogen
C	Carbon
CA	Crude ash
CMRT	Particle mean retention time in the rumen
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
FM	Fresh matter
HPLC	High performance liquid chromatography
LAM	Liquid associated microbes
LW	Live weight
MER	Maintenance energy requirement
MW	Metabolic weight
N	Nitrogen
n.a	Not applicable
n.d	Not determined
NDFom	Neutral detergent fibre – ash free
NH_4-N	Ammonium nitrogen
OM	Organic matter
OPA	*ortho*-phthaldialdehyde

PB	Purine base
PD	Purine derivatives
Per	Period
SAM	Solid associated microbes
SCFA	Short chain fatty acid
SEM	Standard error of the mean
T_{50}	Half time of marker in the rumen
THI	Temperature humidity index
TMRT	Particle mean retention time in the total tract
Trt	Treatment
TT	Time of first marker appearance in faeces
UDN	Undigested dietary nitrogen
WSN	Water soluble nitrogen
Yb	Ytterbium

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Summary

Water is the main challenge facing animal husbandry in arid and semi-arid regions of the globe. These areas are characterized by a long dry season lasting between 6 and 8 months. At certain times during the long dry period, water can be so scarce that animals may face severe water shortages. Many studies have been conducted to highlight how ruminants cope during these times of water scarcity. Yet, livestock are often exposed to mild water shortages most times of the year. Only a few studies have been conducted to investigate the effects of mild water restriction on ruminants. These studies have highlighted the effects of mild water restriction on feed intake and blood parameters. However, the effects of mild water restriction on other physiological processes are yet to be studied. Therefore, the present study aimed at bridging this knowledge gap by investigating whether (a) mild water restriction affects the digestibility, digesta passage and faecal microbial biomass, (b) the rumen microbial yield and turnover are altered by water restriction and (c) nitrogen retention is enhanced and a shift towards faecal nitrogen excretion is achieved. The specific objectives of the study were to:

1) Determine the effects of water restriction on digestibility, digesta passage and faecal microbial biomass (Study 1);

2) Investigate the effects of water restriction on rumen fermentation and microbial composition and yield (Study 2); and

3) Evaluate the effects of water restriction on nitrogen balance and partitioning of excreted nitrogen between urine and faeces (Study 3).

To answer these objectives, two trials were conducted at Sultan Qaboos University, Muscat, Oman, in summer of 2013 (Trial 1) and 2014 (Trial 2). In each trial, a 3 x 3 Latin Square Design was used with the following watering regimes: (a) water offered *ad libitum* (100%), water restricted to (b) 85% and (c) 70% of individual *ad libitum* consumption. Nine adult male Batinah goats were used as the experimental animals. Six animals were intact and three were rumen fistulated in each of the experimental trials. The fistulated animals were used to collect data on rumen fermentation characteristics which are presented in study 2. Rhodes grass hay and barley grains were fed at a ratio of 1:1 at 1.3 times maintenance energy requirement. Trial 1 entailed three periods, each comprising 21 days of adaptation and 6 days of sampling (i.e., experimental period). Trial 2 had three periods with 16 days of adaptation and 8 days of sampling. During the

experimental period, feed offered and refused, urine as well as faeces were quantified and sampled. For all the three studies, feed and faecal samples were analysed for their dry matter (DM), organic matter (OM), nitrogen (N), ash free neutral detergent fibre (NDFom) and ash free acid detergent fibre (ADFom) concentrations using standard procedures. In addition, each study had different measured parameters. For study 1, passage rate was evaluated using an oral pulse dose of ytterbium-labelled Rhodes grass hay. Also, ergosterol and amino sugars concentrations were used to determine the faecal microbial biomass (i.e., bacteria and fungi). For study 2, rumen contents (i.e., fluid and solids) as well as urine samples were additionally collected. Rumen fluid was analysed for concentrations of ammonium-N and short chain fatty acids, whereas, urine was analysed for concentrations of purine derivatives. Rumen solids were used to determine N and purine base concentrations in liquid and solid associated microbes. Lastly in study 3, the N partitioning between urine and faeces was determined based on ^{15}N marked Rhodes grass hay.

Study 1 addressed the question whether mild water restriction improves nutrient utilization and whether it affects the microbial biomass in the hindgut. The results revealed that mild water restriction does not affect feed intake and digesta passage. However, there was an increase in apparent feed digestibility during water restriction but this was not associated with digesta kinetics. While the total faecal microbial mass was not affected by water restriction, the microbial community structure shifted towards fungal C when water was restricted to 70% of *ad libitum* water intake. Hence, mild water restriction seems promising in terms of increasing feed utilization through better digestibility.

Study 2 confirmed that mild water restriction can alter rumen fermentation products. Results from the three fistulated goats, revealed that mild water restriction did not affect feed intake. However, total tract digestibility of ADFom increased when water intake was reduced. Moreover, ammonium-N substantially increased when water was restricted to 70% of *ad libitum* water intake. Also, the proportion of butyrate increased when water was restricted to 70% of *ad libitum* water intake. These phenomena were associated with increases in protozoal counts when water intake decreased. The urinary purine derivatives excretion as well as the duodenal microbial N flow were however not affected by water restriction. Mild water restriction appears to enhance rumen protein

and carbohydrate degradation, which may explain the increased feed digestibility observed.

In study 3, comparisons between the two consecutive years (i.e., the two trials) were made. The results showed that mild water restriction had no effect on feed intake, faecal nitrogen forms, nitrogen balance and partitioning between urine and faeces in both trials. However in trial 2, total tract digestibility of DM, OM, NDFom and ADFom increased when water was restricted to 70% of *ad libitum* water intake. Whereas no differences in total tract digestibility were observed between treatments in trial 1. Mild water restriction did not influence nitrogen partitioning between faeces and urine.

The general discussion summarizes the most important points of study 1, 2 and 3. Firstly, desert adapted goats can easily cope with a mild water restriction without lowering their feed intake. Secondly, mild water restriction slightly improves the digestibility of diets low in nitrogen content, which often prevails in arid and semi-arid areas during the dry season. This improved diet digestibility is explained by an increase in rumen fermentation rather than by prolonged digesta retention in the gastrointestinal tract. Thirdly, when animals are exposed to mild water restriction, fungal microbial biomass is increased in the faeces. Lastly, mild water restriction does not cause a shift in nitrogen excretion between faeces and urine, but increases the concentration of ADFom in faecal matter; this slows down short term nutrient mineralization in the soil, making nutrients plant-available in the longer term.

Zusammenfassung

Die Wasserversorgung ist die größte Herausforderung der Tierhaltung in ariden und semi-ariden Regionen der Welt. Diese Gebiete sind charakterisiert durch eine Trockenzeit von sechs bis acht Monaten. Während dieser langen Trockenperioden kann es zu Wasserknappheit für die Tiere kommen. Viele Studien zeigen, wie Wiederkäuer mit Zeiten starker Wasserlimitierung zurechtkommen. Jedoch sind Nutztiere häufig über große Teile des Jahres lediglich einer milden Wasserrestriktion ausgesetzt. Nur wenige Studien befassen sich mit der Untersuchung von milden Wasserlimitierungen bei Wiederkäuern. Diese Studien heben die Effekte von mildem Wassermangel auf die Futteraufnahme und Blutparameter hervor. Die Wirkung auf andere physiologische Prozesse müssen jedoch noch erforscht werden. Durch die Untersuchung, ob (a) milde Wasserrestriktion die Verdaulichkeit, den Digestadurchgang sowie die mikrobielle Biomasse im Kot beeinflusst, (b) die ruminale mikrobielle Biomasse und Fermentationsparameter verändert und (c) die Stickstoffretention erhöht sowie eine Verschiebung hin zu mehr fäkaler Stickstoffausscheidung erreicht wird, soll die vorliegende Studie dazu beitragen, diese Wissenslücken zu schließen. Die konkreten Ziele dieser Studie waren:

1) Die Bestimmung der Effekte von Wasserrestriktion auf die Verdaulichkeit, die Digestapassage sowie die fäkale mikrobielle Biomasse (Studie 1);

2) Die Untersuchung der Effekte von Wasserrestriktion auf die Pansenfermentation und die mikrobielle Proteinsynthese im Pansen (Studie 2);

3) Die Evaluierung der Effekte von Wasserrestriktion auf die Stickstoffbilanz und die Partitionierung des ausgeschiedenen Stickstoffs in Urin und Kot (Studie 3).

Um diese Ziele zu bearbeiten, wurden zwei Experimente im Sommer 2013 (Versuch 1) und 2014 (Versuch 2) an der Sultan Qaboos Universität in Muscat, Oman, durchgeführt. In beiden Versuchen wurde ein 3 x 3 Latin Square Design mit folgenden Behandlungen angewendet: (a) Wasserversorgung *ad libitum* (100%), eingeschränkte Wasserversorgung auf (b) 85% sowie (c) 70% des individuellen *ad libitum*-Verbrauchs. Die beiden Experimente wurden mit je neun ausgewachsenen männlichen Batinah-Ziegen durchgeführt, von denen jeweils drei eine pansenfistel hatten waren. Die fistulierten Tiere dienten der Erhebung von Daten zu Parametern der Pansenfermentation. Die Futterration bestand aus Rhodesgras-Heu und Gerste im

Verhältnis 1:1 und deckten den 1,3-fachen Erhaltungsenergiebedarf. Das Experiment 1 bestand aus drei Perioden, welche jeweils 21 Tage Adaption und 6 Tage Beprobung (experimentelle Phase) beinhalteten. Das Experiment 2 setzte sich ebenfalls aus drei Perioden, jedoch mit 16 Tagen Adaption und 8 Tagen Beprobung, zusammen. Während der experimentellen Phase wurde das angebotene Futter sowie Futterreste, Urin und Kot quantifiziert und beprobt. Für alle drei Studien wurden die Konzentrationen an Trockenmasse (TM), organische Masse (OM), Stickstoff (N), Asche-freier neutraler Detergenzfaser (NDFom) und die Asche-freie saure Detergenzfaser (ADFom) in Futter- und Kotproben mittels Standardmethoden gemessen. Zusätzlich wurden in jeder Studie weitere Parameter erhoben. In Studie 1 wurde durch Gabe einer oralen Pulsdosis an Ytterbium-markierten Rhodesgras-Heu die gastrointestinale Passage von Futterpartikeln ermittelt sowie Ergsterol- und Aminozuckerkonzentrationen im Kot gemessen, um die mikrobielle Biomasse (Bakterien und Pilze) im Kot zu bestimmen. Für Studie 2 wurden von den fistulierten Tieren der Proben von Panseninhalt (Flüssigkeit und Feststoffe) und Urin einbehalten. In der Pansenflüssigkeit wurden Ammonium-N und kurzkettige Fettsäuren analysiert, während im Urin Purinderivate gemessen wurden. In den Digestaproben wurden ausserdem Stickstoff und Purinbasen der mikrobiellen Biomasse von Flüssigkeit und Feststoffen. In Studie 3 wurde mittels ^{15}N-markiertem Rhodesgras-Heu die Partitionierung der Stickstoffausscheidung in Urin und Kot ermittelt, und die Gesamt N-Bilanz errechnet.

In Studie 1 wurde die Frage, ob eine milde Wasserrestriktion die Nährstoffnutzung verbessert und die mikrobielle Biomasse im hinteren Dünndarm beeinflusst wird, addressiert. Die Ergebnisse zeigten, dass eine milde Einschränkung der Wasserversorgung die Futteraufnahme sowie die Digestapassage nicht beeinflussten. Jedoch war die scheinbare Futterverdaulichkeit bei Wasserlimitierung erhöht, wobei dies nicht mit einer Veränderung in der Digestakinetik einherging. Obwohl die Gesamtbiomasse an fäkalen Mikroorganismen nicht durch eine eingeschränkte Wasserversorgung beeinflusst wurde, war bei einer Wasseraufnahme von 70% der *ad libitum* Versorgung die zusammensetzung der mikrobiellen Masse hin zu pilzlichem Kohlenstoff verschoben. Aufgrund der Ergebnisse erscheint eine milde Einschränkung der Wasserversorgung vielversprechend im Hinblick auf eine erhöhte Futternutzung durch eine verbesserte Verdaulichkeit.

Studie 2 bestätigte, dass milde Wasserrestriktion die Fermentationsprodukte im Pansen verändern kann. Die Ergebnisse zeigten, dass eine milde Einschränkung der Wasserversorgung die Futteraufnahme nicht beeinträchtigte. Die Gesamtverdaulichkeit von ADFom erhöhte sich jedoch bei reduzierter Wasseraufnahme. Zudem nahm im Pansen die Ammonium-N-Konzentration bei einer 70%igen Wasserversorgung deutlich zu, wie auch die Konzentration an Butyrat. Diese Veränderungen gingen mit einer Vermehrung der Protozoenzahl bei abnehmender Wasseraufnahme einher. Dagegen wurde die Konzentration an Purinderivaten im Urin und damit die mikrobielle proteinsynthese nicht durch die Wasserrestriktion beeinträchtigt. Es scheint, dass eine milde Wasserlimitierung den ruminalen Abbau von Proteinen und Kohlenhydraten erhöht, wodurch die verbesserte Futterverdaulichkeit erklärt werden kann.

In Studie 3 wurden die Ergebnisse der beiden aufeinanderfolgenden Jahre (die beiden Versuche) miteinander verglichen. Die Ergebnisse zeigten, dass eine milde Restriktion der Wasserversorgung in beiden Experimenten keinen Einfluss auf die Futteraufnahme, die fäkalen Stickstoffformen, die Stickstoffbilanz und die Partitionierung der Stickstoffausscheidung in Urin und Kot hatte. In Experiment 2 war jedoch die Gesamtverdaulichkeit von TM, OM, NDFom und ADFom bei einer 70%-igen Wasserversorgung erhöht, in Experiment 1 wurden keine Unterschiede in der Gesamtverdaulichkeit zwischen den Behandlungen beobachtet. Milde Wasserrestriktion scheint die Stickstoffpartitionierung in Urin und Kot nicht zu beeinflussen.

Die allgemeine Diskussion fasst die wichtigsten Punkte aus den Studien 1, 2 und 3 zusammen. Zum Einen können an Wüsten angepasste Ziegenrassen sehr gut mit einer milden Wasserlimitierung zurechtkommen, ohne ihre Futteraufnahme zu reduzieren. Zum Zweiten verbessert eine milde Wasserrestriktion leicht die Verdaulichkeit von Futter mit niedrigem Stickstoffgehalt, welches in ariden und semi-ariden Gebieten während der Trockenzeit oft überwiegt. Dabei kann die verbesserte Verdaulichkeit des Futters durch eine erhöhte Pansenfermentation und nicht durch eine verlängerte Verweildauer des Digesta im Magen-Darm-Trakt erklärt werden. Zum Dritten erhöht sich die pilzliche Biomasse im Kot, wenn Tiere einer milden Wasserlimitierung ausgesetzt sind. Letztendlich verursachte eine milde Wasserlimitierung keine Verschiebung der Stickstoffausscheidung zwischen Kot und Urin, führt aber zu einer erhöhten Konzentration von ADFom im Kot; dies verlangsamt die kurzfritige

Nährstoffmineralisation im Boden wodurch Nährstoffe längfristig pflanzenverfügbar werden.

Chapter 1

General introduction

1.1 Background

Water is the main challenge facing agriculture in arid and semi-arid regions of the globe (NRC, 2007). In the wake of climate change, precipitation is fluctuating and rainfall is becoming more erratic as well as unpredictable and thus water availability more limited (Jaber et al., 2013). Therefore it is important to make wise use of the water that is available while aiming at maximum efficiency.

Another key challenge facing livestock and crop production is to recycle nutrients, particularly nitrogen (N) and phosphorus (P), from animal manure back to farmland where they can be used for crop production (Satter et al., 2002). To achieve this, knowledge of the whole production system and its components (soil, crops, animals, feeding, housing and manure management) is important for an effective balance between nutrient supply and requirements in both animal and crop production (Børsting et al., 2003). This will enable the farmer to improve nutrient efficiency and minimize nutrient losses (Halberg, 1999).

In farms, nitrogen is lost to the environment through gaseous emissions of ammonia, nitrous oxide, and the leaching of nitrate to groundwater through soil (Steinfeld et al., 2006). Ammonia is regarded as a major precursor for the formation of atmospheric fine particulates affecting human health (NRC, 2003), contributing to eutrophication of ecosystems, acidification of soils and nitrous oxide formation, which contributes to the greenhouse effect (Sutton et al., 2008). More scientific attention has been given to nitrogen oxide (NO_x) emissions than to NH_x (ammonia and ammonium; Galloway, 1998). Ironically, NH_x deposition dominates over NO_x (Sutton et al., 2008) as NH_x may eventually lead to the formation of NO_x. As much as 23 million tonnes ammonia-N is emitted each year from livestock husbandry globally (Steinfeld et al., 2006). Thus, NH_3 losses should be controlled to minimize environmental impacts.

Over the years, there has been an increase in regulations to minimize the environmental impact of N from agricultural systems. To achieve this goal, extensive research has been done on the different parts of the farm to reduce N losses through soil, crops, feed (Rotz et al., 1999; Satter et al., 2002) and manure management (Jokela and Meisinger, 2008). More recently, the central role of animal husbandry in reducing environmental problems is receiving recognition. Among others, the amount as well as the route of N excretion can have a significant environmental impact (Satter et al., 2002). Studies have shown

that manipulating animals' feed quality influences the amount of N excreted as well as the partitioning of excreted N in urine and faeces (Al-Asfoor et al., 2012; Al-Kindi et al., 2015; Rotz et al., 1999). Because urea readily breaks down to ammonia, decreasing urine-N excretion will eventually lead to a decline in ammonia emissions (Børsting et al., 2003). Nitrogen contained in faeces is more stable and less likely to be volatilized than urine-N, therefore shifting the N excretion towards faeces is one efficient way of reducing ammonia emissions (Korevaar, 1992).

In arid and semi-arid regions, feed resources are limited both in terms of quality and quantity; moreover, water supply is often limited due to erratic rainfall occurring in these areas (Jaber et al., 2013). Short term water shortage has been observed to be beneficial to ruminants in terms of feed digestion and utilization (Misra and Singh, 2002). Furthermore, ruminants excrete less faeces and urine when exposed to water shortage (Qinisa, 2010). As a consequence, loss of N to the environment may be reduced.

These interdependencies between feed quality, water intake and excreta quantity and quality formed the basis of the current study that analyses nutrient digestion and utilization in a desert adapted goat breed exposed to mild water restriction, with particular emphasis on N metabolism and N losses via excreta. Goats were chosen for the experiment because they are the main animals kept in arid and semi-arid environments and are known for their superior ability to adapt to water shortages as will be detailed below.

The study was conducted within the framework of the Research Training Group 1397 "Regulation of soil organic matter and nutrient turnover in organic agriculture" funded by Deutsche Forschungsgemeinschaft (DFG; http://www.uni-kassel.de/fb11agrar/de/fachgebiete -einrichtungen/graduiertenkolleg/graduiertenkolleg-1397.html). The overall research project comprised three cohorts, with this study (RTG 1397-cohort 3-D2) being part of the third cohort.

1.2 Goats

1.2.1 System of production

Goats are found in all livestock production systems be it pastoral, mixed farming or commercial systems (Lebbie, 2004; Peacock, 2005). However, the vast majority of goats are kept in extensive/semi-extensive low-input systems (Steinfeld et al., 2006), especially in rural households of low income countries (Morand-Fehr et al., 2004). This is because goats have a small body size, can walk relatively long distances, and utilize a wide range of feed resources of highly varying quality (Silanikove, 2000). They also reproduce quickly, have a short generation interval (Peacock, 2005) and are economically viable (Bosman et al., 1997). Moreover, they are efficient utilizers of marginal lands (Alexandre and Mandonnet, 2005) and can be herded by younger and older members of the family. When slaughtered their smaller carcasses are conveniently marketed or consumed over a short time - a vital factor in rural areas without cold storage facilities (Lebbie, 2004).

1.2.2 Adaptive traits

Goats are an integral part of farming systems in almost all agro-ecological zones. This is mainly attributed to their wide adaptability to an array of climates and the ability to cope with extreme environmental conditions and stress (Hassan, 1989). Of particular interest here are the mechanisms that have led to the adaptation of goats to arid and semi-arid environments. These areas are characterized by water scarcity, high solar radiation, low quality feed and erratic precipitation. The effect of global warming has worsened the situation in these areas, as rainfall is becoming even more irregular and water availability more limited (Steinfeld et al., 2006). Hence, goats are at last claiming their value as species worthy of serious investment under an increasingly challenging environment.

Behavioural adaptations

Goats exhibit behavioural traits that enable them to survive in harsh environments. It has been documented that desert breeds opt for nocturnal feeding in order to avoid high temperatures during the day (Kay, 1997). Moreover, desert goats have been reported to feed frequently, but eat smaller meals so as to reduce heat production linked to rumen fermentation (Morand-Fehr, 2005). Also, the timing of reproduction is a distinct trait

that desert goats demonstrate to ensure survival of offspring. Kidding mostly occurs when food and climate are more favourable for the new-borns, and usually takes place between February and April, while breeding spans from June to November in the northern hemisphere (Amoah et al., 1996).

Morpho-physiological adaptations

Morphologically, desert adapted goats have developed traits that enable them survive the hot arid environments. Their larger surface area (small body) allows them to better dissipate heat to the environment thus reducing heat load (Jaber et al., 2013). Also, desert breeds have a long narrow body shape which offers a small body surface to the overhead midday sun, hence the animals avoid some of the short-wave solar radiation (Kay, 1997). In addition, desert adapted goats have thin long legs which increase the distance between the female's udder and the ground. This ensures that they absorb less long wave radiation emitted from the ground, thereby reducing heat stress. Moreover, desert breeds have long fleece of hair with an array of bright colours (Zaibet et al., 2004). The fleece insulates the animal by trapping air (creation of a microclimate) and thus enhances thermal stability. Concurrently, the bright colours of their hair reflect solar radiation and absorb less heat (Kay, 1997). This helps to maintain body temperature without directly resorting to evaporative cooling that may lead to excessive water loss (Jaber et al., 2013). Furthermore, desert adapted goats have relatively large ears compared with their non-desert counterparts (Zaibet et al., 2004). The ears function as heat dissipaters to reduce heat stress (Jaber et al., 2013; Kay, 1997).

Physiologically, goats can exhibit adaptive mechanisms that enable them to cope with heat, water shortage and low quality forage. Desert goats are reported to excrete concentrated urine (Qinisa et al., 2011) and faeces (Igbokwe, 1997) as a mode of conserving water. At the initial stages of dehydration, antidiuretic hormone is produced and is responsible for the reabsorption of water from the renal tubules. The production of concentrated urine is linked to the length of the Henlé loops located in the medulla of the kidney (Zervanos, 2002). Desert breeds have longer loops and as a consequence, they produce highly concentrated urine of over 3000 milli-osmol per litre of water without disrupting their homeostasis (Kay, 1997).

Goats' morphology and physiology also enables them to efficiently utilize the available feed resources. They are opportunistic foragers and therefore are able to maintain a high

quality diet across a wide array of forages on offer (Erasmus, 2000; Ramirez, 1999). In addition to tree and shrub leaves, goats often select buds, fruits and flowers which contain less fibre and more protein than grasses (Lu, 1988; Sanon et al., 2007). This selective nature is aided by their mouth idiosyncrasy. They have a narrow muzzle compared to cattle and sheep, hence can efficiently select their diet (van Soest, 1994). Furthermore, goats have a mobile upper lip which enables them to graze as close to the ground as sheep (Lu, 1988; Sanon et al., 2007). Also very helpful is the bipedal stance they employ to pull down branches permitting them to browse horizons of up to 2 m (Sanon et al., 2007; van Soest, 1994). These features enable goats to shift between grazing and browsing hence expand the foraging environment. Yet, their feed resources are often characterised by high-fibre (high cell wall and lignin), low protein and high tannin contents (Silanikove, 2000). Desert adapted goats have been reported to cope well with high fibre forages. This is attributed to the increased mean retention time of feed in their digestive tract and the ability to maintain a high microbial density on the particulate matter (Silanikove et al., 1993).

Lignin and tannins are formed by plants to provide protection against environmental stress. While they are an asset to plants, these compounds have negative effects on the palatability and digestibility of feed (van Soest, 1994). Lignin limits cell wall carbohydrate digestibility rendering it unavailable to herbivores (Givens et al., 2000). Even though lignin is believed to be largely indigestible (Givens et al., 2000), some reports suggest that lignin can be utilized by desert goats after extensive modification, degradation and absorption in the digestive tract (Silanikove, 2000; Silanikove and Brosh, 1989). This owes to the above-mentioned prolonged mean retention time of digesta in the rumen (Brosh et al., 1986a), which enhances the microbial fermentation and release of structural carbohydrate and hemicellulose (Silanikove and Brosh, 1989).

Tannins on the other hand form complexes with proteins in the saliva or in the rumen (Min et al., 2003). Consequently, these complexes by-pass rumen fermentation and become available only in the lower digestive tract. Much of the protein may be undigested therefore, and excreted without being utilized by the animal (Komolong et al., 2001). Desert goats have been found to consume large amounts of tannin-rich browse without exhibiting toxicity. This is related to their ability to neutralize the negative effects of tannins by producing more protein-rich saliva when exposed to tannins (Silanikove et al., 1996). These saliva proteins have a higher affinity to tannins

than proteins in the feed, which therefore are not complexed and can be digested in the rumen. Goats also produce microbial tannase in their rumen mucosa that is effective in neutralizing tannic acid toxicosis (Silanikove et al., 1996).

In addition, desert goats often feed on forages with a low protein content. In these cases, desert goats optimize their N metabolism by reducing the amount of urea voided in urine. To efficiently reduce urea excretion, the kidney retains the urea formed in the liver. Subsequently, the urea is recycled to the rumen via saliva and blood (Harmeyer and Martens, 1980). In the rumen, the recycled urea is then used by the microbial population to synthesise microbial protein which is digested and can then be utilized by the animal. This adaptive strategy helps desert breeds to maintain a balanced N economy (Silanikove, 2000), thus they can thrive in areas where cattle and sheep cannot. The multiple adaptive capacities and efficient resource utilization of goats therefore makes them attention deserving in terms of both rearing and research.

1.3 Water requirements of goats

Water is the most abundant molecule in all living cells and functions as a solvent for numerous compounds (NRC, 2007). It is virtually involved in all physiological functions of animals including body temperature regulation, digestion, absorption of digested nutrients, transport of metabolites, excretion of metabolic wastes as well as production and maintenance of blood volume (Wilson and Brigstocke, 1981). Animals are more sensitive to water deprivation than feed restriction in that 20% loss of body water results in death, whereas animals can still survive after 40% loss of their dry body weight when starved (Bondi, 1987).

In practice, ruminants and specifically goats may experience a negative water balance depending on the watering regimes. In arid areas, watering places are located far from homesteads hence livestock may not be watered daily. Desert goats have been reported to cope with up to four days without drinking water (Silanikove, 2000). Other reports suggest that goats can survive up to 12 days without drinking water (Igbokwe, 1997). Thereby the rumen plays a vital role in maintaining homeostasis when goats are dehydrated (Silanikove, 1994), since it acts as a temporary reservoir, holding water until physiological pools become rehydrated (NRC, 2007; Silanikove, 1994).

Goats obtain their water from three sources: drinking water, food water (preformed water) and metabolic water (Kay, 1997; NRC, 2007). The water requirement for

maintenance of goats in hot climates ranges between 3 kg/kg dry matter intake (DMI) at 23°C to 5 kg/kg DMI at 35°C (Giger-Reverdin and Gihad, 1991). This can be largely met by drinking water but during dry spells preformed and metabolic water may be more important. Grass contains up to 80% water in the wet season and up to 10% during the dry season (Kay, 1997). During wet seasons, goats may obtain their water needs from moist forage hence seek less drinking water. Furthermore, dew on leaves moisten grass and shrubs (Hanisch et al., 2015) and may be beneficial for animals feeding at night or in the early morning. When carbohydrates and fat are oxidized, carbon dioxide and water is formed, thereby contributing to the animals' metabolic water resource. Roughage diets with a digestibility of 55% yield 550 g of metabolic water per kg dry matter digested, which is contributing to about 10-20% of the total water requirement in water conserving species (Kay, 1997).

Water losses by excretion and evaporation also play a key role in the maintenance of the water balance. When goats consume forage rich in fibre, they lose around 60-70% of water through faeces suggesting that faecal excretion can be a major route of water loss (Kay, 1997). In addition, water losses due to urinary excretion as well as sweating and panting may occur when the animal is exposed to heat (Qinisa, 2010). Sweating and panting are sometimes inevitable thus, besides water storage and conservation, tolerance to dehydration is vital for desert breeds.

1.4 Effects of water restriction

Although the role of water in ruminants has been studied since the First World War (Larsen et al., 1917), studies on the effects of water restriction gained momentum only after the Second World War. By the beginning of the 21st Century, different physiological aspects of water restriction and deprivation were extensively studied. These studies focused on the effects of water scarcity especially on feed intake, nutrient utilization, body weight, blood parameters and general animal performance.

Water restriction has been reported to decrease feed intake due to the reduction in the volumetric and osmotic stimuli as the animal adjusts to reduce its water needs (Igbokwe, 1997). It has been established that voluntary feed intake decreases following an increase in water restriction (Abdelatif and Ahmed, 1994; Abioja et al., 2010; Alamer, 2006). The reduction in feed intake (hypophagia) is due to the postprandial increase in the osmolality of the ruminal fluid (Igbokwe, 1997). Water restriction causes

a high substrate concentration in the plasma which continuously stimulates the brain satiety centre and thus reduces feed intake (Igbokwe, 1997).

The reduction in feed intake is partly compensated for by an increase in feed utilization. Water restriction has been reported to increase feed utilization by enhancing digestibility of nutrients (Ahmed and El Shafei, 2001; Ghassemi et al., 2014; Silanikove, 1985). This is due to a slower feed movement through the digestive tract (Jaber et al., 2013), which in turn ensures that more time is available for the microbial community in the gastrointestinal tract to act on the feed (Asplund and Pfandes, 1972; Jaber et al., 2013). Although feed intake influences digestibility (Igbokwe, 1997), it is not regarded as a factor responsible *per se* for the increase in mean retention time of particulate matter (Silanikove, 1992). Factors that lead to the increase in digestibility include decrease in saliva production and flow rate, decrease in feed intake, rumen motility, rumination activity and rate of passage of digesta as well as changes in conditions of the rumen (Igbokwe, 1997).

A physiological consequence of water restriction as well as of the reduced feed intake is weight loss (Ahmed and El Shafei, 2001; Alamer, 2006; Alamer and Al-hozab, 2004). Losses of up to 21% of body weight following three days of water deprivation have been reported from Saudi Arabian goats (Alamer, 2006). Evidence shows that part of the reduction in body weight is due to body water losses (Alamer, 2006; Jaber et al., 2004). The other part is caused by losses in body solids such as mobilization of fat (and in extreme cases muscle) that is used for energy metabolism during water restriction (Jaber et al., 2004). Furthermore, water restriction has been observed to lead to more weight loss as compared to feed restriction (Muna and Ammar, 2001), owing to the ripple effects of water restriction on energy intake and fat metabolism (Jaber et al., 2013).

During water restriction, blood parameters are reported to be elevated (Aganga et al., 1989). The increase in plasma osmolality following water restriction is associated with hemoconcentration due to losses of body fluid (Abdelatif and Ahmed, 1994). As a consequence, the increase in plasma osmolality causes a rise in plasma sodium contributing to the maintenance of plasma volume by inducing water movement into the vascular system (Alamer, 2006). Blood cholesterol and glucose concentrations also increase when ruminants are subjected to water restriction (Casamassima et al., 2008; Jaber et al., 2004; Kaliber et al., 2015). This is related to the decline in feed intake as the

ruminants activate fat mobilization to meet energy demands (Abdelatif and Ahmed, 1994).

Plasma urea and protein concentrations increase under water restriction (Igbokwe, 1993). The elevation in plasma protein could be attributed to loss of water when the animal is dehydrated, causing hemoconcentration as a result of lower blood water level (Casamassima et al., 2008; Jaber et al., 2004). Conversely, the increase in plasma urea is due to the immense water uptake by the kidney as well as a decrease in renal blood flow causing a reduction in glomerular filtration rate (Casamassima et al., 2008). Furthermore, urea recycling is expected to increase with water restriction thus leading to an increase in plasma urea (Jaber et al., 2013).

Water restriction has been shown to decrease milk yield in goats (Mengistu et al., 2007). However, milk production similar to free-watered animals has been reported for Bedouin goats watered every second day (Maltz and Shkolnik, 1984). This is believed to be a result of the improved digestion and retention time of food caused by water restriction (Casamassima et al., 2008). It is suggested that the general reduction in milk yield is partly due to reduced feed intake, increased water conservation (Forbes, 2007) and a decrease in blood flow to the mammary glands leading to a drop in milk volume (Dahlborn et al., 1997).

1.5 Study objectives and research hypotheses

Arid and semi-arid regions are characterized by a long dry season lasting between 6 and 8 months. At certain times during the long dry period, water can be so scarce that animals may face severe water shortages (Jaber et al., 2013). Many studies have been conducted to highlight how ruminants adapt to severe water shortages (Igbokwe, 1997; Jaber et al., 2013; Silanikove, 1992, 2000). Yet, livestock are much more often exposed to mild drinking water shortage – in arid and semi-arid regions often for a long time per year. However, only few studies have been conducted to investigate the effects of mild water restriction on ruminants (Casamassima et al., 2008; Hadjigeorgiou et al., 2000) - these are summarized in figure 1. However, the effects of a mild water restriction on rumen fermentation parameters, the composition of the microbial community in the hindgut and on the partitioning of excreted nitrogen between faeces and urine is unknown. Thus, the research objectives of the present study were to:

1) Determine the effects of mild water restriction on feed digestibility, particulate digesta passage and faecal microbial biomass (Chapter 2).
2) Investigate the effects of mild water restriction on rumen fermentation parameters, microbial composition and yield (Chapter 3).
3) Evaluate the effects of mild water restriction on nitrogen balance and partitioning of excreted nitrogen between urine and faeces (Chapter 4).

Based on these objectives, it was hypothesized that mild water restriction will:

1) Increase feed digestibility, mean retention time of digesta in the gastrointestinal tract and faecal microbial biomass.
2) Increase rumen fermentation characteristics as well as microbial yield and composition.
3) Lead to a positive nitrogen balance and increase faecal nitrogen excretion.

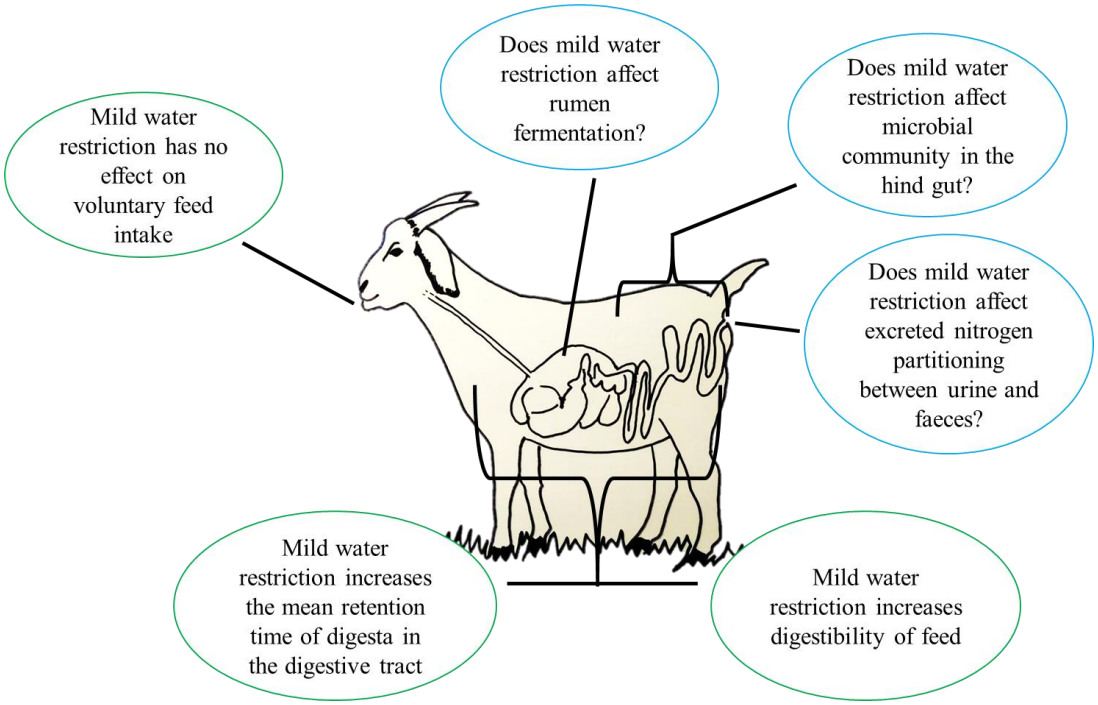

Figure 1: Chart demonstrating the digestive tract of the goat and the known and unknown effects of mild water restriction in ruminants. Circles in green represent known effects while circles in blue indicate the research questions. Sketch by eS, 03.02.2017.

1.6 Thesis outline

To achieve the main objectives of this study, feed, rumen contents, faeces and urine samples were collected in two different trials (summer 2013 and 2014) at Sultan Qaboos University, Muscat, Oman. In Chapter 2, the results of the study on feed digestibility and digesta passage are presented (Figure 2), whereas Chapter 3 analyses the effect of mild water restriction on rumen microbes and discusses the impact of water intake on rumen fermentation. Consequently, Chapter 3 gives a more detailed insight (physiological explanations) on what happens in the animal when exposed to mild water restriction. Chapter 4 evaluates the animals' N-balance, the faecal microbial components and the partitioning of N between urine and faeces, thereby, presenting possibilities of reducing environmental N losses. Results and insights gained in Chapters 2, 3, and 4 as well as the implications with respect to overall N efficiency are discussed in Chapter 5. Finally, conclusions are drawn on how a mild water restriction can be beneficial for N cycling with regards to ruminant nutrition as well as the environment.

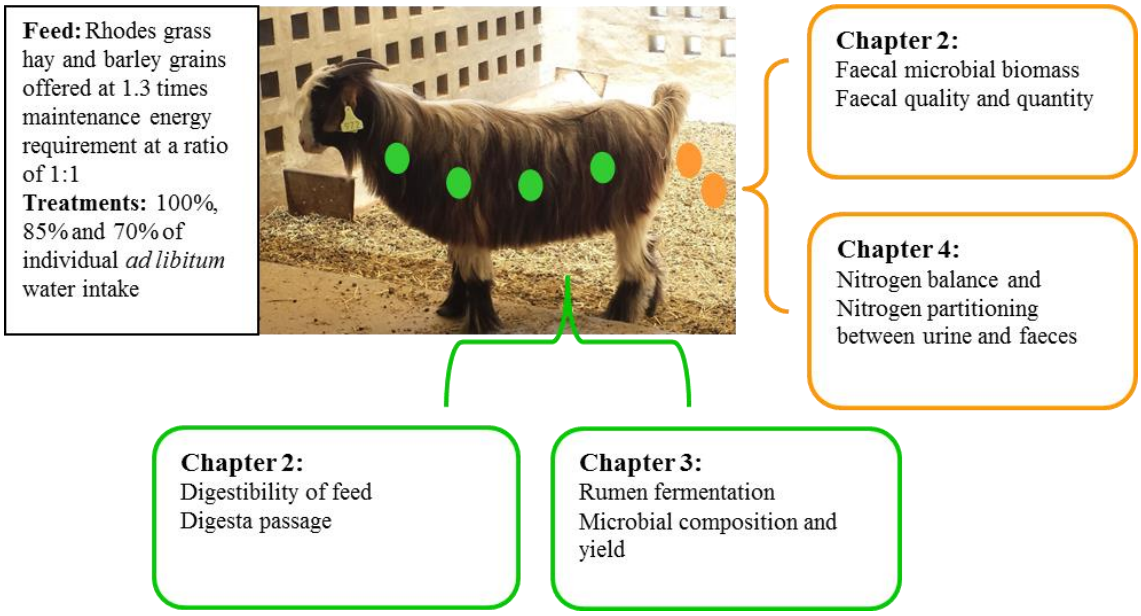

Figure 2: Schematic overview of the thesis structure with reference to the goat. The colours of the boxes represent the main foci of the different chapters. Green colour represents feed, while brown colour represents faeces.

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Chapter 2

Feed digestibility, digesta passage and faecal microbial biomass in a desert adapted goat breed exposed to mild water restriction

Feed digestibility, digesta passage and faecal microbial biomass in a desert adapted goat breed exposed to mild water restriction

Abstract

In arid and semi-arid environments, animals are prone to experience water shortage given that water is often scarce. The present study therefore investigated the effects of no or mild water restriction on feed intake, feed digestibility, passage of digesta particles and the composition of faeces including faecal microbial biomass. A feeding trial was conducted at Sultan Qaboos University, Muscat, Oman, during the dry summer of August to October 2014. Nine adult male Batinah goats were subjected to three watering regimes in a 3 x 3 Latin Square design. The three treatments were: 1) water offered *ad libitum* (100%, W100); 2) water restricted to 85% of individual *ad libitum* consumption (W85); and 3) water restricted to 70% of individual *ad libitum* consumption (W70). The trial entailed three periods, each comprising 16 days of adaptation and 8 days of sampling. During the experimental periods, feed offered and refused as well as faeces were quantified and sampled. Passage rate was determined using an oral pulse dose of ytterbium-labelled Rhodes grass hay; ergosterol and amino sugars were used as markers for faecal microbial biomass that is, fungi and bacteria, respectively. Water restriction had no significant effect on feed intake and the parameters of gastrointestinal passage of feed particles. However, feed dry matter, organic matter and fibre digestibility increased ($p < 0.05$) at W70 as compared to W85. Furthermore, the amount of faecal dry matter, organic matter, nitrogen and neutral detergent fibre excretion decreased ($p < 0.05$) in W70 compared to W85, whereas faecal concentration of acid detergent fibre increased in W70 compared to W85. Even though water restriction did not significantly affect total faecal microbial C concentrations, fungal C concentrations increased ($p < 0.05$) in W70 compared to W85. Therefore, mild water restriction seems advantageous from a physiological and nutrient utilization perspective as it increases feed digestibility and fungal microbial biomass without negatively affecting feed intake.

Keywords: Amino sugars; Batinah goats; mean retention time; ergosterol; faecal microbial composition; water restriction.

2.1 Introduction

Globally, goats constitute the highest share of mammalian livestock numbers in arid and semi-arid environments (Haan et al., 1997). This reflects their adaptation to harsh dryland conditions and is the reason for the animal to be considered as hardy (Silanikove, 2000). Previous studies have shown that compared to the other ruminant livestock species, goats were least affected by successive drought events (Khan et al., 1979; Lu, 1988; Silanikove, 2000). Although goats can survive up to a week with little or no water (Igbokwe, 1997), prolonged water deficiency affects its physiological homeostasis leading to a loss of body weight, low reproductive rates and a decreased resistance to diseases (Jaber et al., 2013).

Many studies have been conducted to highlight the physiological processes that enable goats and other small ruminants to tolerate prolonged and severe water shortage (Hamadeh et al., 2006; Jaber et al., 2004; Jaber et al., 2013; Mengistu et al., 2007). Water shortage has been found to decrease voluntary feed intake (Abdelatif and Ahmed, 1994; Alamer, 2006), enhance feed digestibility (Burgos et al., 2001), increase the mean retention time of feed in the digestive tract (Brosh et al., 1986a; Hadjigeorgiou et al., 2000) and enhance nutrient utilization since more time is made available for the microbes in the gastro intestinal tract (GIT) to act on the feed (Ahmed and El Shafei, 2001).

Much attention has been paid to the effects of severe water shortage on ruminants (Brosh et al., 1986a; Jaber et al., 2013; Silanikove, 2000). However, livestock are exposed to severe water shortage during harsh summer periods (where animals may face several days without water), whereas during the other times of the year ruminants may be watered once a day or on alternate days and are more likely to face mild water shortages. Few studies have been conducted to determine the effects of mild water restriction in ruminants (Casamassima et al., 2008; Hadjigeorgiou et al., 2000). Yet, these studies focused on feed intake and animal performance and were conducted when the environmental temperatures were low.

Against this background, this study aimed to determine the effect of mild water restriction on the digestibility of feed and its proximate constituents, the digesta passage rate and the composition of the faecal microbial biomass during summer in desert adapted goat breeds. We hypothesized that: (1) feed digestibility will increase due to an

increase in the mean retention time of digesta in the gastrointestinal tract when water restriction increases, and (2) faecal microbial biomass will increase when water is restricted.

Faecal quality can be characterised microbially by determining the microbial biomass using amino sugars and ergosterol methods. Amino sugars occur in the cell membrane of fungi and bacteria and have been used as indicators for the presence of microbial residues in soil (Joergensen et al., 2010), roots (Appuhn et al., 2004) and more recently in faeces (Al-Kindi et al., 2015; Jost et al., 2011; Jost et al., 2013). Out of the 26 amino sugars that have been found in micro-organisms (Appuhn and Joergensen, 2006; Sharon, 1965), only muramic acid, glucosamine, galactosamine and mannosamine have been quantified in faeces (Al-Kindi et al., 2015; Jost et al., 2013). Muramic acid is present exclusively in bacterial cell walls (Kortemaa et al., 1997). Fungi are the major source of glucosamine (Appuhn and Joergensen, 2006), although bacteria also contain glucosamine in their peptidoglycan cell wall (Amelung, 2001). Galactosamine and mannosamine are found both in fungal and bacterial cells (Indorf et al., 2011). Ergosterol is an important constituent of fungal cell membrane and has been successfully used as an index for fungal biomass in faeces (Jost et al., 2011).

2.2 Materials and methods

2.2.1 Experimental design

A trial was conducted during the dry summer period (August-October 2014) at the Animal Experimental Station of the Department of Animal and Veterinary Sciences, Sultan Qaboos University, Muscat, Oman. Nine adult male Batinah goats of similar age (13 months) and body weight (25.5 $\pm$ 5.3 kg) were used as experimental animals. The average daily temperature, relative humidity and the temperature humidity index (THI) during the three experimental periods were 31.3°C, 63.5% and 81.9, respectively, with no rainfall occurrence (Table 1).

Table 1: Meteorological data as measured at Sultan Qaboos University, Muscat, Oman during the three experimental periods.

	Experimental periods in 2014		
Parameter	1 (13th-20th Aug)	2 (6th-13th Sep)	3 (30thSep-7th Oct)
Maximum ambient daily temperature (°C)	34.8	35.5	37.2
Minimum ambient daily temperature (°C)	26.7	27.1	24.8
Mean ambient daily temperature (°C)	30.9	31.0	31.3
Relative air humidity (%)	70.5	70.1	50.0
Temperature humidity index*	82.9	82.8	80.0

*The temperature humidity index was calculated using the equation of NRC (1971):
THI = (1.8 * T°C + 32) – [(0.55 – 0.0055 * RH %) * (1.8 * T°C -26)]
where T°C is the average daily air temperature and RH is the relative humidity.

A pre-trial was conducted one month before the commencement of the experiment to determine the *ad libitum* water consumption for the individual animals. Animals were fed at a ratio of 1:1 with concentrate and roughage, respectively and given 4 litres of drinking water in two portions at 08:00 h and 16:00 h for a week. Water and feed refused per animal and day were measured and recorded. Thereafter, the average water intake (ml) per day was calculated and the water intake per unit dry matter intake (ml g^{-1} DMI) was calculated for each animal.

The experiment was subsequently conducted as a complete Latin Square (3 x 3) with the following regimes for the provision of drinking water (treatments): 1) water offered *ad libitum* (100%; treatment W100); 2) water restricted to 85% of individual *ad libitum* consumption (W85); and 3) water restricted to 70% of individual *ad libitum* consumption (W70). Water was offered in two equal portions (at 08:30 h and 16:30 h, each time roughly 30 minutes after start of feeding). The trial entailed three periods, each comprising of 16 days of adaptation and 8 days of experiment. During adaptation, the animals were individually housed in paddocks of ca. 2.25 m² within a large roofed stable with open sides. During the experimental periods, the goats were kept in individual metabolic crates designed to ease collection of urine and feed samples. In addition, faecal bags were used during the sampling period for the collection of faeces. All animals were weighed before morning feeding on two consecutive days before and after each experimental period. Weighing was done using a scale of 0.1 kg accuracy. Animal care and use were in accordance with the country regulations.

2.2.2 Feed and feeding

Two types of feed were used, namely whole barley (*Hordeum vulgare* L.) grains and Rhodes grass (*Chloris gayana* Kunth.) hay at a 1:1 ratio (on dry matter basis). Animals were fed at 1.3 times individual maintenance energy requirement according to NRC feeding standards (NRC, 2007). Before commencing the trial, all rations for every meal and animal were weighed and stored in paper bags until feeding. This was done to ensure that the diet's chemical composition between the experimental periods was similar. Feed was offered in two equal portions at 08:00 h and at 16:00 h, with barley grains offered first. After the barley grains were completely consumed (within 5 - 10 minutes), the Rhodes grass hay was offered. Protein free mineral blocks were made available to each animal throughout the experiment; the chemical composition of the diet is presented in Table 2.

Table 2: Chemical composition (g kg^{-1} DM) of Rhodes grass hay and barley grains as the experimental diet[1] components offered to the goats during the feeding trial. Values are arithmetic means of six samples per feedstuff across the three experimental periods.

Components	Rhodes grass hay	Barley grains
DM (g kg^{-1} FM)	841	927
OM	910	970
CP	44	94
NDFom	631	245
ADFom	358	49

FM = Fresh matter; DM = Dry matter; OM = Organic matter; N = Nitrogen; NDFom = Ash free neutral detergent fibre; ADFom = Ash free acid detergent fibre.
[1]The mineral blocks contained 380,000 mg kg^{-1} sodium, 5000 mg kg^{-1} magnesium, 1,500 mg kg^{-1} iron, 300 mg kg^{-1} copper, 300 mg kg^{-1} zinc, 200 mg kg^{-1} manganese, 150 mg kg^{-1} iodine, 50 mg kg^{-1} cobalt, 10 mg kg^{-1} selenium.

2.2.3 Determination of feed and water intake

During each experimental period, about 250 g fresh matter (FM) of each of the feed offered were collected in duplicate and stored in paper bags at room temperature. There were no refusals of barley. Hay refusals were collected for each animal separately twice daily before every meal during the experimental period. At the end of the experimental period, hay refusals for each animal was pooled and thoroughly mixed. Two representative sub-samples were then taken from the pooled samples and stored in paper bags at room temperature until analysis.

Water was measured using a calibrated cylinder (with reference to the water treatment) and offered to each animal in a bucket in two equal portions at 08:30 h and 16:30 h. One hour before feeding (both morning and evening), the buckets were removed from the metabolic cages and the water refused was recorded for each animal. The buckets were then washed and water was offered according to the treatment for each animal. Water intake was determined as the difference between water offered and refused for each individual animal.

2.2.4 Determination of passage rate of feed particles

Fibre particles marked with ytterbium (Yb) were used to determine the passage rate of feed particles through the gastrointestinal tract. Rhodes grass hay was chopped to about 3 cm long pieces, then sieved through a 2 mm mesh to remove very small particles. Pieces remaining on the sieve were boiled in EDTA-free neutral detergent solution for one hour and then rinsed repeatedly with tap water until all detergent was removed. Washed hay particles were dried at 70°C and afterwards soaked for 24 hours in 12.4 mmol l^{-1} aqueous solution of $Yb(CH_3COO)_3 \cdot 4H_2O$ (Teeter et al., 1984). To ensure that all particles were marked, the soaked fibre was mixed twice within the 24 hours and subsequently thoroughly rinsed with tap water. Afterwards the particles were soaked for 6 hours in a solution of 100 mmol l^{-1} of acetic acid to discard unabsorbed Yb, and again thoroughly rinsed with tap water and dried at 70°C (Figure 1). About 25 g of the thus marked fibre was kept for determination of the Yb concentration of the marked hay.

On the first day of each experimental period, each animal was offered to eat marked fibre particles corresponding to 5.6 mg Yb kg^{-1} live weight (LW). In instances where goats refused to consume the marked fibre immediately, 5 – 30 g hay was mixed with the marked fibre. Starting time (t_0) of marker passage for each goat was defined as the time when the animal had completely ingested the marked fibre. In situations where the ingestion of marked fibre took longer than 30 minutes, t_0 was considered as half time of marker consumption. Faecal bags were emptied at 0, 6, 12, 18, 24, 30, 36, 42, 50, 58, 66, 74, 86, 98, 110, 122, 134, 146, 158 hours after dosing the Yb-marked fiber. Samples were identified by animal, day and time. The total amount of faeces (FM) was recorded at each time of collection. A thoroughly homogenised sub-sample of 50 – 60 g of faecal FM was kept each time the bag was emptied and was dried at 60°C for the

determination of air dry matter; afterwards all samples were stored in sealed paper bags at room temperature until Yb analysis.

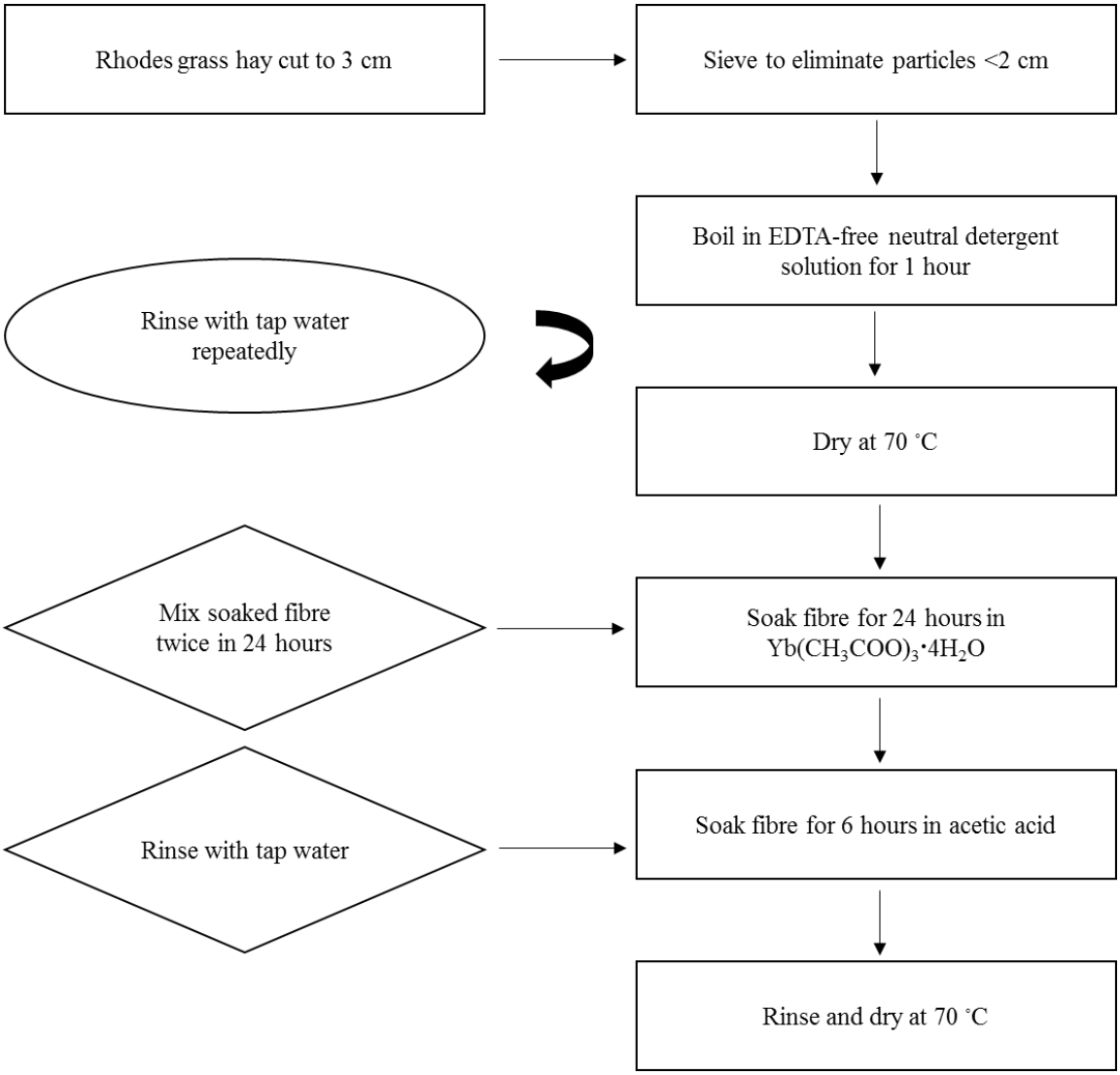

Figure 3: Flow chart illustrating the steps involved in ytterbium marker preparation.

2.2.5 Determination of faecal microbial biomass and total faecal output

To determine ergosterol and amino sugars that serve as markers for faecal microbial biomass, freshly excreted faecal samples were taken by emptying the faecal bags at one hour after morning feeding on days 2, 4 and 6 of each experimental period. After recording the total amount of FM, about 30 g of fresh faeces were collected from the faecal bags and immediately frozen at -20°C. Samples from the three days were pooled, thoroughly homogenized and a sub-sample of 50 g of the pooled material was freeze-dried, ball milled and stored at room temperature until analysis.

The faeces remaining after taking Yb and microbial biomass sub-samples were pooled for each animal and stored at 4°C until the end of the experimental period. After homogenization, two representative sub-samples of about 250 g FM each were taken from the pooled faeces and stored at -20°C for proximate analysis.

2.2.6 Chemical analyses

Proximate analyses of feed and faeces

Faecal samples were thawed before commencement of analysis. Rhodes grass hay offered and refused, offered barley grains and faeces were oven dried at 60°C and ground to pass through a 1mm screen (Retsch ZMI mill; Retsch GmbH, Haan, Germany). Analyses were done according to the methods of VDLUFA (2012) with method numbers represented in parenthesis. The samples were analysed in duplicate for their dry matter (DM) concentration by drying to constant weight for 24 hours at 105°C (method 3.1). Crude ash (CA) concentrations were determined in dried solids after DM analysis by incineration at 550 °C in a muffle furnace for 7 hours (method 8.1). Organic matter (OM) concentrations were calculated as the difference between DM and CA.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) in feed and faecal samples were determined in duplicate using an Ankom^{220} Fibre Analyser (ANKOM Technology, Macedon, NY, USA), thereby following the procedure of van Soest et al. (1991). Alpha-amylase and sodium sulphite were used for NDF analysis. ADFom and NDFom concentrations were expressed without residual ash. Nitrogen (N) contents of oven dried feed and faeces were determined in duplicate by means of a VarioMax CHN (Elementar Analysensysteme GmbH, Hanau, Germany). All analyses were repeated when the results for duplicate samples deviated by more than 5%.

The total tract digestibility (for simplicity termed ‘digestibility’ in the following text) of feed and feed components was calculated from the difference between the quantity of constituent ingested minus the quantity of constituent excreted (in faeces) divided by the quantity of constituent ingested. Digestibility was expressed in grams per kilogram of the specific nutrient.

Faecal ytterbium concentration

The concentration of Yb was determined following the method of Heinrichs et al. (1986) with some modifications. About 200 mg of the oven dried (60°C) samples of

marked fibre and faeces was treated with 0.5 ml double distilled water and later mixed with 1 ml hydrogen peroxide and 3 ml of 65% (v/v) nitric acid. The sample was then digested at 198°C for 1 hour in Teflon® vessels. The residue was rinsed with double distilled water into 50 ml flask and filtered over ashless Whatmann 40 filter paper. One millilitre of the sample was further diluted to 10 ml before analysis. The Yb concentration was determined as the average of three independent readings using an inductively coupled plasma mass spectrometer (ICP-MS; Optimass 9500, GBC Scientific Equipment Australia) and was detected at a wavelength of 396.4 nm.

Microbial biomass analysis

Extraction of ergosterol was done following the method of Zelles et al. (1987), applying modifications as described by Wentzel and Joergensen (2015). Reversed-phase high performance liquid chromatography (HPLC) was used to establish ergosterol concentrations, which were detected at a wavelength of 282 nm. The amino sugars muramic acid, mannosamine, glucosamine and galactosamine were determined by chromatographic separation using *ortho*-phthaldialdehyde (OPA) reagent as described by Indorf et al. (2011). Analyses were repeated for both ergosterol and amino sugars if triplicate determinations for each pool sample deviated by more than 8%. Fungal C was calculated as follows: mmol fungal C = (mmol glucosamine-2 x mmol muramic acid) x 9 (Engelking et al., 2007). Bacterial C was calculated as an index for bacterial residues by multiplying the concentration of muramic acid by 45 (Appuhn and Joergensen, 2006). Microbial C was calculated as the sum of fungal C plus bacterial C.

2.2.7 Statistical analyses

Quantitative outflow of Yb (Yb-concentration in faeces DM times faecal DM excreted at the respective point in time) was used to calculate parameters of solid digesta passage through the gastrointestinal tract, applying the models of Richter and Schlecht (2006). Ytterbium leaching due to disassociation from marked particles was observed in four cases, to which the disassociation model ('Type-D model') was applied. The normal model ('Type-N model') was used in all other cases (Richter and Schlecht, 2006).

SAS 9.3 (SAS Institute Inc., Cary, NC, USA) was used for model computation (PROC NLIN method=dud) to determine time of first marker appearance in faeces (TT), passage rate of fibre-bound marker through the rumen (λ, Gamma-2 parameter), half time of marker in the rumen (T_{50}: 0.8392 x $2\lambda^{-1}$), particle mean retention time in the

rumen (CMRT: $2\lambda^{-1}$) and particle mean retention time in the total tract (TMRT: TT + $2\lambda^{-1}$).

In total, 27 observations were obtained for data on water intake, feed intake, feed digestibility, parameters of digesta passage, faecal quantity and microbial biomass (3 periods x 9 animals). Statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The data was tested for normal distribution using the Shapiro-Wilk-test (UNIVARIATE procedure); all data sets were normally distributed. Analysis of variance was thereafter conducted by means of a mixed model procedure with treatment and period as fixed effects and animal as a random factor. The model used was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + T_k + e_{ijkl}$$

where y_{ijk} is the value of the response variable for a particular ijk case, μ is the overall mean, α_i and β_j are the fixed effects of treatment and period, respectively, $\alpha\beta_{ij}$ is the interaction of treatment and period, T_k the random effect variable (animal), and e_{ijkl} is the residual error.

Interactions between period and treatment were derived from the model using type 3 tests of fixed effects. Means were compared using the Tukey post-hoc test and significance was declared at $p < 0.05$. Spearman correlation statistics and probabilities were computed using the CORR procedure.

2.3 Results

2.3.1 Water intake, feed intake, faecal excretion and digestibility

When water was restricted to W70, water intake of Batinah goats (ml g^{-1} DMI) was lower ($p = 0.029$) compared to W80 (Table 3). Moreover, water consumption (ml $kg^{-0.75}$ LW) was 1.12 times higher in W85 ($p = 0.039$) compared to W70. There were no significant interactions between treatment and experimental period for any of the water intake parameters.

Intake (g $kg^{-0.75}$ LW) of DM, OM, N, NDFom and ADFom was not affected when the Batinah goats were subjected to the watering treatments (Table 3). However, all intake variables were affected by the experimental period, leading to significant treatment

times period interactions for the intake of DM ($p = 0.029$), OM ($p = 0.022$), NDFom ($p = 0.041$) and ADFom ($p = 0.045$).

The quantitative faecal excretion (g $kg^{-0.75}$ LW) of DM and OM decreased significantly in W70 as compared to W85. Also, faecal excretion of NDFom decreased by 14% in W70 compared to W85 (Table 3). No differences among treatments were observed for the faecal excretion of ADFom. As a consequence, there were no differences in the digestibility of ADFom among treatments. In comparison with W85, the digestibility of DM and OM increased by 5% in W70 ($p = 0.03$). Similarly, in W70 the NDFom digestibility was increased by 6% ($p = 0.046$) compared to W85 (Table 3).

2.3.2 Diet composition, faecal quality and digesta passage

The water treatments had no effect on the quality of the actually consumed diet, that is the concentration of N, NDFom and ADFom (g kg^{-1} DM), but enhanced the OM concentration of the ingested diet ($p = 0.036$) in the W85 and W70 as compared to W100 (Table 4). Similarly, the faecal OM concentration (g kg^{-1} DM) increased in W85 and W70 as compared to W100. Faecal ADFom concentration numerically increased when water was restricted at W70 (Table 4). There was no significant interaction between treatment and experimental period for any of the qualitative parameter.

Across treatments, all parameters of particle passage were similar (Table 5), even though the laminar flow (TT) of fibre particles through the lower gastrointestinal tract was fastest in the W70 as compared to the other treatments. As a consequence, the total tract mean retention time (TMRT) was shortest in W70 compared to the other two treatments, but the differences between treatments were insignificant (Table 5), as were treatment times period interactions for all parameters of particle passage.

Table 3: Intake of water and feed, faecal excretion and diet digestibility as measured in adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per).

	Water treatment				*p*-value		
Variable	W70	W85	W100	SEM	Trt	Per	Trt x per
Water intake per day (ml)	965^{b}	1083^{a}	1033^{ab}	35.6	0.08	0.26	0.19
Water intake per day (ml g^{-1} DMI)	1.6^{b}	1.8^{a}	1.8^{a}	2.4	0.07	0.07	0.14
Water intake per day (ml kg$^{-0.75}$ LW)	84^{b}	94^{a}	89^{ab}	0.1	0.10	0.93	0.18
Feed intake per day (g kg$^{-0.75}$ LW)							
DM	52.2	52.2	51.2	0.85	0.54	0.002	0.029
OM	49.3	49.3	48.4	0.92	0.14	0.002	0.022
N	0.67	0.66	0.66	0.010	0.66	0.0001	0.05
NDFom	22.2	22.5	21.8	0.54	0.44	0.0001	0.041
ADFom	9.9	10.2	9.7	0.27	0.33	0.013	0.045
Faecal excretion per day (g kg$^{-0.75}$ LW)							
DM	14.5^{b}	16.5^{a}	14.3^{b}	0.44	0.04	0.58	0.26
OM	13.1^{b}	14.9^{a}	12.8^{b}	0.42	0.04	0.57	0.27
N	0.24^{b}	0.27^{a}	0.24^{b}	0.007	0.06	0.65	0.70
NDFom	7.3^{b}	8.3^{a}	7.2^{b}	0.24	0.03	0.48	0.24
ADFom	4.4	4.8	4.2	0.15	0.15	0.54	0.41
Digestibility (g kg^{-1})							
DM	722^{a}	683^{b}	723^{a}	7.5	0.04	0.21	0.21
OM	734^{a}	696^{b}	737^{a}	7.5	0.05	0.23	0.26
N	637^{a}	581^{b}	632^{a}	13.4	0.05	0.007	0.68
NDFom	668^{a}	630^{b}	667^{a}	8.3	0.08	0.03	0.49
ADFom	553	528	571	10.9	0.32	0.23	0.76

Within rows, means with different superscripts differ at $p < 0.05$ (Tukey post-hoc test).
DMI = Dry matter intake; LW = Live weight; DM = Dry matter; OM = Organic matter; N = Nitrogen; NDFom = Ash free neutral detergent fibre; ADFom = Ash free acid detergent fibre. SEM = Standard error of the mean.

The nonparametric Spearman's rank-correlation analysis indicated that CMRT and TMRT were significantly influenced by the animals' live weight ($r = 0.66$; $p \leq 0.001$ for CMRT and $r = 0.55$; $p \leq 0.01$ for TMRT). Furthermore, TT was positively related to intake (g kg$^{-0.75}$ LW) of DM, OM and ADFom, but was not affected by the intake of N (Table 6). On the other hand, TMRT was negatively correlated with intake (g kg$^{-0.75}$ LW) of DM, OM and ADFom. None of the parameters of particulate passage showed significant relation to digestibility coefficients (data not shown).

Table 4: Feed and faecal composition as measured in adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per).

	Water treatment				*p*-value		
Variable	W70	W85	W100	SEM	Trt	Per	Trt x per
Ingesta composition (g kg^{-1} DM)							
OM	945^{a}	944^{a}	945^{b}	4.3	0.07	0.71	0.95
N	12.0	11.9	12.1	0.18	0.12	0.0001	0.12
NDFom	424	432	425	9.1	0.32	0.0001	0.38
ADFom	189	194	188	2.2	0.62	0.29	0.22
Faecal composition (g kg^{-1} DM)							
OM	902^{a}	905^{a}	894^{b}	1.8	0.02	0.34	0.84
N	16.6	16.6	17.2	0.34	0.13	0.52	0.03
NDFom	504	505	508	8.8	0.96	0.86	0.21
ADFom	305	291	289	4.8	0.25	0.88	0.33

Within rows, means with different superscripts differ at $p < 0.05$ (Tukey post-hoc test).
DM = Dry matter; OM = Organic matter; N = Nitrogen; NDFom = Ash free neutral detergent fibre; ADFom = Ash free acid detergent fibre; SEM = Standard error of the mean.

2.3.3 Faecal microbial composition

The faecal ergosterol concentration was numerically highest in W70 as opposed to the other two treatments (Table 7). Also, the faecal concentration of fungal glucosamine was higher ($p = 0.010$) in W70 compared to W85. On the other hand, there were no differences between treatments for faecal concentrations of muramic acid, galactosamine and mannosamine.

Faecal microbial C concentration tended to be higher in W70 as opposed to the other two treatments (Table 7). Whereas the bacterial C concentration was not affected by the treatments, the fungal C concentration significantly increased by 1.79 mg g^{-1} DM in W70 compared to W85. Fungal to bacterial C ratio was numerically higher in W70 than in the other treatments (Table 7). Interactions between treatment and period were only significant for fungal C ($p = 0.013$) and fungal glucosamine ($p = 0.012$).

Table 5: Parameters of gastrointestinal passage of feed particles in adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per).

	Water treatment				*p*-value		
Parameter	W70	W85	W100	SEM	Trt	Per	Trt x Per
TT (h)	16.7	17.1	17.3	0.63	0.94	0.68	0.25
λ (h^{-1})	0.053	0.054	0.050	0.0023	0.49	0.14	0.67
T_{50} (h)	32.5	33.4	35.1	1.53	0.46	0.04	0.78
CMRT (h)	38.7	39.8	41.9	1.82	0.46	0.04	0.80
TMRT (h)	55.4	56.9	59.1	1.86	0.55	0.10	0.98

TT = Time of first marker appearance in faeces; λ = Passage rate of fibre-bound marker through the rumen; T_{50} = Half time of marker in the rumen; CMRT = particle mean retention time in the rumen; TMRT = particle mean retention time in the total tract; SEM = Standard error of the mean.

Table 6: Spearman correlation coefficients (r_s) and significance levels[1] of the individual relationships between diet composition, quantitative intake and digestibility of diet constituents[2] with passage rate parameters in adult male Batinah goats exposed to treatment of no or mild restriction of water intake. All coefficients are based on 27 observations per variable.

	Passage rate parameter				
Variable	TT (h)	λ (h^{-1})	T_{50} (h)	CMRT (h)	TMRT (h)
Diet composition (g kg^{-1} DM)					
OM		0.56**	-0.56**	-0.56**	-0.52**
ADFom	0.38*	0.46*	-0.46*	-0.46*	-0.39*
Intake (g $kg^{-0.75}$ LW)					
DM	0.44*	0.54**	-0.54**	-0.54**	-0.44*
OM	0.41*	0.61***	-0.61***	-0.61***	-0.50**
NDFom		0.45*	-0.45*	-0.45*	
ADFom	0.41*	0.56**	-0.56**	-0.56**	-0.47*

[1]Significance levels: *p $\leq$ 0.05; **p $\leq$ 0.01; ***p $\leq$ 0.001; empty cells depict insignificant relationships between variables.
[2]DM = Dry matter; OM = Organic matter; NDFom = Ash free neutral detergent fibre; ADFom = Ash free acid detergent fibre.
LW = Live weight; TT = Time of first marker appearance in faeces; λ = Passage rate of fibre-bound marker through the rumen; T_{50} = Half time of marker in the rumen; CMRT = particle mean retention time in the rumen; TMRT = particle mean retention time in the total tract.

Table 7: Concentration of ergosterol, amino sugars, microbial C, fungal and bacterial C in faeces excreted by adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per).

	Water treatment				*p*-value		
Variable	W70	W85	W100	SEM	Trt	Per	Trt x per
Ergosterol (μg g^{-1} DM)	1.84	1.67	1.74	0.11	0.64	0.025	0.54
Amino sugars (mg g^{-1} DM)							
Mannosamine	0.22	0.28	0.25	0.024	0.39	0.84	0.58
Muramic acid	0.62	0.65	0.61	0.035	0.89	0.58	0.16
Galactosamine	1.65	1.65	1.51	0.07	0.44	0.29	0.13
Glucosamine	2.28	2.11	2.12	0.10	0.51	0.22	0.29
Fungal glucosamine	1.41^{b}	1.18^{a}	1.28^{ab}	0.08	0.031	0.004	0.012
Microbial C (mg g^{-1} DM)							
Fungal C	12.2^{b}	10.4^{a}	11.7^{ab}	0.74	0.07	0.009	0.013
Bacterial C	28.0	29.1	27.7	1.64	0.93	0.52	0.20
Microbial C	41.0	40.1	38.9	1.72	0.87	0.58	0.40
Fungal to bacterial C ratio	0.51	0.38	0.46	0.038	0.22	0.09	0.23
Fungal to microbial C ratio	0.33	0.27	0.30	0.017	0.28	0.14	0.16

Within rows, means with different superscripts differ at $p < 0.05$ (Tukey post-hoc test).
SEM = Standard error of the mean.
Ergosterol = Constituent of fungal cell membrane.
Mannosamine and galactosamine = Found in both fungi and bacteria cell wall.
Muramic acid = Found exclusively in bacterial cell wall.
Glucosamine = Found mostly in fungi but also in some bacterial cell wall.

2.4 Discussion

Ruminants reared in arid and semi-arid areas continuously face infrequent water supply and high ambient temperatures that may affect their behaviour, physiology and productivity (Silanikove, 1992). Water scarcity and heat stress affect appetite and digestive responses and an interaction of the two environmental factors may be detrimental to animals if prolonged (Silanikove, 1992). According to Silanikove and Koluman (2015), goats exposed to a temperature humidity index (THI) of 80 to 85 are subjected to moderate heat stress. The goats in the present study were able to cope with a moderate heat stress (THI 80 - 83) while facing a reduction in their water intake, without showing any signs of impeded well-being.

2.4.1 Effects of mild water restriction on feed intake, digesta passage and feed digestibility

It has been long established that water restriction reduces voluntary feed intake due to changes in energy and water fluxes (Kaliber et al., 2015; Silanikove, 1989). Brosh et al. (1986a) reported a drop in feed intake by 40% when Bedouin goats fed with lucerne hay decreased water intake by 109 ml $kg^{-0.75}$ LW. However, in the present study there were no changes in feed intake when water intake was reduced. This may be because treatment W70 decreased water intake by 10 ml $kg^{-0.75}$ LW compared to treatment W85 and this may not have been so severe as to have a negative impact on feed intake. Our findings are in line with those obtained in Comisana sheep fed a diet consisting of mixed field hay, alfalfa pellets and pelleted concentrate, and being subjected to 60% of *ad libitum* water consumption (Casamassima et al., 2008). Feed intake was also not affected in Karagouniko sheep fed lucerne hay when water was restricted to 65% of *ad libitum* water consumption (Hadjigeorgiou et al., 2000). It therefore seems that mild water restriction has no effect on feed intake of small ruminants regardless of the type of feed offered.

Reduction of drinking water intake did not affect the mean retention time of feed in the gastrointestinal tract in the present study. A probable explanation of the observed results may be the positive relationship between feed intake and reticulo-rumen fill (Clauss et al., 2016). Since feed intake was not affected in the present study, the gastrointestinal fill may have been unaltered, leading to a constant mean retention time even if water intake was reduced. This may also explain the positive relation observed between quantitative intake of DM, OM, NDFom and ADFom and the rate of particle passage through the rumen (λ). Contrary to our findings, the mean retention time of particulate matter in the gastrointestinal tract has been shown to increase when water intake was reduced (Brosh et al., 1986a; Hadjigeorgiou et al., 2000). An explanation for the contradiction with Brosh et al. (1986a) may be due the water restriction level imposed to the Bedouin goats (watered once every 4 days) as well as the high ambient temperatures (35 ºC) and outdoor weather conditions during their study. However, the average TMRT of 57 hours recorded in the present study was very similar to the values reported for desert adapted goats watered once daily and fed Rhodes grass hay (Silanikove et al., 1993).

The digestibility values of DM, OM and NDFom were observed to increase when water intake reduced in the present study. This is consistent with results obtained by Muna and Ammar (2001) who reported an increase in the digestibility of DM, OM and crude fibre in Sudanese desert goats. Yet, these authors fed high quality lucerne hay and reported a much lower water intake (570 ml per day) compared to our study. The enhanced digestibility following water restriction is believed to be associated with depressed feed intake which consequently leads to an increase in the mean retention time (Ghassemi et al., 2014; Singh et al., 1976). However, in the current study neither did feed intake drop nor was there an increase in the mean retention time of digesta when water intake was reduced. Furthermore, passage rate parameters did not directly correlate with the digestibility of proximate diet constituents (data not shown). This disputes our first hypothesis that feed digestibility will increase due to an increase in mean retention time of digesta in the gastrointestinal tract when water restriction increases. Our results indicate that, at the watering levels used in the present study, water restriction affected the digestibility of feed in other ways than through altered digesta kinetics. A factor that may have been involved in the increased feed digestibility could be increased rumen fermentation. However, further studies are needed to verify this.

2.4.2 Effects of mild water restriction on quantitative faecal excretion

Total faecal DM, OM, N and NDFom excretion significantly decreased when water intake was reduced in the present study. This was expected considering the increased digestibility of the above mentioned parameters when water intake was reduced. Similarly, quantitative faecal OM and N excretion decreased when South African Mutton Merino sheep were restricted to 50% of their *ad libitum* water intake and fed a low nitrogen diet (van der Walt et al., 1999). The decrease in quantitative faecal excretion is thought to be a water conserving mechanism adopted by ruminants facing drinking water shortage (Qinisa, 2010). During water restriction the hindgut plays a regulatory role by re-absorbing moisture from boli, thereby reducing the amount of faeces (Bohra and Ghosh, 1977).

2.4.3 Effects of mild water restriction on faecal microbial biomass

The present faecal concentrations of the different amino sugars as well as the calculated values of bacterial, fungal and total microbial C were similar to those reported for Boer

goats fed temperate meadow grass hay and a concentrate mix and having *ad libitum* access to drinking water (Al-Kindi et al., 2015). Furthermore, differences in total faecal microbial biomass between our watering treatments did not reach the significance level, which contradicts our second hypothesis that faecal microbial biomass will increase when water intake is restricted. This is most likely due to the relatively mild level of water restriction imposed on the animals in the present study.

Despite the unaltered faecal microbial biomass concentration in water-restricted goats, the faecal microbial community structure shifted towards fungi (indicator: increased fungal C) when water intake was reduced. This was also confirmed by the numerical increase in the fungal to bacterial C ratio in the W70 treatment. A probable explanation for this may be the high fibre concentration in the diet, as fungi have been reported to prefer C-rich diets (Rezaeian et al., 2006). In the large intestine, digestion of so-far undigested diet components depends on the availability of unfermented and undigested carbohydrates (van Vliet et al., 2007). Due to the high NDFom concentration in the diet offered to the goats in the present study, a certain share of this fraction may not have been fully fermented in the rumen, rendering it available to microbial fermentation in the hindgut. The fibre entering the hindgut is much harder to degrade and the time for degradation is shorter due to the volume differences between rumen and hindgut (Gressley et al., 2011; Zeitz et al., 2016). Consequently, fibrolytic bacteria may not establish nor proliferate in the hindgut (Zeitz et al., 2016). This was affirmed by de Oliveira et al. (2013) who reported an absence of *Fibrobacter* spp. in faeces of Brazilian Nelore steers. As water restriction is affiliated with an increased feed digestibility (Burgos et al., 2001), the digestion of the difficult to degrade fibre fractions may be associated with an increase of fungi in the hindgut. Further, since bacteria grow well in the presence of N-rich substrates (van Vliet et al., 2007), the increase in fungal biomass in the faeces of water restricted animals reflects a decreased N availability to the microbial population in the hindgut and hence lowered N incorporation into its microbial flora (Jost et al., 2013). This may eventually have positive consequences on animal performance as more N is apparently utilized by the animal.

2.4.4 Potential benefits of mild water restriction on faecal quality

When taking into account that goat faeces is an important soil amendment in Oman (Siegfried et al., 2013) and other subtropical countries, fibre fractions present in the

faeces are of importance since they will be decomposed to different extents by soil microorganisms when faeces are used as fertilizer (Al-Asfoor et al., 2012). The faeces excreted by goats in the W70 treatment were characterized by a high concentration of ADFom. As this component essentially consist of lignified cellulose, its decomposition in the soil will be relatively slow and will lead to stronger N-immobilisation (Jost et al., 2013) as compared to faeces with a lower ADFom concentration, as for example excreted by goats watered *ad libitum*. However, studies have shown that N in ruminant faeces is released by soil microbial decomposition in the long term and is eventually taken up by plants (Chadwick et al., 2000; Morvan and Nicolardot, 2009; Peters and Jensen, 2011). Thus, the decelerated decomposition of faecal N and C constituents may reduce CH_4/CO_2 as well as N_2O emissions and NO_3^- leaching from faeces of water restricted goats, even under the high temperatures and regular irrigation regimes that characterize crop farming in the semi-arid and arid areas of the Near and Middle East and beyond (Siegfried et al., 2011; 2013).

2.5 Conclusions

Our data suggests that desert adapted goats can easily cope with a 30% water restriction without compromising their feed intake. The goats were even able to improve their feed utilization through increased digestibility when restricted to 70% of their *ad libitum* water intake. The increase in slowly decomposable carbohydrate fractions (ADFom) in the faeces of water restricted animals may contribute to a stable soil organic carbon pool if the faeces are used as manure. Mild water restriction can therefore be considered beneficial to the animals in terms of feed utilization and to the farming systems in terms of manure quality and soil fertility.

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Chapter 3

Urinary excretion or purine derivatives, microbial protein synthesis and rumen fermentation in desert adapted goats exposed to mild water restriction

Urinary excretion of purine derivatives, microbial protein synthesis and ruminal fermentation in desert adapted goats exposed to mild water restriction

Abstract

Severe water restriction has been observed to modify the rumen physico-chemical conditions by decreasing the rumen volume, the osmotic concentration of the rumen fluid and the volatile fatty acids concentration. However, livestock inhabiting semi-arid areas are often exposed to mild water restriction for several months of the year. The focus of this study was therefore to determine the effects of mild water restriction on feed intake, total tract digestibility of feed, rumen fermentation, microbial composition and microbial yield. A 3 x 3 Latin Square design feeding trial was conducted at Sultan Qaboos University, Muscat, Oman, during the dry summer months in 2014. The trial comprised three periods each consisting of 16 days of adaptation and 8 days of sampling. Three watering regimes (100%, 85% and 70% of individual *ad libitum* consumption) were imposed on three rumen fistulated male Batinah goats. Feed offered and refused, urine as well as rumen contents were sampled and quantified. Urine and rumen fluids were analysed for purine derivatives (PD) and short chain fatty acids, respectively. Nitrogen (N) and purine base (PB) concentrations were determined in liquid-associated microbes (LAM) and solid-associated microbes (SAM). Water restriction to 70% of *ad libitum* water intake had no significant effect on feed intake, PD, LAM and SAM. Also, no significant effect was observed on the estimated microbial N flow from PD, LAM and SAM when water was restricted to 70% of *ad libitum* water intake. However, the total tract digestibility of ADFom increased ($p < 0.05$) when water was restricted to 70% of *ad libitum* water intake. Similarly, the proportion of butyrate and the concentration of ammonium-N increased ($p < 0.05$) when water was restricted to 70% of *ad libitum* water intake. Hence, a mild water restriction which is expected to occur frequently in arid and semi-arid regions may positively affect rumen fermentation, thereby improving nutrient utilization in desert adapted goats.

Key words: Batinah goats, microbial nitrogen flow, protozoa, purine bases, purine derivatives, short chain fatty acids, water restriction

3.1 Introduction

Lack of drinking water is a major limiting factor for livestock production in arid and semi-arid regions of the globe. These areas have sparsely distributed water sources and forage is sparse and mostly of low quality consisting of scattered thorny trees and low desert scrubs (Silanikove, 2000). In order to satisfy their nutritional requirements, livestock may have to walk long distances, and drinking may often be limited to once a day most times of the year or to once every four days during harsh dry summer periods (Brosh et al., 1986a).

Many studies have been conducted to establish how ruminants adapt to severe water restriction (Igbokwe, 1997; Jaber et al., 2013; Silanikove, 1992). Water deprivation and restriction have been reported to decrease feed intake in ruminants (Abioja et al., 2010; Alamer and Al-hozab, 2004; Silanikove, 1985). The decrease in feed intake is believed to be compensated for by an increase in the mean retention time of feed in the digestive tract (Jaber et al., 2013). As a result, there is an increase in feed digestibility as more time is made available for the micro-flora in the rumen to act on the feed (Silanikove et al., 1993). Nevertheless, rumen conditions are likely to be modified especially when feed is of low quality and water intake is limited (Brosh et al., 1988). When ruminants are exposed to 3 to 4 days of total water deprivation, rumen volume as well as the osmotic concentration of the rumen fluid were observed to drop (Brosh et al., 1988; Silanikove and Tadmor, 1989). Also, volatile fatty acids concentration was reported to decrease consistently following a four day water deprivation (Brosh et al., 1988). However, livestock herds in arid and semi-arid areas often face mild water restriction during most times of the year as opposed to a complete water deprivation which mostly occurs in the late summer period. Therefore, we wanted to ascertain whether mild water restriction affects total tract digestibility of feed, rumen fermentation and microbial composition as well as microbial yield. We postulated that mild water restriction will (1) increase digestibility of feed, (2) increase proportions of short chain fatty acids thereby increasing rumen fermentation, and (3) alter yield and composition of microbial mass thus enhancing the estimated microbial nitrogen flow.

3.2 Materials and methods

3.2.1 Experimental animals and site

A trial was conducted during the dry summer period (August-October 2014) at the Animal Experimental Station (AES) of the Department of Animal and Veterinary Sciences, Sultan Qaboos University, Muscat, Oman. Three adult male Batinah goats of body weight 22.9 kg (SD 3.30) were used as experimental animals. The goats had been fitted with rumen cannulae and were held indoors throughout the experiment. During the trial, the average daily temperature was 31°C and the relative humidity 63%. No rainfall occurred during the trial. The meteorological data collected during the trial is presented in Table 8.

Table 8: Meteorological data as measured at Sultan Qaboos University, Muscat, Oman, during the three experimental periods.

	Experimental periods in 2014		
Parameter	1 (13^{th}-20^{th} Aug)	2 (6^{th}-13^{th} Sep)	3 (30^{th}Sep-7^{th} Oct)
Maximum ambient daily temperature (°C)	34.8	35.5	37.2
Minimum ambient daily temperature (°C)	26.7	27.1	24.8
Mean ambient daily temperature (°C)	30.9	31.0	31.3
Relative air humidity (%)	70.5	70.1	50.0
Temperature humidity index*	82.9	82.8	80.0

*The temperature humidity index was calculated using the equation of NRC (1971):
THI = (1.8 * T°C + 32) – [(0.55 – 0.0055 * RH %) * (1.8 * T°C -26)]
where T°C is the average daily air temperature and RH is the relative humidity.

3.2.2 Experimental design

A pre-trial was conducted one month before the commencement of the experiment to determine the *ad libitum* water consumption of each animal. Animals were fed a ration consisting of entire barley grains (*Hordeum vulgare* L.) and Rhodes grass hay (*Chloris gayana* Kunth.) at a dry weight ratio of 1:1. They were offered 4 litres of drinking water in two portions at 08:00 h and 16:00 h for a week. Water and feed refusals per animal and day were quantified. Thereafter, the average water intake (ml) per day as well as the water intake per unit of dry matter (DM) intake (ml g^{-1} DMI) was calculated for each animal. The *ad libitum* water consumption for each animal was then defined at that level.

The experiment was subsequently conducted as a complete Latin Square (3 x 3) with the following regimes for the provision of drinking water (treatments): 1) water offered *ad libitum* (100%; treatment W100); 2) water restricted to 85% of individual *ad libitum* consumption (W85); and 3) water restricted to 70% of individual *ad libitum* consumption (W70). Water was offered in two equal portions at 08:30 h and 16:30 h (each time roughly 30 minutes after feeding). The trial entailed three periods, each comprising of 16 days of adaptation and 8 days of experiment. During adaptation, the animals were individually housed in paddocks of ca. 2.25 m² within a large roofed stable with open sides. During the experimental periods, the goats were kept in individual metabolic crates designed to ease collection of urine and rumen samples. All animals were weighed before morning feeding on two consecutive days before and after each experimental period. Animal care and use adhered to all legal and institutional animal welfare guidelines of the country.

3.2.3 Diet

Barley (*Hordeum vulgare* L.) grains and Rhodes grass (*Chloris gayana* Kunth.) hay were offered to the goats at a ratio of 1:1 (on dry matter basis). Animals were fed at 1.3 times individual maintenance energy requirement (MER) according to NRC feeding standards (NRC, 2007). The hay was derived from the AES stock; it was chopped to about 10 cm length and thoroughly mixed before the start of the trial. Thereafter, rations for every meal and animal were weighed and stored until feeding for each experimental period. The daily amount of feed was divided into two equal portions and offered at 08:00 h and 16:00 h. At each meal, barley grains were offered first, followed by hay. The barley grains were consumed completely within 5 - 10 minutes. Mineral blocks were made available *ad libitum* throughout the experiment. The chemical composition of the diet is shown in Table 9.

Table 9: Chemical composition (g kg^{-1} DM) of Rhodes grass hay and barley grains as the experimental diet[1] components offered to the goats during the feeding trial. Values are arithmetic means of six samples per feedstuff across the three periods.

Components	Rhodes grass hay	Barley grains
DM (g kg^{-1} FM)	841	927
OM	910	970
CP	44	94
NDFom	631	245
ADFom	358	49

FM = Fresh matter; DM = Dry matter; OM = Organic matter; N = Nitrogen; NDFom = Ash free neutral detergent fibre; ADFom = Ash free acid detergent fibre.
[1]The mineral blocks contained 380,000 mg kg^{-1} sodium, 5000 mg kg^{-1} magnesium, 1,500 mg kg^{-1} iron, 300 mg kg^{-1} copper, 300 mg kg^{-1} zinc, 200 mg kg^{-1} manganese, 150 mg kg^{-1} iodine, 50 mg kg^{-1} cobalt, 10 mg kg^{-1} selenium.

3.2.3 Sample collection and analyses

Feed

About 250 g fresh matter (FM) of the offered hay and barley were sampled per experimental period and stored in sealed paper bags at room temperature until analysis. Hay refusals for each animal were collected twice daily during the experiment and pooled at the end of each sampling period. There were no refusals of barley. Barley and the pooled hay samples (offer and refusals) were oven dried at 60°C and ground to pass through a 1mm screen (Retsch ZMI mill; Retsch GmbH, Haan, Germany). Analyses of dry and organic matter contents were done according to the methods described in VDLUFA (2012). Dry matter (DM) content was determined in duplicate by drying to constant weight for 24 hours at 105°C (method 3.1). Crude ash (CA) concentrations were determined in dried solids after DM analysis by incineration at 550°C in a muffle furnace for 7 hours (method 8.1). Organic matter (OM) concentrations were calculated as the difference between DM and CA.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) in feed samples were determined in duplicate using an Ankom^{220} Fibre Analyser (ANKOM Technology, Macedon, NY, USA) following the procedure of van Soest et al. (1991). Alpha-amylase and sodium sulphite were used for NDF analysis. ADFom and NDFom concentrations were expressed without residual ash. Total N in feed samples was determined in duplicate using a Vario max CHN analyser (Elementar Analysensysteme GmbH, Hanau, Germany)

according to the procedures described by Naumann and Bassler (1997). All analyses were repeated if duplicate determinations for each pool sample deviated by more than 5%.

Rumen contents

Rumen samples were obtained from the 3 cannulated animals at -1, 1, 2, 4 and 8 hours after the morning feeding on day 8 of the experimental period. To determine rumen pH, ammonia and short chain fatty acid (SCFA) concentrations, 120 ml of rumen fluid were collected from the ventral rumen sac. The pH of the fluid was measured immediately after collection and the sample was then stored at -20°C until analysis. Ammonium-N was determined by the Berthelot colour reaction using phenol plus sodium nitroprusside and alkaline hypochlorite as reagents as described by Weatherburn (1967). Absorbance was measured at 625 nm after 30 minutes of incubation at 25°C using a Varian Cary 50 spectrophotometer (Agilent Technologies, Böblingen, Germany). The SCFA concentrations were measured using gas chromatography (GC 14A; Shimadzu Corp., Tokyo, Japan). About 70 µL of internal standard solution was added to 630 µl of rumen fluid. The mixture was incubated for 16 hours at 4°C and afterwards centrifuged at 20,000 x g for 10 minutes at 4°C. The supernatant was then used to determine the concentrations of the SCFA acetate, propionate, butyrate, valerate, isobutyrate and isovalerate by gas chromatography (GC 14A; Shimadzu Corp., Tokyo, Japan) and Permabond column (0.35 µm; 30 m by 0.32 mm; Shimadzu Corp.)

Solid rumen contents were collected from the reticulum, from the top of the particulate mat of the rumen and the caudal part of the ventral rumen sac. These were pooled and immediately stored at -20°C for analysis of the concentrations of nitrogen and of the purine bases (PB) adenine and guanine in the microbial matter associated to the liquid and solid phase. For the determination of liquid associated microbes (LAM) and solid associated microbes (SAM), solid rumen samples were thawed and gently homogenized. About 80 g of the three samples per sampling time and animal (i.e., from the reticulum, the top of the particulate mat and the caudal part of the ventral rumen sac) were combined, thoroughly mixed and pressed through four layers of cheesecloth. The filtrates were centrifuged at 500 x *g* for 10 minutes at 4°C. The resulting pellet (i.e. protozoa and feed particles) was combined with the residues in the cheesecloth and an equal amount of salt solution (0.9%

wt/vol NaCl) was added. Thereafter, the material was thoroughly mixed for 30 seconds and pressed again through the 4 layers of cheesecloth. This filtrate was centrifuged for the second time (500 x *g* for 10 minutes at 4°C) and the supernatant was decanted; the latter was then added to the supernatant from the first centrifugation and centrifuged at 22,000 x *g* at 4°C for another 8 minutes. The pellet of the third centrifugation was washed with distilled water and lyophilized. This was considered as LAM. The pellet from the second 500 x *g* centrifugation was then combined with the residuals in the cheesecloth. Twice the amount (vol/wt) of 0.9% sodium chloride and 0.1% methylcellulose solution was added and the material was homogenized for 15 minutes at 39°C. After incubation at 4°C for 16 h, the sample was pressed again through a cheesecloth. The filtrate was centrifuged at 22,000 x *g* at 4°C for 8 minutes and the resulting pellet was washed with distilled water and lyophilized. This represented SAM. Adenine and guanine concentrations in lyophilized microbial pellets were measured according to Balcells et al. (1992) by means of HPLC (Merck KGaA, Darmstadt, Deutschland) in duplicate using reversed phase C18 Hypersil Gold column (250 x 4 mm; Thermo Fisher Scientific GmbH, Dreieich, Germany). The N and carbon (C) concentrations in microbial pellets were measured by Dumas combustion using a Vario max CN analyser (Elementar Analysensysteme GmbH, Hanau, Germany) in duplicate following procedures of Naumann and Bassler (1997).

To enumerate protozoa, additional 100 ml of rumen fluid and 100 g of solid rumen contents were collected 2 hours after the morning feeding on day 8 of the sampling period. The liquid and solid rumen samples were combined, mixed thoroughly and pressed through a layer of cheesecloth. For the preservation of the protozoa, 5 ml of methyl green formalin saline solution was added to the 5 ml aliquots (Ogimoto and Imai, 1981). The samples were stored in darkness at room temperature until counting. Protozoal numbers were microscopically determined under x100 magnification using Fuchs-Rosenthal counting chambers (Glaswarenfabrik Karl Hecht KG, Sondheim/Rhön, Germany). Counts were repeated when the CV between duplicate records exceeded 10%.

Urine

For the collection of urine, containers were fitted beneath the metabolic crates during each sampling period. The containers were filled with approximately 10 ml 10% sulphuric acid

to maintain urine pH below 3 hence avoid bacterial degradation of purine derivatives (George et al., 2006). Sampling was done twice daily at 06:00 h and 15:00 h. The urine collected in the afternoon was weighed and stored at 4°C until the next morning. Immediately after the morning feeding, the pH, volume and weight of the evening and the morning samples were recorded. The two were then combined and thoroughly mixed. About 50 ml of the mixed urine was filtered over Whatmann no. 1 1001-185 filter paper and then diluted with distilled water (1:5). Three aliquots of 10 ml each were obtained from the diluted samples and stored at -20°C for the analysis of purine derivatives (PD). Two aliquots were thawed and used for the analysis of PD concentrations (creatinine, allantoin, uric acid, xanthine and hypoxanthine). Urinary PD concentrations (mmol/l) were analysed in duplicate by reversed-phase HPLC (Varian 1920LC) according to Balcells et al. (1992). Analyses were repeated if duplicate determinations for each pool sample deviated by more than 6%.

The microbial N flow was estimated from urinary PD excretion, LAM and SAM according to Chen and Ørskov (2004) using the body weight of the three experimental animals per treatment and a purine base to nitrogen ratio in microbial matter of 0.116 g N in purines per g total N (Chen and Ørskov, 2004).

3.2.4 Statistical analyses

A total of 9 observations were obtained for water intake, feed intake, total tract digestibility, urinary PD concentrations and protozoal counts (3 periods x 3 animals), and 45 observations were obtained for rumen parameters (3 periods x 3 animals x 5 sampling times). Statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The data was tested for normal distribution using the Shapiro-Wilk-test (UNIVARIATE procedure); all data sets were normally distributed. For water intake, feed intake, PD concentrations and protozoal counts the mixed model procedure was used to conduct ANOVA, with treatment as the fixed effect and animal considered as a random effect. The model used was:

$$y_{ij} = \mu + TRT_i + a_j + e_{ijk} \qquad \text{[Eq. 1]}$$

where y_{ij} is the value of the response variable for a particular ij case, μ is the overall mean, TRT_i is the fixed effect of treatment i, a_j is the random effect of animal j, and e_{ijk} is the

residual error. Treatment was not included in the model because each treatment was represented once in each period.

For the other rumen parameters (SCFA, ammonia-N, pH, LAM and SAM) the mixed model procedure was used to conduct ANOVA, with treatment as the fixed effect, time as the repeated measure and animal considered as a random effect. The model used was:

$$y_{ijk} = \mu + TRT_i + T_j + TRT_i \times T_j + a_k + e_{ijkl} \qquad \text{[Eq. 2]}$$

where y_{ijk} is the value of the response variable for a particular ijk case, μ is the overall mean, TRT_i is the fixed effect of treatment i, T_j is the fixed effect of sampling time (j = -1, 1, 2, 4 and 8 h after morning feeding), a_k is the random effect of animal k, and e_{ijkl} is the residual error. The Tukey-Kramer post-hoc test was used to detect significant differences between individual treatment means. Significance was declared at $p < 0.05$.

3.3 Results

3.3.1 Water intake, feed intake and digestibility of diet components

When water was restricted to W70, Batinah goats drunk less water both in terms of ml g^{-1} DMI and ml $kg^{-0.75}$ LW compared to W85 and W100 (Table 10). Yet, these differences were not statistically different among the treatments. Similarly, feed intake (g $kg^{-0.75}$ LW) was not affected by watering treatment (Table 10). However, the total tract digestibility (g kg^{-1}) of ADFom increased ($p < 0.05$) in W70 goats compared with the other two treatments. Similarly, the total tract digestibility of DM, OM and NDFom tended to be higher at W70 compared to the other two treatments, but these differences were not statistically significant (Table 10).

3.3.2 Rumen fermentation parameters

The W70 treatment was associated with an increase ($p < 0.05$) in the ammonium-N concentration in rumen liquid (Table 11). Across all watering treatments, ammonium-N concentrations were lower ($p < 0.05$) at 4 and 8 hours after feeding compared to the other sampling times. Rumen pH did not differ among the watering treatments, but it was highest at 1 hour after feeding and lowest at 4 hours after feeding (as compared with the other sampling times). Although total SCFA concentration in rumen liquid was not affected by

watering treatment, a drastic drop in total SCFA concentration was observed one hour after feeding and watering. Further, the proportion of butyrate increased ($p < 0.05$) in W70 goats as compared to W85. Across all water treatments, the proportion of acetate, isobutyrate and isovalerate were highest at -1 hour after feeding compared to the other sampling times. Treatment and sampling time interactions were not significant for any of the rumen fermentation parameters (Table 11).

Table 10: Daily water intake, feed intake and digestibility of diet components by adult male Batinah goats exposed to no or mild restriction of water intake. Values are arithmetic means of three animals across three experimental periods.

	Water treatment				
Variable	W70	W85	W100	SEM	*p*-value
Metabolic weight ($kg^{-0.75}$ LW)	10.4	10.6	10.4	0.38	0.97
Water intake per day (ml)	840	998	892	34.3	0.15
Water intake per day (ml g^{-1} DMI)	81.5	95.3	87.0	4.09	0.27
Water intake per day (ml $kg^{-0.75}$ LW)	1.5	1.7	1.7	0.07	0.31
Feed intake per day (g $kg^{-0.75}$ LW)					
DM	55.1	55.2	51.3	1.19	0.39
OM	52.0	52.0	48.4	1.34	0.41
N	0.70	0.68	0.67	0.023	0.88
NDFom	23.6	24.2	21.8	0.89	0.60
ADFom	10.7	11.2	9.6	0.42	0.29
Total tract digestibility (g kg^{-1})					
DM	746	688	698	14.5	0.15
OM	758	696	712	14.6	0.14
NDFom	687	631	637	19.0	0.51
ADFom	598[a]	518[b]	494[b]	17.8	0.02

Within rows, means with different superscripts differ at $p < 0.05$ (Tukey-Kramer post-hoc test).
LW = Live weight; DMI = Dry matter intake; DM = Dry matter; OM = Organic matter; N =Nitrogen; NDFom = Ash-free neutral detergent fibre; ADFom = Ash-free acid detergent fibre.
SEM = Standard error of the mean.

3.3.3 Microbial composition and yield

The water treatment had no significant effect on C, N and PB concentrations in LAM and SAM pellets (Table 12). Still, N and PB concentrations were higher in LAM than in SAM pellets. Across all water treatments, higher N concentrations ($p = 0.004$) and therefore lower C to N ratios ($p = 0.021$) were observed in LAM pellets at -1 hour after feeding

compared to the other sampling times. In SAM pellets, C ($p = 0.026$) and N ($p = 0.041$) concentrations were highest at -1 and 8 hours after feeding compared with the other times. Moreover, C to N ($p = 0.027$) as well as adenine to guanine ($p = 0.003$) ratios were lowest at -1 and 8 hours after feeding in the SAM pellets. Protozoal counts tended to be higher in W70 compared to the other treatments, but no statistical differences were observed (Table 12). There were no significant interactions between treatment and sampling time for all microbial parameters.

Table 11: Ammonium-nitrogen (NH_4-N) and short-chain fatty acid (SCFA) concentrations in rumen fluid collected at five sampling times from adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means of three goats across three experimental periods.

	Water treatment				*p*-value		
Variable	W70	W85	W100	SEM	Trt	Time	Trt x Time
pH	6.5	6.6	6.6	0.03	0.56	0.0025	0.86
NH_4-N (mg ml^{-1})	0.11^{a}	0.08^{b}	0.09^{b}	0.008	0.03	0.003	0.17
SCFA (mmol l^{-1})	55.6	52.4	49.4	1.59	0.33	0.55	0.97
SCFA profile (% total SCFA)							
Acetate	68.0	67.3	68.0	0.57	0.86	0.19	0.99
Propionate	15.3	17.7	16.7	0.63	0.16	0.0005	0.97
Butyrate	13.5^{a}	11.7^{b}	12.1^{ab}	0.38	0.05	0.0004	0.99
Isobutyrate	0.86	0.86	0.87	0.019	0.97	0.0001	0.98
Isovalerate	1.00	1.11	1.07	0.057	0.31	0.95	0.99
Valerate	1.41	1.32	1.18	0.030	0.19	0.0003	0.96

	Time after morning feeding (h)				
Variable	-1	1	2	4	8
pH	6.68^{a}	6.74^{ab}	6.55^{ac}	6.41^{c}	6.69^{a}
NH_4-N (mg ml^{-1})	0.15^{a}	0.12^{a}	0.10^{ac}	0.05^{b}	0.05^{b}
SCFA (mmol l^{-1})	53.8	46.8	53.7	55.3	52.6
SCFA profile (% total SCFA)					
Acetate	70.3^{a}	68.8^{ab}	66.3^{ab}	65.9^{b}	67.5^{ab}
Propionate	13.1^{b}	14.3^{b}	16.5^{a}	19.4^{a}	19.6^{a}
Butyrate	13.2^{a}	13.7^{a}	13.9^{a}	11.6^{b}	9.8^{b}
Isobutyrate	1.00^{a}	0.92^{ab}	0.84^{b}	0.74^{c}	0.79^{c}
Isovalerate	1.58^{a}	1.33^{ab}	1.25^{ab}	1.14^{b}	1.21^{b}
Valerate	0.86^{b}	0.97^{ab}	1.10^{a}	1.26^{ac}	1.10^{a}

Within rows above, treatment means with different superscripts differ at $p < 0.05$ (Tukey-Kramer post-hoc test).
Within rows below, means of different sampling times carrying different superscripts differ at $p < 0.05$ (Tukey-Kramer post-hoc test).
SEM = Standard error of the mean.

Table 12: Composition of microbial pellets isolated from rumen contents collected at five sampling times from adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means of three goats across three experimental periods.

	Water treatment				*p*-value		
Variable	W70	W85	W100	SEM	Trt	Time	Trt x Time
Liquid associated microbes							
C (g 100g^{-1} DM)	49.2	48.7	48.7	0.22	0.63	0.33	0.99
N (g 100g^{-1} DM)	7.2	7.2	7.0	0.11	0.69	0.033	0.59
C-to-N ratio	7.0	6.8	6.9	0.14	0.75	0.14	0.57
PB (mmol 100g^{-1} DM)	0.86	0.93	0.91	0.026	0.55	0.21	0.56
PB-to-N ratio	0.12	0.13	0.13	0.003	0.22	0.011	0.44
Adenine-to-guanine ratio	0.79	0.78	0.78	0.005	0.59	0.99	0.58
Solid associated microbes							
C (g 100g^{-1} DM)	49.0	48.8	49.2	0.19	0.68	0.013	0.99
N (g 100g^{-1} DM)	5.6	5.0	5.3	0.13	0.18	0.022	0.72
C-to-N ratio	8.9	10.2	9.6	0.28	0.15	0.08	0.79
PB (mmol 100g^{-1} DM)	0.40	0.34	0.37	0.018	0.40	0.39	0.58
PB-to-N ratio	0.07	0.07	0.07	0.002	0.72	0.16	0.54
Adenine-to-guanine ratio	0.86	0.83	0.84	0.008	0.25	0.004	0.74
Protozoal count (x100)	22493	21670	15489	2280	n.s	n.a	n.a

	Time after morning feeding (h)				
Variable	-1	1	2	4	8
Liquid associated microbes					
C (g 100g^{-1} DM)	49.3	49.2	48.4	48.0	49.2
N (g 100g^{-1} DM)	7.5^{a}	7.4^{a}	7.1^{ab}	6.5^{b}	7.2^{a}
C-to-N ratio	6.6^{b}	6.7^{b}	6.9^{ab}	7.7^{a}	6.9^{ab}
PB (mmol 100g^{-1} DM)	0.81	0.94	0.85	0.91	1.00
PB-to-N ratio	0.11^{a}	0.13^{ab}	0.12^{ab}	0.14^{b}	0.14^{b}
Adenine-to-guanine ratio	0.78	0.78	0.78	0.78	0.79
Solid associated microbes					
C (g 100g^{-1} DM)	50.1^{a}	48.8^{bc}	48.2^{b}	48.45^{bc}	49.4^{ac}
N (g 100g^{-1} DM)	5.9^{a}	5.2^{b}	4.8^{b}	4.9^{b}	5.5^{a}
C-to-N ratio	8.4^{b}	9.4^{ab}	10.3^{a}	10.6^{a}	9.2^{ab}
PB (mmol 100g^{-1} DM)	0.38	0.34	0.32	0.40	0.42
PB-to-N ratio	0.06	0.06	0.07	0.08	0.08
Adenine-to-guanine ratio	0.83^{b}	0.83^{b}	0.90^{a}	0.84^{b}	0.81^{b}

Within rows above, treatment means with different superscripts differ at $p < 0.05$ (Tukey-Kramer post-hoc test). Within rows below, means of different sampling times carrying different superscripts differ at $p < 0.05$ (Tukey-Kramer post-hoc test).
C = Carbon; N = Nitrogen; PB = Purine bases (sum of adenine and guanine)
SEM = Standard error of the mean;
n.a = Not applicable. Protozoal counts were determined once per treatment at 2 h after feeding.

3.3.4 Purine derivatives and microbial nitrogen flow

The W70 treatment decreased the daily urinary output (g d^{-1}) by 45% and 25% compared to the W85 and W100 treatments, respectively, although these differences were not statistically significant. Across all treatments, allantoin excretion was highest followed by uric acid, then hypoxanthine and lastly xanthine. Yet, water restriction had no significant effect on the daily urinary PD excretion (Table 13). Similarly, there were no effects of water restriction on the microbial N flow as estimated from the PD, LAM, SAM and the average of LAM and SAM (Table 14). The estimated values of microbial N flow from PD and LAM were similar, while values obtained from LAM+SAM were higher than those obtained from PD.

Table 13: Daily urinary excretion of purine derivatives (PD) and creatinine (Cre) in adult male Batinah goats exposed to treatments of no or mild restriction of water intake. Values are arithmetic means of three goats across the three experimental periods.

	Water treatment				
Variable	W70	W85	W100	SEM	*p*-value
Urine (g d^{-1})	249	450	328	52.4	0.35
Urine (g $kg^{-0.75}$ LW)	24.1	43.9	33.2	5.71	0.41
Urine nitrogen excretion (g $kg^{-0.75}$ LW)	0.12	0.13	0.13	0.015	0.86
Creatinine excretion (mmol d^{-1})	6.9	7.7	7.1	0.76	0.66
Urinary PD excretion (mmol d^{-1})					
Allantoin	6.9	8.0	6.9	0.49	0.65
Uric acid	0.7	0.7	0.6	0.04	0.53
Hypoxanthine	0.4	0.4	0.4	0.02	0.87
Xanthine	0.02	0.03	0.02	0.002	0.31
Total PD excretion	8.0	9.1	7.9	0.52	0.71
PD/Cre ratio	1.2	1.3	1.2	0.12	0.60

LW = Live weight; SEM = Standard error of the mean.

Table 14: Duodenal microbial nitrogen flow as estimated from the urinary excretion of purine derivatives and purine base to nitrogen ratio in rumen microbial biomass of adult male Batinah goats exposed to treatments of no or mild restriction of water intake. Values are arithmetic means of three goats across the three experimental periods.

	Water treatment				
Variable	W70	W85	W100	SEM	*p*-value
Urinary PD excretion (mmol d^{-1})	8.0	9.1	7.9	0.52	0.71
PD absorption[1] (mmol d^{-1})	8.9	10.4	8.8	0.66	0.63
Estimated duodenal microbial N flow (g d^{-1})					
PD	6.3	7.4	6.3	0.47	0.63
LAM	6.2	6.6	5.6	0.49	0.74
SAM	10.3	12.6	10.3	0.85	0.50
LAM + SAM	8.3	9.6	7.9	0.64	0.59

PD = Purine derivatives; LAM = Liquid-associated microbes; SAM = Solid-associated microbes; SEM = Standard error of the mean.

3.4 Discussion

3.4.1 Effects of mild water restriction on feed intake and digestibility

In ruminants, water intake has been observed to be inversely related to feed intake (Igbokwe, 1997; Silanikove, 1989). This is attributed to the essential role of water in chewing, rumination, digestion and elimination of metabolic waste products (NRC, 2007). Imposing a reduction in water intake to once every 72 hours reduced intake of lucerne hay by 31% in Sudanese desert sheep (Abdelatif and Ahmed, 1994). Also, an 85% reduction in feed intake was observed when Saudi Arabia indigenous goats were deprived of water for three days and fed a diet consisting of a concentrate mix and alfalfa hay (Alamer, 2006). However, feed intake was not affected by water restriction in the current study. This may be attributed to the only mild restriction in water intake, the latter thus being too high to cause such effects. Further, the diet given to the animals in the present study was of relatively low quality compared to the diets offered in the above mentioned studies. Although the present study was not designed to determine effects of water restriction in response to feed quality, previous work shows that the decline in feed intake under water restriction is also dependent on the type of feed offered to the animals (Brosh et al., 1986b; Jaber et al., 2013). For example, Bedouin goats watered once every 4 days and fed Rhodes grass hay (i.e., medium quality feed) did not reduce their feed intake (Brosh et al., 1986b).

Thus, watering level and quality of the diet in combination seem to be the main factors affecting feed intake during water restriction.

Several researchers have consistently reported an increase in feed digestibility following water restriction in ruminants (Burgos et al., 2001; Muna and Ammar, 2001; van der Walt et al., 1999). Our results partly agree with these findings as water restriction at 70% of *ad libitum* water intake increased the total tract digestibility of ADFom. The increase in digestibility is argued to be due to an increase in the mean retention time of feed particles in the rumen, which provides more time for the microflora to act on the feed (Brosh et al., 1986a). However, complementary measurements (Chapter 2) showed that the passage rate of feed particles did not change under the current watering regimes, suggesting that digestibility was affected in other ways than through altered digesta passage. Thus, it is possible that changes in the chemical conditions of the rumen caused the increased digestibility recorded in the present study, as is outlined below (see 3.4.2).

3.4.2 Effects of mild water restriction on rumen fermentation parameters

The results of this study confirm those of Brosh et al. (1988) who demonstrated that a four day water deprivation in Bedouin goats did not affect the rumen pH. However, a drop in pH at 4 hours after feeding was observed in the present study. This is most likely due to the elevated production of SCFA that follows feed consumption (Li et al., 2009; Wora-anu et al., 2007). Nevertheless in the present study, the pH did not fall below 6.41 indicating that rumen function was not negatively affected by pH at any time (de Veth and Kolver, 2001; Orskov, 1994).

Short chain fatty acids are the end products of rumen microbial fermentation and represent the main supply of metabolizable energy for ruminants (Busquet et al., 2006). Therefore, a considerable increase in their production would be nutritionally favourable for the animal. The SCFA profile was altered with W70 towards higher proportions (in % of total SCFA) of butyrate. Butyrate is an end product of carbohydrate fermentation by rumen protozoa (Williams and Coleman, 1997) and cellulolytic bacteria (Li et al., 2009). Since cellulolytic bacteria have been reported to be insensitive to feed and water restriction (Fluharty et al., 1996), the increase in butyrate when water intake was reduced may result from the numerically higher rumen protozoa observed in our study. The increase in the proportion of

butyrate may explain the above-discussed increase in total tract digestibility of ADFom (see 3.4.1) observed in the current study. Also, when water intake was reduced, rumen ammonium-N concentration was increased in our study. This may be due to the increase in protozoal numbers (mostly by trend) when water intake was reduced. Protozoa are responsible for extensive ammonia production in the rumen, therefore these results indicate a higher proteolytic activity in the rumen (Zeitz et al., 2016). It seems that reduced water intake is linked to increased rumen protein and carbohydrate degradation, which agrees with our hypothesis 2 that rumen fermentation processes are enhanced when water intake is reduced.

The concentrations of SCFA and ammonium-N in rumen fluid varied with regards to the time elapsed after feeding in this study. Moreover, the concentration of SCFA was observed to drastically drop one hour after feeding. This drop may be due to the dilution of the rumen fluid that results from water ingestion (Brosh et al., 1988; Silanikove et al., 1993). Therefore rumen fermentation varies according to feeding and watering rhythms.

3.4.3 Effects of mild water restriction on rumen microbial composition and yield

Higher concentrations of N and PB have been reported in LAM compared to SAM pellets (Dickhoefer et al., 2016; Ipharraguerre et al., 2007; Martin-Orue et al., 2000) which is in line with the results of our study. The differences in N and PB concentrations between LAM and SAM have been attributed to differences in abundance of microbial species, growth rates and micro-environmental conditions in the liquid and solid phases of digesta (Ipharraguerre et al., 2007). However, no effect of water restriction was observed with respect to the microbial composition in LAM and SAM, which contradicts our hypothesis 3 that water restriction will alter the rumen microbial composition. Further, no interdependencies of linear regression were observed between the current water treatments and LAM (r^2= 0.010) as well as SAM (r^2= 0.013, data not shown).

Effects of sampling time were observed for the LAM and SAM pellets in this study. Nitrogen increased with increasing time after feeding, whereas C to N ratios decreased with increasing time after feeding in both pellets. Similarly, Cecava et al. (1990) and Dickhoefer et al. (2016) found that composition of rumen microbial matter was affected by time after

feeding. This is probably due to variation in the release of energy and N during fermentation which in turn affects the microbial growth (Cecava et al., 1990).

Consistent with previous reports (Carro et al., 2012; Yanez Ruiz et al., 2004), the endogenous PD was almost completely composed of allantoin (86%) in the present study. However, the mean urinary PD excretion (8.3 mmol d^{-1}) was lower than values reported from studies with goats fed mixed diet of rye grass hay and concentrate at 1.5 times MER (Al-Kindi et al., 2016) and alfalfa hay and concentrate at 1.3 times MER (Carro et al., 2012). On the other hand, the present values were higher than those observed in Barbari goats fed a diet consisting of wheat straw and concentrate mix at maintenance level (George et al., 2011). The contradictions may be due to differences between studies in the diet of the animals and the feeding levels. Further, water restriction had no effect on the urinary PD excretion in this study. Urinary PD excretion has been observed to increase when feed intake increases (Braga et al., 2012; George et al., 2011; Singh et al., 2007). Since feed intake was not affected by water restriction in the present study, the unaltered PD excretion is to be expected.

Urinary PD excretion has been extensively used for estimating microbial N flow to the duodenum in ruminants (Funaba et al., 1997). Also, PB in microbial mass have been used as internal markers to quantify the duodenal microbial N flow (Chen and Ørskov, 2004). Water restriction in the present study did not affect the microbial N flow as estimated from PD, LAM and SAM, which disagrees with our hypothesis 3. This may be due to the unaffected values recorded for the individual parameters when water was restricted. Also, when assuming a PB-to-N ratio in the microbial mass of 0.116 g PB g^{-1} N, the mean duodenal microbial N flow in our study ranged between 6.3 and 7.4 g N d^{-1} from urinary PD excretion. The absolute estimates of duodenal microbial N flow from LAM were similar to those obtained from PD, but values from SAM were considerably higher. This may be due to overestimation of duodenal microbial N flow from feed particles in SAM which may limit its use as a microbial marker. Although, further studies are necessary to estimate the variability and contribution of microbial mass to duodenal microbial flow.

3.5 Conclusions

Our data suggests that feed intake, rumen microbial composition and yield are to be unaffected by mild water restriction. However, ADMom digestibility as well as rumen fermentation products (ammonia-N and butyrate) were enhanced when desert adapted goats were subjected to mild water restriction. Therefore, mild water restriction seems to slightly improve the utilization of low quality diets (with low N contents) which are often predominating in arid and semi-arid areas for most times of the year.

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Chapter 4

Effects of mild water restriction on feed digestibility, nitrogen balance, faecal nitrogen fractions and nitrogen partitioning between urine and faeces in desert adapted goats

Effect of mild water restriction on feed digestibility, nitrogen balance, faecal nitrogen fractions and nitrogen partitioning between urine and faeces in desert adapted goats

Abstract

When exposed to reduced drinking water intake, nitrogen (N) retention in ruminants is increased whereas N excretion via urine is reduced. This may have beneficial environmental effects as N in urine is unstable and easily volatilized, while faecal N is much more slowly degraded, even under semi arid tropical conditions. However, little is known about the proportion of N excreted via faeces and urine during water restriction. This study therefore determined the effects of mild water restriction on feed intake, diet digestibility, N retention, partitioning of excreted N between urine and faeces and the fractionation of N in the faeces. Two feeding trials were conducted at Sultan Qaboos University, Muscat, Oman, during the dry summer months (August-October) in 2013 and 2014. In each trial, three watering regimes (100%, 85% and 70% of individual *ad libitum* consumption) were imposed on nine adult male Batinah goat breed in a 3 x 3 Latin Square design. Water intake, feed offered and refused, and excretion of urine and faeces were quantified and their respective quality determined using standard procedures. Water restriction at 70% had no significant effect on feed intake, N retention and urinary N excretion in both trials. However, in trial 2 the apparent total tract digestibility of dry matter, organic matter, N and neutral detergent fibre increased ($p<0.05$) when water was restricted to 70% of *ad libitum* water intake. Furthermore, quantitative faecal N excretion decreased ($p<0.05$) when water was restricted at 70% in trial 2. There was no absolute or relative shift in N excretion from urine to faeces during water restriction. Also, the proportion of major faecal N fractions was not affected by water restriction in both trials. Therefore, a restriction of the *ad libitum* water intake to 70%, which is expected to occur more frequently with current climate change phenomena observed in the semi-arid tropics and subtropics, may be anticipated with serenity because it improves nitrogen utilization in agro-pastoral farming systems without compromising the performance of regionally adapted livestock breeds.

Keywords: Batinah goats, faecal nitrogen fractions, nitrogen partitioning, nitrogen retention, water restriction.

4.1 Introduction

Arid and semi-arid regions comprise a third of the total land area of the world (FAO, 1989) and host 80% of global ruminant livestock (Silanikove, 2000). Arid and semi-arid regions located in the tropics and subtropics experience extended dry periods of between 6 to 8 months and erratic rainfall in the remaining period (Jaber et al., 2013). Due to high inter- and intra-annual rainfall variability, amount and quality of the available forage resources, such as natural vegetation, crop residues and fruits and leaves of ligneous species, are also very variable. Especially towards the end of the dry season, forage supply is often scarce and of mostly low quality (Silanikove, 2000). For ruminants reared under such environmental conditions, the maintenance of a balanced water and energy intake is a challenge especially with regards to animal productivity (Jaber et al., 2013).

Ruminants in general and goats in particular can be exposed for longer periods to water restriction without negative consequences for their physiological processes (Silanikove, 1992, 2000). During summer and periods of prolonged droughts, goats are grazed far from watering sources in order to find enough forage and meet their nutritional requirements (Abdelatif and Ahmed, 1994). As a consequence, watering is done once every 2 - 4 days, depending on the distance to watering points. Under such conditions, goats are likely to suffer from a certain level of dehydration (Silanikove, 2000). Therefore, their ability to economize water at levels lower than their voluntary water intake is crucial.

Goats can tolerate water restriction and deprivation even when it causes a remarkable decrease in their feed intake (Choshniak et al., 1995; Hassan, 1989), body weight (Mengistu et al., 2007; More and Sahni, 1978) and overall performance (Alamer, 2009). This is largely due to the increased feed digestibility brought about by slower digesta passage when goats are exposed to water restriction (Jaber et al., 2013). Furthermore, water restriction and deprivation in goats is reported to increase nitrogen (N) retention and reduce N excretion (Silanikove, 2000). The kidneys thereby play a critical role by retaining the urea formed in the liver and recycling it back to the rumen and the gut (Silanikove, 2000). As a result, less N is excreted via urine.

Reduced urinary N excretion during water restriction periods may have environmental benefits with regards to N stability in the soil to which grazing animals' excreta are recycled. Nitrogen excreted via urine is easily lost as ammonia (James et al., 1999; Petersen et al., 1998), particularly at high ambient temperatures prevailing in the tropics, whereas N in faeces is relatively stable and less likely to be volatilized (Satter et al., 2002).

Although many studies have been conducted in ruminants to examine the effects of water deprivation on N balance and retention (Bohra and Ghosh, 1977; Ikhatua et al., 1985; Misra and Singh, 2002; Muna and Ammar, 2001), ruminants are more often exposed to mild water restriction during most times of the year. Thus, the current study was conducted during summer of two subsequent years to determine the effect of mild water restriction on feed and nutrient intake, digestibility, N retention, faecal N fractions and the partitioning of excreted N between urine and faeces. We thereby postulated that a mild restriction of drinking water intake will (1) increase the digestibility of feed, (2) enhance N retention and (3) induce an absolute and relative shift of N excretion from urine to faeces.

4.2 Materials and methods

4.2.1 Experimental site and animals

Two feeding trials were conducted at the Animal Experimental Station of the Department of Animal and Veterinary Sciences, Sultan Qaboos University, Muscat, Oman. The trials took place during the dry summer period August to October 2013 (Trial 1) and August to October 2014 (Trial 2). Both trials lasted for 3 months each with no rainfall occurring during these months. The meteorological data collected during both trials are presented in Table 15.

In each of the two trials, nine adult male Batinah goats aged 13 - 18 months were used as experimental animals. In each trial, six animals were intact and three were fistulated at the rumen. The fistulated animals were used to collect data on rumen fermentation characteristics which are presented in Chapter 3). The live weight (LW) of the goats averaged 32.0 kg (SD 4.09; Trial 1) and 26.3 kg (SD 5.03; Trial 2). The goats were weighed in the morning prior to feeding and watering using a scale of 0.1 kg accuracy. This was done for two consecutive days before and after each experimental period. Each trial consisted of three periods of 27 (trial 1) and 24 (trial 2) days, namely 21 and 16 days of adaptation, respectively, and 6 and 8 days of quantifying feed and water intake as well as faecal and urinary excretion (experimental period). During adaptation periods, animals were individually housed in paddocks of about 2.25 m² within an open roofed stable; they were transferred to individual metabolic crates within the same stable for each of the experimental periods. Animal care and use was in accordance with the country regulations.

Table 15: Meteorological data registered at the experimental stable during the two trials. Values are daily means for the three experimental periods (P).

	Trial 1			Trial 2		
Variable	P1	P2	P3	P1	P2	P3
Maximum ambient temperature (°C)	41.9	35.7	40.1	34.8	35.5	37.2
Minimum ambient temperature (°C)	28.7	26.7	27.1	26.7	27.1	24.8
Mean ambient temperature (°C)	33.3	30.3	31.4	30.9	30.9	31.3
Relative air humidity (%)	62.3	75.3	60.6	70.5	70.1	50.0
Temperature Humidity Index*	84.8	82.6	81.9	82.9	82.8	80.0

Exact dates of experimental periods are, in 2013: P1 = 11th-17th Aug, P2 = 8th-13th Sept, P3 = 5th-10th Oct; in 2014: P1 = 13th-20th Aug, P2 = 06th-13th Sept, P3 = 30thSept - 07th Oct.
* The temperature humidity index was calculated using the equation of NRC (1971):
THI = (1.8 * T°C + 32) – [(0.55 – 0.0055 * RH %) * (1.8 * T°C -26)]
where T°C is the average daily air temperature and RH is the relative humidity.

4.2.2 Experimental design

A pre-trial experiment was conducted one month before the commencement of each of the two trials to determine the *ad libitum* water consumption for each animal. Animals were fed at a ratio of 1:1 (amount, g DM/day) with barley grains (*Hordeum vulgare* L.) and Rhodes grass hay (*Chloris gayana* Kunth.), and were offered 4 litres of drinking water in two portions at 08:00 h and 16:00 h for a week. Water and feed refusal per animal and day were quantified. Thereafter, the average water intake (ml) per day as well as the water intake per unit of dry matter (DM) intake (ml g^{-1} DMI) was calculated for each animal. The *ad libitum* water consumption for each animal was then defined at that level.

Each of the two trials was conducted as a complete Latin Square (3 x 3) with the following treatments for provision of drinking water: 1) water offered *ad libitum* (100%, treatment W100); 2) water restricted to 85% of individual *ad libitum* consumption (W85); 3) water restricted to 70% of individual *ad libitum* consumption (W70).

4.2.3 Feed and feeding

Rhodes grass hay and barley grains were fed a ratio of 1:1 (on dry matter basis) in both trials. The barley grains were rolled in the first trial but were offered as whole grains in the second trial due to technical difficulties. The animals individually were fed at 1.3 times maintenance energy requirements according to NRC (2007). Feed was supplied twice per day (08:00 h and 16:00 h) whereby the total amount of feed was divided into two equal meals. The barley was offered first, and mostly consumed in 15 minutes; afterwards the hay was supplied. In addition, mineral blocks were made available *ad libitum* throughout each trial. The chemical composition of the diet offered to the goats is presented in Table 16.

Table 16: Chemical composition (g kg^{-1} DM) of Rhodes grass hay and barley grains as the experimental diet[1] offered to the goats during two feeding trials. Values depict arithmetic means of six samples per feedstuff across the three experimental periods.

	Trial 1		Trial 2	
Components	Rhodes grass hay[2]	Barley grains	Rhodes grass hay	Barley grains
DM (g kg^{-1} FM)	886	922	841	927
OM	920	980	910	970
CP	70	91	44	94
NDFom	599	321	631	245
ADFom	314	53	358	49

FM = Fresh matter; DM = Dry matter; OM = Organic matter; N = Nitrogen; NDFom = Ash free neutral detergent fibre; ADFom = Ash free acid detergent fibre.
[1]The mineral blocks contained 380,000 mg kg^{-1} sodium, 5000 mg kg^{-1} magnesium, 1,500 mg kg^{-1} iron, 300 mg kg^{-1} copper, 300 mg kg^{-1} zinc, 200 mg kg^{-1} manganese, 150 mg kg^{-1} iodine, 50 mg kg^{-1} cobalt, 10 mg kg^{-1} selenium.
[2]In trial 1, Rhodes grass hay was marked with ^{15}N.

4.2.4 Production of ^{15}N labelled Rhodes grass hay

In trial 1, ^{15}N labelled Rhodes grass hay was given to the animals to determine partitioning of N excretion in urine and faeces. The hay was produced at the experimental farm of Sultan Qaboos University in Al Khoudh, Muscat. According to year-long practice, Rhodes grass hay is harvested every 35 days (cutting cycle). During one cutting cycle, Rhodes grass hay was fertilized with 6 kg urea-$^{15}N_2$ per hectare with a ^{15}N abundance of 10 atom% as foliar application on day 21 and 25 after the previous cutting. To this end, the urea solution was mixed with 1.2 g l^{-1} of urease inhibitor N-(n-butyl)-thiophosphoric triamide (NBTT) and 450 mg l^{-1} of a surfactant containing lecithin and propionic acid (Li 700) to improve the absorption of nitrogen through the leaves. The hay was harvested on day 35, thoroughly air-dried and bailed (8 – 10 kg bales) and stored in a closed and dry shed until usage. The enrichment of the ^{15}N in the labelled hay was 0.675 atom% compared with 0.369 atom% of unlabelled hay.

4.2.5 Quantification of feed and water intake

Two samples of about 250 g fresh matter (FM) of the offered hay and barley, respectively, were sampled per experimental period. There were no refusals of barley grains. Hay refusals were collected for each animal separately twice daily before every meal during the experimental periods. At the end of each experimental period, hay refusals were pooled per animal and thoroughly mixed. Two representative sub-samples were taken from the pooled samples and stored in paper bags at room temperature until analysis.

Water was measured using a calibrated cylinder (with reference to the water treatment) and offered to each animal in a bucket in two equal portions at 08:30 h and 16:30 h. One hour before feeding (both morning and evening), the buckets were removed from the metabolic cages and the water refused was recorded for each animal. The buckets were then washed and water was offered to each animal according to their respective treatment. Water intake was determined as the difference between water offered and refused for each individual animal.

4.2.5 Quantification of faeces and urine excretion

During each experimental period, faeces were quantitatively collected into faecal bags that were fitted to the goats. In trial 1, faecal bags were emptied twice daily (at 07:00h and 15:00h), while in trial 2 faecal bags were emptied at specific hours due to the parallel determination of particle passage rate (Chapter 2). In trial 2, about 50 g fresh matter (FM) of the faeces collected at any specific time was air-dried at 60°C and used for marker determination. All faeces collected in trial 1 and faeces remaining after subtraction of 50 g FM in trial 2 were stored at 4°C until the end of each experimental period. The total amount of faeces excreted per animal during an entire experimental period was then pooled and thoroughly homogenized. Two representative sub-samples of about 250 g FM each were taken from every pool and stored at -20°C until analysis.

For the collection of urine, containers (volume: 20 litres) were placed beneath the metabolic crates in which the animals were housed during the experimental periods. The containers contained approximately 10 ml 10% (v/v) sulphuric acid to lower urine pH to <3 and to thereby avoid ammonia losses. The containers were emptied twice daily before feeding at 06:00 h and at 15:00 h, washed, dried and refilled with sulphuric acid. The urine collected in the afternoon was weighed and stored at 4°C until the next morning. The urine excreted overnight was collected immediately after morning feeding, weighed, mixed with the urine collected in the afternoon of the preceding day, and again total weight was recorded. About 50 ml of the mixed urine was filtered over Whatmann no. 1 1001-185 filter paper and then diluted with distilled water (1:4). Three aliquots of 10 ml each were obtained from the diluted samples and stored at -20°C for the analysis of purine derivatives (PD). The remaining urine was stored at 4°C until the end of the experimental period; then all urine was pooled per animal and thoroughly homogenized. Three subsamples of 500 ml each of the pooled urine were retained per animal and period and stored at -20°C for analysis.

4.2.6 Chemical analyses

Proximate analyses of feed, faeces and in urine

Two thoroughly homogenised samples each (100 g FM) of Rhodes grass hay offered and refused, barley grains offered and of freshly thawed faeces were weighed, dried in a forced draught oven at 60°C until weight constancy, weighed again and then ground to pass through a 1 mm screen (Retsch ZMI mill; Retsch GmbH, Haan, Germany). Analyses were done according to the methods of VDLUFA (2012). The samples were analysed in duplicate for their dry matter (DM) concentration by drying to constant weight for 24 hours at 105°C (VDLUFA, 2012). Crude ash (CA) concentration was determined in dried solids after DM analysis by incineration at 550°C in a muffle furnace for 7 hours (VDLUFA, 2012). Organic matter (OM) concentrations were calculated as the difference between DM and CA.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) in feed and faecal samples were determined in duplicate using an Ankom^{220} Fibre Analyser (ANKOM Technology, Macedon, NY, USA), thereby following the procedure of van Soest et al. (1991). Alpha-amylase and sodium sulphite were used for NDF analysis. ADFom and NDFom concentrations were expressed without residual ash. Total nitrogen (N) in oven dried feed and faecal samples was determined in duplicate using a VarioMax CHN (Elementar Analysensysteme GmbH, Hanau, Germany). All samples were finely ground using a ball mill (Retsch GmbH, Germany) prior to nitrogen analyses. Nitrogen in urine was determined using the Kjeldahl method with Vapodest Vap 50s (Gerhardt Analytical Systems, Königswinter, Germany). All the analyses were repeated when duplicate analyses deviated by more than 5%.

Two aliquots of the above-mentioned diluted urine samples (section 4.2.5) were thawed and used for the analysis of PD concentrations. Urinary PD concentrations (mmol/l) were analysed in duplicate by reversed-phase HPLC (Varian 1920LC) according to Balcells et al. (1992). Analyses were repeated if duplicate determinations for each pool sample deviated by more than 6%. Urinary PD excretion was then used to estimate the microbial N synthesis according to Chen and Ørskov (2004).

Fractionation of nitrogen in faeces

Samples of freshly thawed faeces were subjected to the analytical procedures of Mason (1969) in order to determine the following fractions: (i) undigested dietary nitrogen (UDN), (ii) bacterial and undigested nitrogen (BUN), (iii) water soluble nitrogen (WSN) and (iv) bacterial

and endogenous debris nitrogen (BEDN). The N fractions were determined in duplicate subsamples of either fresh (BUN, WSN) or oven dried (total N, UDN, BEDN) faeces (Figure 4). The UDN fraction was determined as the nitrogen resistant to a modification of the neutral detergent fibre extraction. The extraction was done using the Ankom^{220} Fibre Analyser (ANKOM Technology, Macedon, NY, USA) following the procedure described above for NDF but without using amylase and sodium sulphite. The BUN fraction was determined by mixing 5 g of fresh faeces with 20 ml sodium chloride (0.9%) / methyl cellulose (0.1%) solution for 5 minutes. From the homogenised mixture, 50 ml were centrifuged at 22,000 x *g* for 40 minutes at 4°C. The supernatant was carefully discarded, the sediment was then lyophilized. All samples were analysed for their N content using a VarioMax CHN (Elementar Analysensysteme GmbH, Hanau, Germany). The WSN fraction was calculated as the difference between total N and BUN. The BEDN fraction was estimated as the difference between total N and WSN + UDN. All analyses were repeated when results for duplicate samples deviated by more than 5%.

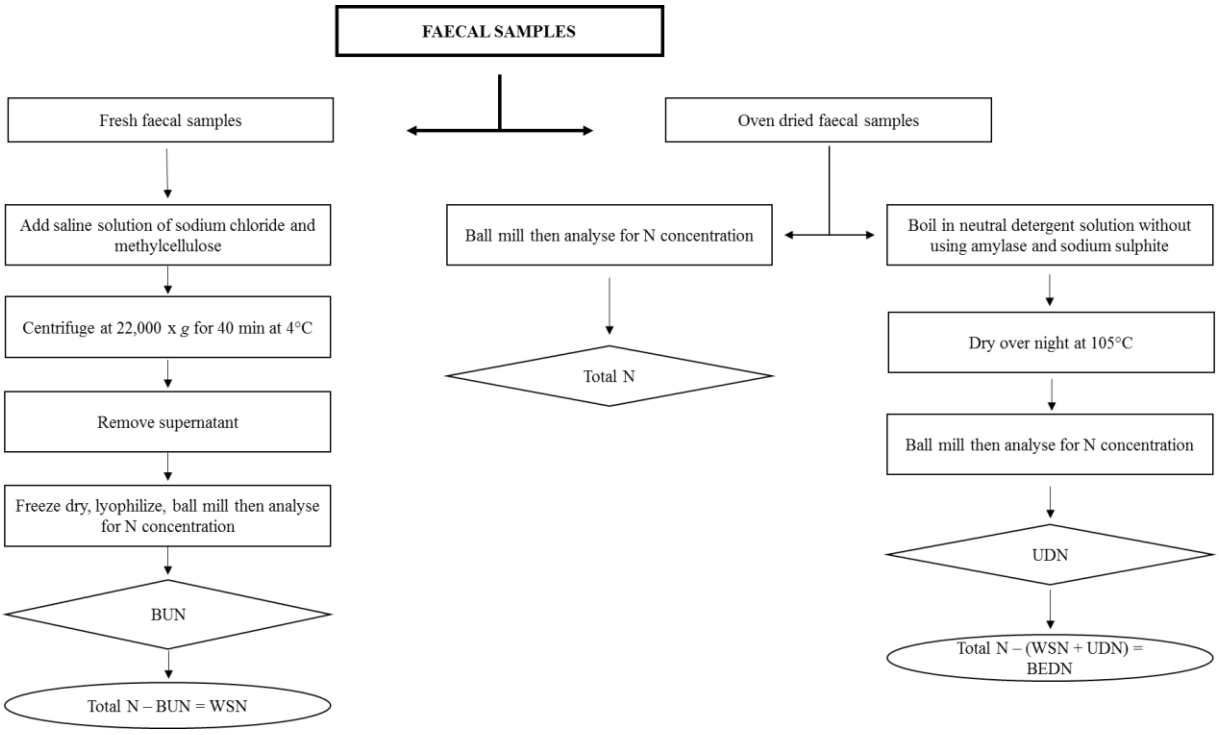

Figure 4: **:** Flow chart outlining the principal steps used to determine faecal nitrogen fractions as described by Mason (1969).
BUN = Bacterial and undigested nitrogen; WSN = Water soluble nitrogen; UDN = undigested dietary nitrogen; BEDN = Bacterial and endogenous debris nitrogen; N = Nitrogen

Determination of ^{15}N in hay, urine and faeces

The solid samples containing ^{15}N (hay offered and refused, faeces) from trial 1 were oven dried and ball milled before their analysis. About 5 mg of oven dried (60°C) material was weighed

into tin capsules (IVA Analysetechnik GmbH & Co. KG, Meerbusch, Germany) and analysed by means of an elemental analyser (CE Instruments, Rodano, Milano, Italy) coupled to a Delta Plus isotope ratio mass spectrometer via a Conflo III interface (Finnigan MAT, Bremen, Germany). The ^{15}N-containing urine samples were thawed and 50 g were dried for 24 hours at 105°C to determine their DM concentration. The thus dried samples were then analysed by elemental analysis- isotope ratio mass spectrometry for their ^{15}N atomic concentrations as described above.

4.2.7 Statistical analyses

In each of the two trials, 27 observations were obtained for data on water intake, feed intake and digestibility of diet constituents, nitrogen balance and faecal composition (3 periods x 9 animals). Statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The data was tested for normal distribution using the Shapiro-Wilk-test (UNIVARIATE procedure); all data sets were normally distributed. Analysis of variance was conducted by means of a mixed model procedure with treatment and period as the fixed effects and animal as a random factor. The model used was:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_k + \alpha\beta_{ij} + \alpha\lambda_{ik} + \beta\lambda_{jk} + \alpha\beta\lambda_{ijk} + T_l + e_{ijklm}$$

where y_{ijkl} is the value of the response variable for a particular ijkl case, μ is the overall mean, α_i, β_j and λ_k are the fixed effects of treatment, period and year, respectively, $\alpha\beta_{ij}$ is the interaction of treatment and period, $\alpha\lambda_{ik}$ is the interaction of treatment and year, $\beta\lambda_{jk}$ is the interaction of period and year, $\alpha\beta\lambda_{ijk}$ is the interaction of treatment, period and year, T_l is the random effect of the animal and e_{ijklm} is the residual error. Arithmetic means were calculated for main effects and compared using the Tukey-Kramer post hoc test. Significance was declared at $p < 0.05$.

4.3 Results

4.3.1 Water intake, feed intake, faecal excretion and digestibility of feed

Water intake per unit of feed dry matter ingested (ml g^{-1} DMI) was significantly reduced at W70 when compared to W100 in both trials. Similarly, water intake per unit of metabolic weight (ml $kg^{-0.75}$ LW) was lowest for W70 compared with the other treatments. Goats in trial 2 drunk less water both in terms of ml g^{-1} DMI and ml $kg^{-0.75}$ LW compared to the goats in trial 1. There was a significant interaction between year and treatment (Table 17).

Quantitative intake (g $kg^{-0.75}$ LW) of feed DM, OM, NDFom and ADFom was not affected when the Batinah goats were subjected to the different watering treatments in both trials (Table 18). Faecal excretion (g $kg^{-0.75}$ LW) of DM, OM, NDFom and ADFom was not significantly different between the watering treatments in trial 1, whereas the quantitative faecal excretion of DM and OM decreased (p=0.03) in W70 as compared to W85 in trial 2. Similarly, quantitative faecal excretion of NDFom decreased by 14% in W70 as compared to W85 (Table 18). Whereas in trial 1 the apparent total tract digestibility of ADFom was lower for W70 than for W100 (p=0.008), in trial 2 the apparent total tract digestibility of DM, OM, NDFom and ADFom was higher for W70 than for W85 (Table 18).

Table 17: Daily water intake of adult male Batinah goats exposed to three treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per) per trial.

Trial, water treatment	Water intake (ml $kg^{-0.75}$ LW)	Water intake (ml g^{-1} DMI)
Trial 1 (2013)		
W70	83^{b}	1.9^{b}
W85	94^{a}	2.1^{ab}
W100	100^{a}	2.4^{a}
SEM	4.0	0.09
Fixed variable effects		
Trt	0.0002	0.007
Per	0.0007	0.023
Trt x Per	0.09	0.56
Trial 2 (2014)		
W70	84^{B}	1.7^{B}
W85	94^{A}	1.9^{A}
W100	89^{AB}	1.9^{A}
SEM	2.4	0.05
Fixed variable effects		
Trt	0.11	0.07
Per	0.93	0.07
Trt x Per	0.18	0.15
Comparison of trials		
Year x Trt	0.029	0.029
Year x Per	0.017	0.14
Year x Trt x Per	0.14	0.37

DMI = Dry matter intake; LW = Live weight.
Within columns means with different superscripts differ at $p \leq 0.05$ (Tukey-Kramer test).
Lowercase letters are used for trial 1, whereas uppercase letters are used for trial 2.

4.3.2 Diet composition and faecal quality

The water treatments had no effect on the quality (g kg^{-1} DM) of N, NDFom and ADFom of the ingested diet in both trials (Table 19). However, in trial 2, the OM concentration of the ingested diet was increased in W85 and W70 ($p = 0.036$) as compared to W100 (Table 19). Similarly, the faecal OM concentration (g kg^{-1} DM) increased in W85 and W70 as compared to W100 in trial 2 ($p = 0.0417$). Faecal ADFom concentration numerically increased when water was restricted at W70 (Table 19). There were no significant interactions between treatment, experimental period and year for all the qualitative parameters.

4.3.3 Nitrogen balance, faecal nitrogen fractions and microbial nitrogen flow

In both trials, the quantitative intake of N was not affected by the watering treatments and a positive nitrogen balance was found for all animals (Table 20). Also, the total amount of N excreted via urine and faeces was unaltered when the goats were exposed to water restriction. However, in trial 2 goats on treatment W70 excreted less ($p=0.03$) faecal N (g $kg^{-0.75}$ LW) compared with W85. In consequence, the ratio of faecal N to urinary N excretion was lower in W70 than in W85. The watering treatments did not affect the faecal nitrogen fractions in both trials, whereby the proportion of BEDN accounted for about two thirds, UDN for a fifth and WSN for 13.2% of total faecal N in both years (Table 20). Water restriction had no significant effect on the daily urinary PD excretion in both years (Table 20). In consequence, no effect of mild water restriction was observed on the microbial N flow from the rumen to the duodenum.

4.3.4 ^{15}N balance, faecal nitrogen fractions and microbial nitrogen flow

In trial 1, water restriction had no significant effect on the ^{15}N intake via Rhodes grass hay, ^{15}N total excretion and ^{15}N retention (Table 21). Although the proportion of ^{15}N was generally higher in faeces than in urine, there were no differences between treatments (Table 21). There were no significant interactions between treatment and experimental period for all the variables measured with respect to ^{15}N intake and metabolism.

Table 18: Intake, faecal excretion and apparent total tract digestibility of diet components by adult male Batinah goats exposed to three treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per).

	Feed intake per day (g $kg^{-0.75}$ LW)				Faecal excretion per day (g $kg^{-0.75}$ LW)				Digestibility (g kg^{-1})			
Trial, water treatment	DM	OM	NDFom	ADFom	DM	OM	NDFom	ADFom	DM	OM	NDFom	ADFom
Trial 1 (2013)												
W70	43.1	40.7	20.8	8.5	12.7	11.4	6.4	4.1	706	720	691	510^{b}
W85	43.7	41.2	21.4	8.8	12.2	11.0	6.2	3.8	722	735	713	567^{a}
W100	41.5	39.1	20.3	8.4	11.7	10.5	5.9	3.6	724	737	717	579^{a}
SEM	0.84	0.87	0.43	0.27	0.57	0.51	0.27	0.18	9.7	8.8	9.2	13.4
Fixed variable effects							*p*-values					
Trt	0.32	0.34	0.27	0.36	0.66	0.66	0.42	0.19	0.50	0.49	0.16	0.02
Per	0.51	0.52	0.22	0.13	0.02	0.03	0.05	0.02	0.008	0.009	0.004	0.003
Trt x Per	0.32	0.35	0.26	0.25	0.30	0.28	0.20	0.22	0.53	0.47	0.49	0.38
Trial 2 (2014)												
W70	52.2	49.3	22.2	9.9	14.5^{B}	13.1^{B}	7.3^{B}	4.4	721^{A}	734^{A}	668^{A}	553^{A}
W85	52.2	49.3	22.5	10.2	16.5^{A}	14.9^{A}	8.3^{A}	4.8	683^{B}	696^{B}	630^{B}	528^{B}
W100	51.2	48.4	21.8	9.7	14.3^{B}	12.8^{B}	7.2^{B}	4.2	722^{A}	737^{A}	667^{A}	570^{A}
SEM	0.85	0.92	0.54	0.27	0.44	0.42	0.24	0.15	7.5	7.5	8.3	10.9
Fixed variable effects							*p*-values					
Trt	0.54	0.14	0.44	0.33	0.04	0.04	0.03	0.15	0.04	0.05	0.07	0.32
Per	0.002	0.002	0.0001	0.01	0.58	0.57	0.48	0.54	0.21	0.23	0.03	0.23
Trt x Per	0.03	0.02	0.04	0.04	0.26	0.27	0.24	0.41	0.22	0.26	0.49	0.76
Comparison of trials												
Year x Trt	0.70	0.67	0.81	0.75	0.12	0.11	0.07	0.24	0.056	0.056	0.036	0.09
Year x Per	0.18	0.11	0.0001	0.54	0.32	0.41	0.49	0.46	0.12	0.18	0.0003	0.07
Year x Trt x Per	0.07	0.07	0.054	0.043	0.22	0.23	0.14	0.30	0.64	0.62	0.81	0.59

LW = Live weight; DM = Dry matter; OM = Organic matter; NDFom = Ash-free neutral detergent fibre; ADFom = Ash-free acid detergent fibre; SEM = Standard error of the mean. Within columns, means with different superscripts differ at $p \leq 0.05$ (Tukey-Kramer test). Lowercase letters are for trial 1, whereas uppercase letters are for trial 2.

Table 19: Proximate composition of ingesta and faeces as measured in adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per).

	Ingesta composition (g kg^{-1} DM)				Faecal chemical composition (g kg^{-1} DM)			
Trial, water treatments	OM	N	NDFom	ADFom	OM	N	NDFom	ADFom
Trial 1 (2013)								
W70	943	13.0	482	196	896	16.0	505	324
W85	942	12.9	489	201	900	16.1	508	317
W100	941	12.9	492	202	900	15.6	508	313
SEM	4.2	0.19	9.5	2.5	1.2	0.24	5.1	4.9
Fixed variable effects								
Trt	0.32	0.34	0.27	0.36	0.57	0.11	0.99	0.43
Per	0.51	0.52	0.22	0.13	0.28	0.03	0.27	0.37
Trt x Per	0.32	0.35	0.26	0.25	0.58	0.71	0.99	0.81
Trial 2 (2014)								
W70	945	12.0	424	189	902^{A}	16.6	504	305
W85	944	11.9	432	194	905^{A}	16.6	505	291
W100	945	12.1	425	188	893^{B}	17.2	508	289
SEM	4.3	0.18	9.1	2.2	1.8	0.34	8.8	4.8
Fixed variable effects								
Trt	0.07	0.12	0.32	0.62	0.02	0.13	0.96	0.25
Per	0.71	0.0001	0.0001	0.29	0.34	0.52	0.86	0.88
Trt x Per	0.95	0.12	0.38	0.22	0.84	0.03	0.21	0.33
Comparison of trials								
Year x Trt	0.49	0.09	0.19	0.28	0.029	0.016	0.98	0.96
Year x Per	0.0001	0.0001	0.0001	0.10	0.16	0.039	0.79	0.41
Year x Trt x Per	0.58	0.08	0.29	0.33	0.84	0.029	0.33	0.61

DM = Dry matter; OM = Organic matter; NDFom = Ash-free neutral detergent fibre; ADFom = Ash-free acid detergent fibre; SEM = Standard error of the mean. Within columns means with different superscripts differ at $p \leq 0.05$ (Tukey-Kramer test). Lowercase letters are used for trial 1, whereas uppercase letters are used for trial 2.

Table 20: Nitrogen balance, excretion of purine derivatives and faecal nitrogen fractions as measured in adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per).

	Nitrogen balance (g $kg^{-0.75}$ BW)						Microbial N synthesis		Faecal N fractions (% of total N in faeces)		
Trial, water treatments	N intake	Total N excretion	Faecal N excretion	Urine N excretion	N retention	Faecal N/ Urinary N	PD excretion (mmol d^{-1})	Microbial N flow (g d^{-1})	BEDN	UDN	WSN
Trial 1 (2013)											
W70	0.64	0.35	0.20	0.14	0.29	1.48	15.1	13.3	65.6	24.7	9.7
W85	0.64	0.37	0.20	0.18	0.27	1.20	13.4	11.3	66.7	24.7	8.7
W100	0.62	0.34	0.18	0.16	0.28	1.20	13.1	10.8	66.2	24.4	9.4
SEM	0.011	0.015	0.010	0.009	0.010	0.078	0.59	0.51	0.01	0.003	0.01
Fixed variable effects						*p*-values					
Trt	0.39	0.09	0.45	0.08	0.41	0.22	0.29	0.27	0.78	0.75	0.77
Per	0.0001	0.0002	0.02	0.001	0.02	0.41	0.52	0.59	0.0003	0.51	0.0004
Trt x Per	0.30	0.19	0.42	0.32	0.90	0.79	0.25	0.24	0.57	0.52	0.49
Trial 2 (2014)											
W70	0.67	0.36	0.24^{B}	0.12	0.31	2.10^{AB}	10.0	7.9	65.1	19.3	15.7
W85	0.66	0.39	0.27^{A}	0.12	0.27	3.11^{B}	9.1	7.6	63.3	19.5	17.2
W100	0.66	0.38	0.24^{B}	0.14	0.28	1.90^{A}	9.4	7.8	61.9	19.5	18.5
SEM	0.014	0.010	0.007	0.010	0.017	0.231	0.34	0.30	0.01	0.01	0.01
Fixed variable effects						*p*-values					
Trt	0.66	0.18	0.06	0.50	0.12	0.06	0.88	0.86	0.24	0.92	0.30
Per	0.0001	0.007	0.64	0.02	0.0001	0.33	0.33	0.31	0.64	0.05	0.95
Trt x Per	0.05	0.67	0.70	0.84	0.77	0.67	0.11	0.11	0.51	0.60	0.62
Comparison of trials											
Year x Trt	0.36	0.58	0.18	0.54	0.63	0.031	0.35	0.33	0.26	0.66	0.41
Year x Per	0.0001	0.10	0.22	0.35	0.0001	0.55	0.93	0.93	0.0004	0.03	0.0048
Year x Trt x Per	0.08	0.33	0.66	0.11	0.93	0.80	0.11	0.09	0.68	0.47	0.75

N = Nitrogen; PD = Purine derivatives; BEDN = Bacteria and endogenous debris nitrogen; UDN = Undigested dietary nitrogen; WSN = Water soluble nitrogen; SEM = Standard error of the mean. Within columns means with different superscripts differ at $p \leq 0.05$ (Tukey-Kramer test).

Table 21: Partial balance of ^{15}N as a fraction of total N balance, determined from ^{15}N labelled Rhodes grass hay fed to adult male Batinah goats exposed to treatments (Trt) of no or mild restriction of water intake. Values are arithmetic means across three experimental periods (Per) in 2013.

	^{15}N Balance (g $kg^{-0.75}$ LW)				^{15}N partitioning (% of total N excreted)	
Trial, water treatments	^{15}N intake	Total ^{15}N excretion	^{15}N faecal excretion	^{15}N retention	Faeces	Urine
Trial 1 (2013)						
W70	0.163	0.064	0.064	0.099	31.5	0.053
W85	0.167	0.061	0.061	0.106	31.3	0.046
W100	0.163	0.059	0.060	0.104	32.5	0.049
SEM	0.0044	0.0019	0.0029	0.004	0.62	0.0019
Fixed variable effects						
Trt	0.47	0.72	0.72	0.18	0.32	0.36
Per	0.038	0.04	0.04	0.0016	0.006	0.07
Trt x Per	0.27	0.26	0.26	0.40	0.81	0.32

LW = Live weight; SEM = Standard error of the mean.

4.4 Discussion

4.4.1 Effect of water restriction on feed intake and diet digestibility

When ruminants are faced with water shortage, they activate several water saving mechanisms to minimize water losses and maintain essential physiological processes. One of these strategies is to reduce feed intake so as to cut on water expenditure associated with feed utilization (Silanikove, 1994). Three days of water deprivation was documented to cause an 85% reduction in feed intake in Saudi Arabian desert goats fed a diet made up of a concentrate mix and alfalfa hay (Alamer, 2006). Moreover, at 50% of *ad libitum* water intake, DM intake was reduced by 18% in Saudi Arabian Aardi goats fed a concentrate mix and alfalfa hay (Alamer, 2009). Contrary to these findings, feed intake did not change when Batinah desert goats were only offered 70% of their *ad libitum* water intake in the present study. This contradiction may be due to the level of watering as well as the relatively low hay quality given to the animals in the present study compared with the above mentioned studies. Our findings are in line with those obtained in Black Bedouin goats subjected to 48 hours of complete water deprivation and fed a pure Rhodes grass hay diet (Maltz and Shkolnik, 1984). Also, DM intake remained unaffected in Indian desert goats drinking water once every 48 hours and fed a diet consisting of pigeon pea straw, green grass and a concentrate mix (Misra and Singh, 2002). Taken together, the findings indicate that the effect of drinking water shortage on quantitative feed intake depends on the quality of the feed (good quality feed leads to higher reduction than poor quality) and the duration or degree of water restriction (more severe restriction having a clear impact on feed intake).

Restricting the access to drinking water was reported to lead to an increase in feed utilization by improving the digestibility of the diet (Ahmed and El Shafei, 2001; Igbokwe, 1997; Silanikove, 1985). This is believed to be due to an increase in the mean retention time of digesta in the gastrointestinal tract when ruminants are exposed to water restriction (Jaber et al., 2013). Our study was in line with these findings as reduced water intake was observed to increase the apparent total tract digestibility of the feed (DM, OM, NDFom and ADFom) in trial 2. However in trial 1, the apparent total tract digestibility of feed (DM, OM and NDFom) did not change when water intake was reduced. This may be due to the differences in water intake between trials: in trial 1, goats exposed to W70 treatment drunk on average 190 ml per animal and day more than

goats on W70 in trial 2. Thus the water intake level in trial 1 may have been still too high to induce significant increases in feed digestibility for the desert adapted goats.

4.4.2 Effect of water restriction on nitrogen balance, rumen microbial protein synthesis and nitrogen partitioning in urine and faeces

Water restriction has been documented to improve nitrogen retention and decrease N excretion via urine and faeces (Ghassemi et al., 2014; Ikhatua et al., 1985). In these studies, the improved N retention during water restriction was attributed to a higher N intake compared to the present study, an elevated CP digestibility and a low total N excretion. In contrast, the current study did not record a decrease in total N excretion when water intake was reduced. Therefore, the unchanged N retention between treatments in both trials was a logical consequence, which contradicts our initial hypothesis that water restriction enhances N retention. Our findings are however in line with Muna and Ammar (2001) who reported no changes in N intake or excretion in Sudan desert goats restricted to 40% of *ad libitum* water intake. Furthermore, Indian desert goats given water once every 48 hours did not improve their N retention (Misra and Singh, 2002).

In trial 2, the excreted amount of faecal N was significantly reduced with treatment W70, while the N excretion via urine remained unaltered. Similarly, South African Merino sheep subjected to 50% water restriction and fed a low-nitrogen diet were observed to reduce their faecal N excretion without altering their urine N excretion (van der Walt et al., 1999). Moreover in their study, van der Walt et al. (1999) observed that the glomerular filtration rate was not affected by water restriction. It is therefore assumed that under water restriction, the first response to nitrogen retention may be through the gastrointestinal tract rather than through the kidney, because of the reduced faecal N and unaffected urine N.

Improved N utilization is stated to be the most attractive strategy to reduce N losses as it affects the route of N excretion in ruminants (Marini and van Amburgh, 2005). From this perspective, faecal N excretion is preferred to urinary N excretion, since less N losses are linked to faecal as compared to urine excretion (Mason, 1969). The ^{15}N data shows that more ^{15}N was excreted via faeces. However, no changes were observed between the three treatments with respect to nitrogen partitioning which refutes our

hypothesis that water restriction will lead to a shift in nitrogen excretion from urine to faeces.

The mean urinary PD excretion values recorded in the present study were comparable to values reported from studies with goats fed rye grass hay and concentrate mix at 1.5 times maintenance energy requirement (Al-Kindi et al., 2016) and alfalfa hay at 1.3 times maintenance energy requirement (Carro et al., 2012). However, mild water restriction had no effect on the urinary PD excretion in this study. This may be due to the linear relationship between feed intake and urinary PD excretion (Braga et al., 2012; George et al., 2011; Singh et al., 2007). Since feed intake remained unaffected by the tested watering regimes, the unaltered PD excretion was to be expected.

4.4.3 Effect of water restriction on faecal excretion and nitrogen fractions

When examining the data on quantitative faecal excretion of DM, OM and NDFom (in g $kg^{-0.75}$ LW), it emerges that response to water restriction followed the expected pattern of an increasing apparent total tract digestibility and therefore a decreasing amount of faeces excreted in trial 2. This is in line with results obtained by van der Walt et al. (1999) who observed a decrease in faecal OM excretion when desert adapted sheep were subjected to 50% water restriction and fed a low nitrogen diet. Furthermore, a 50% water restriction reduced the total faecal output in cattle fed a mixed diet of grass silage, corn silage and hay (Burgos et al., 2001). The decrease in faecal output following water restriction is attributed to an increased re-absorption of moisture from the lower gastrointestinal tract (Bohra and Ghosh, 1977; van der Walt et al., 1999), so as to minimize water losses.

Water restriction had no effects on the total faecal N concentration (g kg^{-1} DM) in the present study. Similarly, no differences between treatments were observed in the different fractions of faecal N in both trials. This may be due to the low nitrogen content in the feed given to the animals. However, the values recorded for the faecal N fractions followed a similar pattern as reported for sheep (Bosshard et al., 2011), whereby BEDN was made up for the major proportion of faecal N followed by UDN and WSN, respectively.

4.5 Conclusions

Water restriction to 70% of the *ad libitum* water intake did not affect feed intake in desert breed goats. Also, there was no shift observed in nitrogen excretion from urine to faeces, neither in absolute nor relative terms. However, diet digestibility (DM, OM, NDFom, ADFom) and N retention were improved at 70% water restriction in both trials, and water losses through faeces and urine as well as faecal nitrogen excretion were reduced. Thus, a restriction of water intake to 70% of the *ad libitum* water intake seems to slightly improve feed utilization while reducing N losses via excreta and subsequent volatilisation.

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Chapter 5

General discussion and conclusions

5.1 General Discussion

This chapter summarizes the most important points of the previous three research chapters. It departs from a critical discussion of methodological aspects of the two trials and then elaborates on key insights and the implications for nutrient recycling. It closes with the perspectives of the study, testing of the research hypotheses and some concluding remarks.

5.2 Discussion of methodology

5.2.1 Experimental design

In order to determine the effects of water restriction, a 3 x 3 Latin square design was used in the current study, as illustrated in figure 5. The two trials conducted in 2013 and 2014 comprised nine animals exposed to three treatments of drinking water allowance and three experimental periods. This design has been used in a few other water restriction studies in South Korea on Corriedale sheep (Ghassemi et al., 2014), in Greece on Karagouniko sheep (Hadjigeorgiou et al., 2000) and in Sudan on Sudanese desert goats (Muna and Ammar, 2001). However, other studies on ruminants exploring the effects of water deprivation and restriction frequently employed a completely randomized design as the experimental approach (Abdelatif and Ahmed, 1994; Al-Ramamneh et al., 2012; Fluharty et al., 1996; van der Walt et al., 1999). Randomization procedures do not balance residual effects (Kim and Kim, 2010) and enable isolation of only one nuisance variable (Kirk, 1982). A Latin square design goes beyond the randomized design and can isolate two nuisance variables (in the present case: the period and the animal effect), therefore the Latin square design is considered more powerful than the randomized procedure (Kirk, 1982). Furthermore, the Latin square design allows animal scientists to reduce the required number of animals for detecting statistical differences in animal experiments and for this reason it is a more efficient approach (Kim and Kim, 2010).

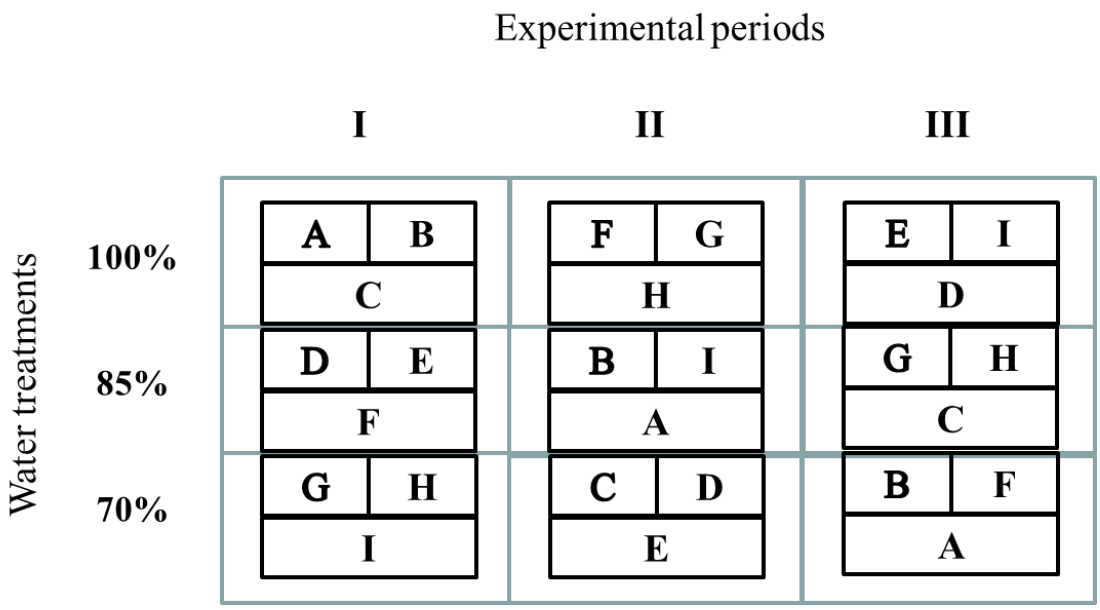

Figure 5: Experimental design of two water restriction trials conducted in 2013 and 2014 at Sultan Qaboos University, Muscat, Oman. The letters (A-I) represent the nine goats which were allocated each time to three water treatments (left-hand label) across three experimental periods (top label).

5.2.2 Quantification of faecal microbial biomass

Ruminant faeces contain a highly variable community of bacteria, archaea and fungi (Jost et al., 2011), which stems from the animal's digestive tract. Throughout the digestive tract, faecal material is exposed to anaerobic conditions. However when voided, faeces are exposed to oxygen-rich conditions. Thus the activity of the anaerobic microbes in faeces cease as soon as aerobic conditions occur, whereas facultative anaerobes switch to aerobic metabolism (van Vliet et al., 2007), rendering the activity of the microbial community in faeces highly dynamic (Jost et al., 2011; van Vliet et al., 2007). Methods of analysis of faecal microbial communities should therefore be able to accommodate this variability.

The classical most probable number and plate count techniques underestimate the total bacterial population and discriminate against non-cultivable microbes (Ouwerkerk and Klieve, 2001). Furthermore, the unknown representation of cultivable microbes has raised doubt in converting counts into microbial biomass (Ritz, 2007). Direct microscopic approaches underestimate fungal microbial biomass (Joergensen and Wichern, 2008). Deoxyribose nucleic acid (DNA) extraction accompanied by analysis of its composition provide important information on the microbial community in substrates (Sekhavati et al., 2009; van Vliet et al., 2007). However, DNA approaches do not provide information on faecal microbial biomass due to losses during extraction and

unknown or highly variable DNA concentrations within microbial species (Joergensen and Emmerling, 2006; Wentzel and Joergensen, 2015). The adenosine triphosphate (ATP) method would be an ideal alternative. This method measures microbial adenylate content in soil and plant substrates (Dyckmans et al., 2006). However, as faecal samples are variable in terms of the anaerobic and micro-aerobic environment, ATP concentration within faecal microbial biomass is more variable than in other substrates (Dyckmans et al., 2006). Therefore, the methods relying on determining the amino sugars and ergosterol were used in the present study to quantify the faecal microbial biomass (Chapter 2). The advantage of this approach is that there are less extraction losses (Jost et al., 2011) and no biases are reported against certain microbial species when using the amino sugars and ergosterol methods (Jost et al., 2013). The analyses also allow a fast, quantitative and reproducible determination of faecal microbial biomass (Indorf et al., 2011; Wentzel and Joergensen, 2015). Moreover, the thus obtained values are comparable with those obtained with other methods (Jost et al., 2011), such as chitin concentration (Sekhavati et al., 2009).

5.3 Effect of water restriction on digestion and nitrogen metabolism

Water is essential for life and should be available to livestock in good quality and sufficient amounts. However, in arid and semi-arid areas of the tropics and subtropics, rainfall has always been and is highly variable, and with climate change rainfall is believed to become even more variable and water availability more limited in these regions (Jaber et al., 2013). Animals in arid and semi-arid regions of sub-Saharan Africa and in the drylands of the Near and Middle East, for example, may face mild water shortage during mid-dry season or complete water shortage during late dry season when watering sources have fallen dry. Water shortage is thus a normal phenomenon for livestock species reared in such region.

There are two types of water shortage that ruminants experience, (1) water deprivation and (2) water restriction. Water deprivation is usually associated with time (i.e. temporal water scarcity), whereas water restriction is related to volume (i.e. volumetric water control). Water deprivation and restriction have been observed to cause similar effects in ruminant species with respect to feed intake and utilization (Mousa et al., 1983; Silanikove, 1992). For instance, three days of water deprivation were reported to cause an 85% reduction in feed intake in Saudi Arabia indigenous goats (Alamer, 2006),

while water restriction to 50% of *ad libitum* water intake reduced DM intake by 18% in Aardi goats (Alamer, 2009). We see that both water deprivation and restriction suppress feed intake. However, the magnitude as well as the physiological basis underlying the changes/effects differ (Silanikove, 1992). Water deprivation induces a decrease in feed intake as a result of depletion of body fluids, whereas water restriction causes a decline in feed intake due to food related drinking (Silanikove, 1992). Food related drinking is derived from a basic relationship of the proportional exchange of energy and water (Silanikove, 1989). Thus, variation of one of the variables (food or water) will lead to a proportional change of the other (Silanikove, 1992). Eating less during water restriction helps to maintain the osmotic balance because a decrease in feed intake reduces the impact of an osmotic load brought about by food (Burgos et al., 2001). Failure of ruminants to suppress feed intake during water restriction might compromise the osmotic buffer function of the rumen, resulting in an increase in rumen fluid osmolality. This may prevent the use of rumen water to alleviate the systemic hypertonicity that occurs during water restriction. The drop in feed intake therefore reflects a homoeostatic mechanism that reduces the negative consequences of water restriction (Burgos et al., 2001).

Despite the established linear relationship between feed and water intake, it has been reported that desert adapted Bedouin goats did not reduce their intake of Rhodes grass hay when subjected to 48 hours of complete water deprivation (Maltz and Shkolnik, 1984). Also, DM intake remained unaltered in Indian desert goats drinking water once every 48 hours and fed a diet consisting of pigeon pea straw, green grass and a concentrate mix (Misra and Singh, 2002). A similar effect was observed in Batinah goats exposed to 70% of *ad libitum* water intake and fed Rhodes grass hay and barley grains (Chapters 2, 3 and 4). On the other hand, the same level of water restriction (73% of *ad libitum* water intake) was found to reduce feed intake by 11% in cross-bred goats (75% German Fawn and 25% Turkish Hair Goat) fed concentrate and alfalfa hay (Kaliber et al., 2015). The ability of desert adapted goats to maintain feed intake during water restriction and deprivation is due to their ability to alleviate an increase in plasma osmolality (Silanikove and Tadmor, 1989) through desalting occurring in the kidney which enables the use of rumen water (Silanikove, 1992). In addition, the maintenance energy requirement of desert breeds is low therefore they are able to maintain a level of feed consumption which is well above their maintenance requirement (Silanikove,

1985). It therefore appears that the relationship between feed and water intake depends on the adaptability of the breed to water scarcity.

The increase in feed digestibility when ruminants are exposed to water restriction is believed to be due to an increase in the mean retention time of digesta in the gastrointestinal tract (Brosh et al., 1986a; Silanikove, 1992), as more time is made available for the microflora in the digestive tract to act on the feed (Brosh et al., 1986a; Hadjigeorgiou et al., 2000). According to their review, Jaber et al. (2013) proposed that further research is needed to ascertain this hypothesis, as reports seem to be inconclusive (Chapter 2). Other factors that might play a role for the increase in diet digestibility during water restriction include the decrease in saliva production and flow rate (Igbokwe, 1997; Silanikove and Tadmor, 1989), the decrease in feed intake (Freudenberger and Hume, 1993), an increase in rumen motility and rumination activity (Igbokwe, 1997) as well as increased rumen fermentation (Chapter 3).

The bacterial community in the rumen remained unaffected, both in terms of concentration and numbers when calves were deprived of water for 72 hours (Fluharty et al., 1996). Likewise, the bacterial community in the hindgut was not affected when Batinah goats were restricted to 70% of *ad libitum* water intake (Chapter 2). It is therefore not surprising that the faecal bacterial and endogenous debris nitrogen (BEDN) fraction was not affected when Batinah goats were exposed to 70% of *ad libitum* water intake (Chapter 4). Further, since bacteria thrive in the presence of N-rich substrates (van Vliet et al., 2007) and that fungi thrive in the presence of C-rich substrates, the increase in faecal fungal biomass of water restricted animals reflects a decreased N-availability to the microbial population in the hindgut and hence lowered N-incorporation into its microbial flora (Jost et al., 2013). This may ultimately have positive consequences on animal performance as more N will apparently be utilized by the animal.

5.4 Relation of the study to nutrient recycling

As mentioned in Chapter 1, the current study was embedded in the framework of the Research Training Group 1397 “Regulation of soil organic matter and nutrient turnover in organic agriculture”. The main aim of the overarching project was to study the underlying processes of soil organic matter and nutrient turnover, as affected by

management in general and fertilization in particular, and thus highlight suitable strategies for long-term preservation of soil fertility in agricultural systems.

For organic fertilizers, Lashermes et al. (2010) identified lignin, cellulose, initial N content and soluble organic fractions as the main determinants for potentially mineralizable N in soils. The faeces excreted when goats were constrained to 70% of *ad libitum* water intake were characterized by a high concentration of ADFom (Chapters 2 and 4). This fraction essentially contains lignified cellulose, therefore faeces from water restricted animals may be slowly degradable (Al-Asfoor et al., 2012). Moreover, N that is bound to the ADFom fraction may not be available to soil micro-organisms and plants in the short term, due to the slow decomposition of lignin (Al-Asfoor et al., 2012). Whereas nitrogen that is rapidly mineralised from organic fertilizers is prone to leaching and/or volatilization losses, the N bound to ADFom will not be easily lost to the environment and may thus be available for plant uptake in the long term (Chadwick et al., 2000).

Even though the anticipated shift in nitrogen excretion towards faeces (Chapter 1, Hypothesis 3) was not observed (Chapter 4), mild water restriction may still play a vital role in reducing environmental nitrogen losses from goat excreta: One distinct outcome of mild water restriction (70% of *ad libitum* water intake) is the shift in the faecal microbial community towards fungi (Chapter 2). Faeces dominated by fungal communities were reported to emit less carbon dioxide and nitrous oxide than faeces with less fungal communities (Jost et al., 2013). Nitrous oxide is considered a major contributor to global warming having a 310 times more harmful mass-specific effect than carbon dioxide as a global warming agent (IPCC, 1995). The prospect that faeces with a relatively higher fungal mass emit less carbon dioxide and nitrous oxide than faeces with a relatively lower fungal mass should be investigated further in view of reducing (climate relevant) emissions from ruminant holdings.

5.5 Perspectives of the study

Information on the ability of ruminants to withstand water shortage is of practical importance for situations in which the animals are not watered daily or experience temporary water shortage, even in temperate regions. In temperate regions, water may at times be insufficient particularly in the course of weaning and during early lactation in high yielding dairy animals. Moreover, pathological situations such as diarrhoea, rumen

acidosis and other diseases can cause mild systemic dehydration (Burgos et al., 2001). In arid and semi-arid areas, water supply is infrequent and there are periods during the year when animals are exposed to mild water shortage. Besides, in pastoral rangelands, concentration of large livestock herds around spatially scattered water resources can create situations where water is not available to all livestock. Therefore the present study highlights the processes that occur when animals are exposed to such situations.

The increase in feed digestibility (Chapter 2) and rumen protein and carbohydrate degradation (Chapter 3) during mild water restriction ensure that the animal can best utilize the available feed resources. This is crucial especially in arid and semi-arid areas where feed is of poor quality i.e., high in fibre and low in nitrogen contents. Moreover, the maintenance of feed intake during mild water restriction (Chapter 4) guarantees that the nutrient requirement of the animal is not compromised. Thus the unaltered feed intake and increased feed digestibility during mild water restriction denotes the ability of ruminants to minimize the negative consequences of an imbalanced water economy.

5.6 Testing the hypotheses

In the following section, the hypotheses guiding the present research are revisited and checked for validity. A summary of the responses (R) to each of the three hypotheses is provided in Figure 6.

Hypothesis 1: Mild water restriction increases (a) feed digestibility, (b) mean retention time of digesta in the gastrointestinal tract and (c) faecal microbial biomass.

This hypothesis is partly accepted (R1a), since mild water restriction (to 70% of *ad libitum* intake) increased feed digestibility (Chapters 2 and 4). However, the mean retention time of digesta and the faecal microbial biomass did not increase under mild water restriction (R1 b and c).

Hypothesis 2: Mild water restriction will increase (a) rumen fermentation characteristics as well as (b) rumen microbial yield and composition.

This hypothesis is partly accepted (R2a) since mild water restriction (to 70% of *ad libitum* intake) increased rumen concentrations of butyrate and ammonium-N (Chapter 3). Nevertheless, rumen microbial yield and composition were not affected when drinking water was restricted (R2b).

Hypothesis 3: Mild water restriction will (a) lead to a positive nitrogen balance and (b) increase faecal nitrogen excretion.

This hypothesis is in part accepted (R3a) as a positive nitrogen balance was observed when goats were exposed to mild water restriction (Chapter 4). However, no increase in faecal nitrogen excretion was observed when the goats were subjected to a mild restriction of drinking water (R3b).

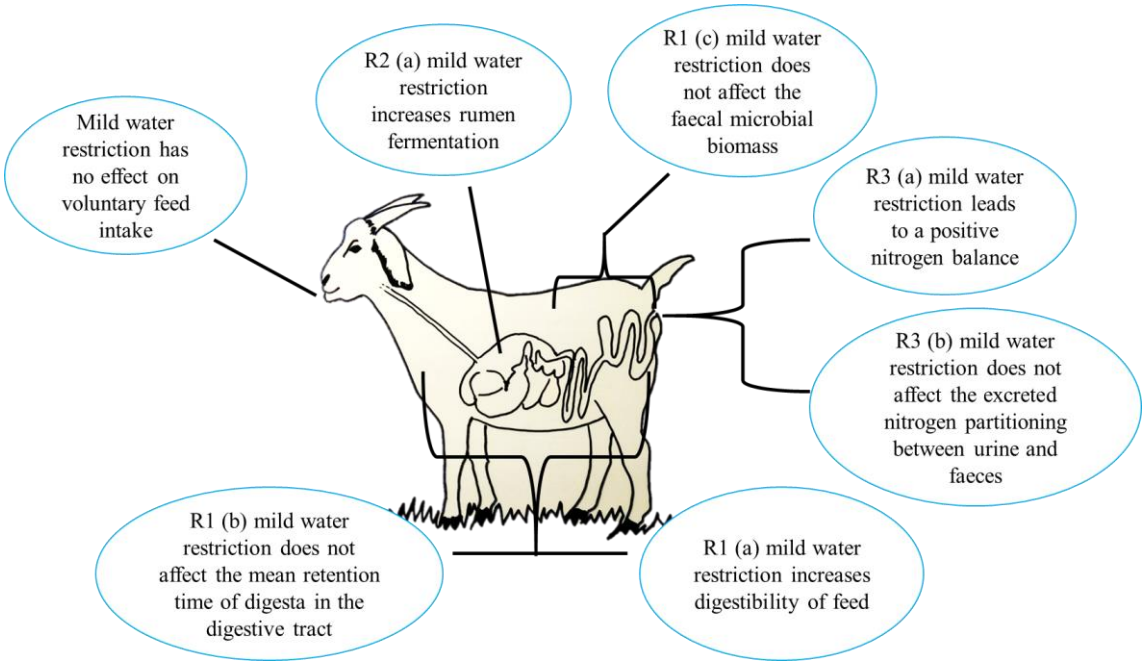

Figure 6: Chart demonstrating the digestive tract of the goat and depicting the results (R) that emerged from testing the three hypotheses on the effects of mild water restriction in desert-adapted goats. Sketch by eS, 03.02.2017.

5.7 Concluding remarks

Livestock in arid and semi-arid areas are sometimes exposed to water shortage in the form of total or partial restriction of access to (good quality) drinking water. This may have positive or negative consequences on the animals' physiology, depending on the extent of dehydration, the adaptability of breed and the environmental conditions. The results of the present study allow concluding that:

- Desert adapted goats can easily cope with a mild water restriction without lowering their feed intake.
- Mild water restriction slightly improves the digestibility of diets low in nitrogen content, which often prevail in arid and semi-arid areas during the dry season.

- Under mild water restriction, the improved diet digestibility is explained by an increase in rumen fermentation rather than by prolonged digesta retention in the gastrointestinal tract.
- When animals are exposed to mild water restriction, faecal fungal microbial biomass is increased.
- Mild water restriction does not cause a shift in nitrogen excretion between faeces and urine, but increases the concentration of ADFom in faecal matter; this slows down short term nutrient mineralization in the soil making nutrients plant-available in the longer term.

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