Influence of quebracho tannin extract and activated charcoal on nutrient intake and digestibility, digesta passage, nitrogen balance, and quality of faecal excreta in goats

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This work has been accepted by the Faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree of Doktor der Agrarwissenschaften (Dr. agr.).

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الإهداء

إلى من علموني علم الحياة: والدي سعيد الكندي ووالدتي شمسة الجابرية

إلى وطني وسكر حياتي أختاي: شريفة وفاطمة

إلى عزوتي في الحياة إخواني: موسى عبدالله ومحمد

Pour la personne qui a donné le sens à ma vie

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Chapter 2

Abbreviations

λ	Rate of particle passage through the mixing compartment
AC	Activated charcoal
ANOVA	Analysis of variance
ADF	Acid detergent fibre
ADIN	Acid detergent insoluble N
C	Carbon
CH_4	Methane
CMRT	Particle mean retention time in the mixing compartment
CO_2	Carbon dioxide
CP	Crude protein
DM	Dry matter
DOMR	Digestible OM apparently fermented in the rumen
EDTA	Ethylene diamine tetraacetic acid
EMPS	Efficiency of microbial protein synthesis
FM	Fresh matter
GHG	Greenhouse gas
H_2	Hydrogen
HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric acid
HPLC	High-performance liquid chromatography
KOH	Potassium hydroxide
ME	Metabolizable energy
MNS	Microbial N supply
N	Nitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NO_3^-	Nitrate
NDF	Neutral detergent fibre
NDIN	Neutral detergent insoluble N
OM	Organic matter
OPA	o-phthalaldehyd
PD	Purine derivatives
PEG	polyethylene glycol
QTE	Quebracho tannin extract
SEM	Standard error of the mean
SOC	Soil organic carbon
SOM	Soil organic matter
THF	Tetrahydrofuran
TT	Time of first marker appearance in faeces
T_{50}	Halftime of the marker in the mixing compartment
TMRT	Particle mean retention time in the total gastrointestinal tract
Yb	Ytterbium
$ZnCl_2$	Zinc chloride

Summary

The current study investigated the possibilities of producing manure that meets the specific requirements of irrigated organic cropping systems under arid sub-tropical conditions. In such environments maintaining soil organic matter requires substantial application of manure since year round high temperatures and flood irrigation induce high carbon (C) and nitrogen (N) turnover. Feeding ruminants with condensed tannins (CT) will lower ruminal protein degradation, reduce urinary N excretion and might increase the faecal fraction of slowly decomposable N; the inclusion of activated charcoal (AC) into the diet might enrich the animals' manure with long-lived C species to be sequestered in the soil. Such manure might also help reducing emission of greenhouse gases and provide a suitable environment for microbial growth in soil. Microbial biomass in faeces is also expected to change upon AC and CT feeding to ruminants, due to alteration in their digestion processes. Faecal microbial biomass plays a critical and direct role for the build-up of soil organic matter and the subsequent cycling of C and N.

Against this background two completely randomised feeding trials with goats were conducted to evaluate the effects of adding 2% and 4% quebracho tannin extract (QTE, a source of CT; QTE2, QTE4), 1.5% and 3.0% AC (AC1.5, AC3.0) and a mixture of 2% QTE plus 1.5% AC (QTEAC) to the total diet (on dry matter basis; DM) on intake of feed and nutrients and their digestibility, passage rate of digesta, N balance and partitioning of N excretion between urine and faeces, rumen microbial protein synthesis and faecal microbial biomass.

The first trial was conducted in Oman (February - April 2011) with 24 male Jabal Akhdar goats (37 kg ±3.1) for three subsequent periods, in which 21 adaptation days were followed by 7 sampling days. Goats were offered 800 g DM of a basal diet (control) which consisted of 50% Rhodes grass hay (Chloris gayana Kunth), 46.5% crushed maize and 3.5% soybean meal (on DM basis). The parameters determined from samples of feed, refusals and faeces were intake of feed and nutrients and their digestibility, and passage rate of digesta. The latter was computed with a one compartment age-dependent Gamma-2 model based on a pulse dose application of Ytterbium-mordanted fibre.

Inclusion of QTE and AC had no significant effects on intake of feed and nutrients. While the digestibility of DM and organic matter (OM) as well as crude protein were reduced by QTE2 and QTE4 ($P<0.001$), the digestibility of neutral and acid detergent fibre (NDF, ADF) were decreased in QTE4, QTEAC and the two AC treatments ($P<0.001$). The outflow of particles from the rumen was unaltered by AC and QTE

inclusion. Moreover there was no influence of digesta passage parameters on the quality of faeces except for the correlation between total tract mean retention time and faecal C/N ratio ($r = -0.35$, $P<0.05$). Feeding QTE and AC did not enhance the concentration of faecal N ($P>0.05$). However, AC increased the faecal concentrations of NDF, ADF and C ($P<0.001$), and QTE4 in tendency also led to an increase in the faecal concentration of slowly decomposable carbohydrates in the form of ADF.

The second trial with 12 male Boer goats (28 kg ±3.9) was also composed of three subsequent periods: each of 21 adaptation days were followed by 6 sampling days. This trial was conducted at the premises of Göttingen University from September to November 2011. The control diet consisted of 50% grass hay (Lolium perenne L.) and 50% pelleted concentrate feed (35% barley, 35% wheat, 15% rape seed meal, 15% sugar beet molasses chips; all on DM basis), which was offered at 1.5 times maintenance energy requirements. In addition to quantifying intake of feed and nutrients and their digestibility, N was measured in both urine and faeces to compute the N balance. Microbial nitrogen supply (MNS) from the rumen was calculated from urine excretion of purine derivatives (PD), while the concentrations of ergosterol and amino sugars were used to determine fungal and bacterial communities in faecal microbial biomass.

Again QTE and AC had no significant effect on quantitative intake of feed and nutrients, however the digestibility of OM, NDF, and ADF were reduced ($P<0.05$). QTE induced a shift in N excretion from urine to faeces ($P<0.001$) without altering N retention despite reduced N digestibility ($P<0.01$) as compared to the control and the AC treatments. MNS and efficiency of microbial protein synthesis were also not influenced by feeding QTE and AC. Faecal concentration of slowly decomposable carbohydrates increased with QTE (mainly ADF) and AC feeding ($P<0.001$). Total microbial C was not altered by the dietary inclusion of QTE and AC, but QTE shifted the microbial community structure towards fungi ($P<0.01$).

From the above results it was concluded that including to up to 4% QTE into the diet of goats will increase the faecal concentration of slowly degradable N (tannin-protein complexes) by lowering protein degradation in the rumen without affecting N retention. Feeding QTE also enhances the concentration of slowly decomposable C (in the form of ADF) in manure, and shifts microbial biomass towards fungi. The latter is an extra merit since such manure might grant a longer-term N supply in the soil due to lower gaseous emissions and stronger N immobilization. Feeding up to 3% AC has also the potential to produce manure that meets the specific requirements of irrigated arid sub-tropical cropping systems through its content of long-lived C species and slowly decomposable carbohydrate fractions. In long term this might also help reducing N losses through

gaseous emissions or leaching, as slowly decomposable C will help stabilizing soil organic matter. Simultaneous QTE and AC feeding at 2% and 1.5% resulted in a combination of the beneficial effects of both components.

Zusammenfassung

Die vorliegende Arbeit untersuchte Möglichkeiten zur Bereitstellung von tierischem Dung der den spezifischen Anforderungen des bewässerten ökologischen Anbaus unter ariden subtropischen Bedingungen genügt. Hierbei sind insbesondere eine verlangsamte Freisetzung von Kohlenstoff (C) und Stickstoff (N) von Bedeutung, da die ganzjährige Oberflächenbewässerung in Kombination mit hohen Temperaturen zu hohen C und N Emissionen sowie N Auswaschung führen. Eine Verfütterung kondensierter Tannine (CT) reduziert beim Wiederkäuer den Proteinabbau im Pansen und damit die renale Ausscheidung von Harnstoff, während die Konzentration an langsam abbaubaren Stickstoffverbindungen im Kot steigt. Durch eine Verfütterung von Aktivkohle (AC) wird der Kot mit langlebigen Kohlenstoff-Verbindungen angereichert, was durch verlangsamte Abbauprozesse im Boden auch zur Minderung der Emission von Treibhausgasen beiträgt. Die Fütterung von CT und AC wirkt sich außerdem auf das mikrobielle Wachstum im Pansen und Dickdarm aus; die mit dem Kot ausgeschiedene mikrobielle Biomasse spielt eine entscheidende Rolle für Auf- und Umbau organischer Substanz im Boden und den C und N Kreislauf. Vor diesem Hintergrund war es Ziel der vorliegenden Arbeit, die Wirkung der Zugabe von CT und AC zur Ration von Ziegen auf Aufnahme und Verdaulichkeit des Futters und der Nährstoffe, der Passagerate von Futterpartikeln, der Stickstoffbilanz und der mikrobiellen Proteinsynthese im Pansen sowie der Qualität des Kotes und der darin enthaltenen Gruppen an Mikrobiota zu bestimmen.

Zu diesem Zweck wurden zwei vollständig randomisierten Fütterungsversuche mit Ziegen durchgeführt. Als Quelle von CT wurde pulverisierter Quebrachotannin-Extrakt (Schinopsis balansae Engl.) verwendet, welcher einer Kontrollration in Konzentrationen (in der Trockensubstanz, TS) von 2% (QTE2) und 4% (QTE4) beigemischt wurde. Außerdem wurden zwei Rationen mit einer Konzentration von 1,5% und 3,0% AC erstellt (AC1.5, AC3.0), sowie eine Mischung aus 2% QTE plus 1,5% AC (QTEAC). In einem ersten Versuch in Oman (Februar bis April 2011) wurden 24 Jabal Akhdar Böcken (37 kg ± 3,1) täglich je 800 g TM einer Ration aus 50% Rhodesgras-Heu (Chloris gayana Kunth), 46,5% Maisschrot und 3,5% Sojaschrot angeboten (Kontrolle), die genannten Konzentrationen an QTE und AC wurden der Kraftfutterkomponente beigemischt und pelletiert. In den drei experimentellen Perioden (je 7 Tage), denen jeweils eine Anfütterungsphase (je 21 Tage) vorausging, wurden Futter- und Nährstoffaufnahme sowie deren Verdaulichkeit bestimmt. Die Passagerate der Futterpartikel durch den Verdauungstrakt wurde anhand einer oralen Pulsdosis von Ytterbium-markierter Faser,

der quantitativen Ausscheidung von Ytterbium im Kot und einer Gamma-2-Funktion ermittelt.

Die Inklusion von QTE und AC hatte keine wesentlichen Auswirkungen auf die Futter- und Nährstoffaufnahme. Während die Verdaulichkeit der TS und der organischen Substanz (OS) sowie des Rohproteins (XP) durch QTE2 und QTE4 reduziert wurde ($P<0,001$), war die Verdaulichkeit der Neutralen und der Sauren Detergenz-Faser (NDF, ADF) in den Behandlungen QTE4, QTEAC, AC1.5 und AC3.0 herabgesetzt ($P<0,001$). Die Partikelpassage durch den Pansen wurde durch die AC und QTE Aufnahme nicht beeinflusst. Mit Ausnahme der Korrelation zwischen der Gesamtverweildauer der Partikel im Verdauungstrakt und des C/N-Verhältnisses im Kot

($r = -0,35$; $P<0,05$) ergab sich kein Einfluss von Parametern der Partikelpassage auf die Kotqualität. Die Fütterung von QTE und AC hatte auch keinen Einfluss auf die N-Konzentration im Kot ($P>0,05$), allerdings erhöhte AC dessen Konzentration an NDF, ADF und C ($P<0,001$). Auch QTE4 führte zu einem geringfügigen Anstieg ADF-Konzentration im Kot.

Der zweite Versuch war von der Anstellung her identisch mit dem ersten, er wurde mit 12 männlichen Burenziegen (28 kg ± 3,9) an der Universität Göttingen durchgeführt (September bis November 2011). Die Grundration (TS-basiert) aus 50% Heu (Lolium perenne L.) und 50% pelletiertem Kraftfutter (35% Gerste, 35% Weizen, 15% Rapssaat, 15% Zuckerrübenschnitzel) wurde in einer Menge angeboten die individuell dem eineinhalbfachen energetischen Erhaltungsbedarf entsprach. Neben der Bestimmung der Futter- und Nährstoffaufnahme und deren Verdaulichkeit wurde die N-Ausscheidung in Urin und Kot quantifiziert, um die N-Bilanz zu bestimmen. Die mikrobielle Stickstoffversorgung aus dem Pansen wurde anhand der Ausscheidung von Purinderivaten (PD) im Harn ermittelt, die Charakterisierung der pilzlichen und bakteriellen Biomasse im Kot basierte auf den Konzentrationen von Ergosterol und Aminozuckern.

Auch in diesem Versuch hatten QTE und AC keinen signifikanten Einfluss auf die quantitative Aufnahme an Futter und Nährstoffen, allerdings wurde die Verdaulichkeit der OS, NDF und ADF reduziert ($P<0,05$). Die Verabreichung von QTE induziert eine Verschiebung der N-Ausscheidung von Urin nach Kot ($P<0,001$), dennoch war trotz einer verringerten N-Verdaulichkeit ($P<0,01$) im Vergleich zur Kontrolle und den AC Behandlungen die Stickstoffretention nicht reduziert. Die mikrobielle Proteinsynthese im Pansen und deren Effizienz wurde durch QTE und AC nicht beeinflusst. Im Kot bewirkten QTE und AC Zulage eine Erhöhung ($P<0,001$) der Konzentration an langsam abbaubaren Strukturkohlenhydraten (hauptsächlich ADF). Die Kot-Konzentration an

mikrobiellem Kohlenstoff wurde durch Zugabe von QTE und AC nicht verändert, allerdings bewirkte QTE ein Verschiebung der mikrobiellen Biomasse im Kot hin zu Pilzen ($P<0,01$).

Die Ergebnisse lassen die Schlussfolgerung zu, dass die Inklusion von bis zu 4% QTE in die Ration von Ziegen die Kot-Konzentration von schwer abbaubaren Tannin-Protein-Komplexen erhöht; dies erfolgt durch Reduktion des Proteinabbaus im Pansen welche jedoch die N-Verwertung des Tieres nicht beeinträchtigt. Gleichzeitig steigt durch QTE-Gabe die Konzentration an langsam abbaubarem C im Kot (in Form von ADF); dies sowie die ebenfalls mit der Fütterung von QTE verbundene Verschiebung der mikrobiellen Biomasse im Kot hin zu Pilzen kann im Boden zu einer initialen Immobilisierung von N sowie langsamerem N und C Abbau aus Dung führen und so zur Emissionsreduktion beitragen. Die Inklusion von bis zu 3% AC in die Ration von Ziegen hat ebenfalls das Potenzial einen Dung zu generieren, der den spezifischen Anforderungen des Bewässerungslandbaus in ariden subtropischen Regionen genügt: durch seinen Gehalt an langsam oder kaum abbaubaren Kohlenstoffverbindungen (ADF und karbonisierte Partikel) stabilisiert er den A-Horizont des Bodens. Bei regelmäßiger Ausbringung eines solchen Dungs kann mittelfristig auch mit einer Verringerung der Stickstoffverluste durch gasförmige Emissionen oder Auswaschung gerechnet werden. Bei gleichzeitiger Verfütterung von 2% QTE und 1,5% AC konnte eine Kombination der vorteilhaften Effekte beider Komponenten beobachtet werden.

1. General introduction

1.1 Background

Sustainability of irrigated organic cropping systems under the arid sub-tropical conditions of Oman, in which crop residues are removed, requires high application of manure to maintain soil organic matter (Buerkert et al., 2010; Sradnick et al., 2014). In such environment nitrogen (N) and carbon (C) from organic manures are prone to losses through leaching and gaseous emissions due to elevated temperatures and frequent wet-dry cycles associated with flood irrigation (Siegfried et al., 2013). Under such conditions, organic fertilizers should therefore contain slowly degradable N as well as slowly decomposable carbohydrates with a long-term stabilizing effect on soil organic matter (Hassink, 1994; Janssen, 1996; Handayanto et al., 1997; Kyvsgaard et al., 2000; Van Kessel et al., 2000). Previous studies have shown that the quality of feed ingested by ruminants influences faecal N and fibre concentrations as well as the relative partitioning of N into faecal fibre fractions (Powell et al., 2006, 2009; Petersen et al., 2007; Van Vliet et al., 2007; Al-Asfoor et al., 2012). It furthermore affects manure-related C and N cycling in soils and hence the accumulation of soil organic matter and the availability of nutrients for plant uptake (Somda and Powell, 1998; Powell et al., 2006; Van Vliet et al., 2007). Moreover, diet characteristics have an impact on the faecal concentration and diversity of microorganisms, which together with the macro- and mesofauna as well as the microbial population of the soil affect the decomposition of faeces, the subsequent cycling of C and N, and the utilization of nutrients for crops' growth (Handayanto et al., 1997; Chadwick et al., 2000; Delve et al., 2001; Petersen et al., 2007; Van Vliet et al., 2007; Jost et al., 2013). Faecal microbial biomass has also a direct influence on the autochthonous soil microbial biomass and microbial residues that will subsequently contribute to soil organic matter (Sradnick et al., 2014). The current study was conducted within the framework of the Research Training Group 1397 "Regulation of soil organic matter and nutrient turnover in organic agriculture" (http://www.uni-kassel.de/fb11/dec/research-training-group-1397_en.html) funded by Deutsche Forschungs-gemeinschaft (German Research Foundation). The current research project (RTG 1397-D2) focused on the impacts on soil organic matter and nutrient turnover by slowing down the release of C and N from goat manure through feed amendments. These manures should meet the specific requirements of irrigated crop farming under arid sub-tropical conditions (Siegfried et al., 2013), namely containing slowly decomposable C and N fractions; they were tested for these characteristics in field experiments with radish and sweet corn by the partner vegetable production project (RTG 1397-F1). Goats were chosen as experimental animals because they are the main source of meat and milk in Oman, and are preferred by Oman's citizens over cattle and sheep. For this reason, goats

constitute two thirds of the national livestock herd and are considered as an important source of income for smallholders (El Tahir and Nair, 2011).

1.2. Slowing down N and C release from manure

1.2.1 Feeding condensed tannins (CT)

Tannins are water- soluble polyphenolic polymers that occur in many feedstuffs including fodder legumes, browse leaves, fruits, cereals and grains. They have the ability to form complexes with proteins and to a lesser extent with carbohydrates, due to the presence of ortho-phenolic hydroxyl groups (Makkar, 2003). They are of relatively high molecular weight and are subdivided into hydrolysable tannins (HT) and condensed tannins (CT) (Min et al., 2003; Smith et al., 2005). The hydrolysable tannins are characterized by the presence of polyols, such as glucose, glucitol, quinic acids, quercitol and shikimic acid, as a central core that is partially or totally esterified with a phenolic group (gallic acid (3,4,5-trihydroxy benzoic acid); gallotannins or gallic acid dimer hexahydroxydiphenic acid (ellagitannins)) to form complex molecules (Patra and Saxena, 2011). The complexity of HT is due to the continuous esterification or oxidative cross-linkage of the resultant phenolic groups (Mueller-Harvey, 2001). Proanthocyanidins (CT) are polymers of flavonol molecules usually formed by C4-C8 and C4-C6 interflavonoid linkages between flavan-3-ol (epi)catechin and (epi)gallocatechin units; however they can also exist as monomers, such as profisetinidins (Quebracho tannins), probinetidins and proguibortinidins (Patra and Saxena, 2011). They exhibit different chemical and biological properties, which are determined by the number of monomeric units that form polymers from di-, tri-and tetra-flavonoids to higher oligomers (Waghorn, 2008). Nevertheless, condensed tannins share one distinctive characteristic, namely their ability to form stable and insoluble CT-protein complexes at pH 3.5-7.0 and to dissociate and release proteins at pH <3.5 (Patra and Saxena, 2011). The CT and HT interact with proteins by forming hydrogen bonds between the phenolic groups of tannins and carboxyl groups of aliphatic and aromatic side chains of proteins and through hydrophobic interaction. However in the rumen HT-protein complexes are degraded by the enzymes of rumen microbes (tannin acylhydrolases and esterases), which to some extent can be toxic when consumed by ruminants as their degradation products can be absorbed from the gastrointestinal tract (Mueller-Harvey, 2006).

The distinct ability of CT to form complexes with dietary proteins in the rumen, whereby increased outflow of non-ammonia N to the abomasum and lower digestive tract of ruminants occurs, has been extensively studied (Komolong et al., 2001; Frutos et al., 2002; Min et al., 2003; Mueller-Harvey, 2006). The lower ruminal degradation of dietary

proteins has been found to enhance N retention and induce a shift in N excretion from urine to faeces (Mueller-Harvey, 2006; Waghorn, 2008; Patra and Saxena, 2011). However the approach of slowing down N release from manures applied to crop fields upon feeding CT, due to the presence of tannin-protein complexes in faeces, has not yet been investigated intensively at the field level (Makkar, 2003, Makkar et al., 2007; Waghorn, 2008; Grainger et al., 2009). Undigested tannin-bound N degrades much more slowly than faecal endogenous and microbial N after application to the soil (Sørensen et al., 1994; Powell et al., 1999). Laboratory experiments have shown that feeding up to 18 g kg^{-1} of feed dry matter (DM) of a mixture of dietary tannin extract that consisted of red quebracho (*Schinopsis lorentzii*) and chestnut (*Castanea sativa*) reduced the emission of ammonia from faecal excreta when applied to soil-containing lab-scale chambers and when applied to lab-scale ventilated chambers with concrete floors (Powell et al., 2011). The same group has also reported that direct application of the tannin mixture to the barn floor inhibited urease activity and decreased ammonia emission by 20%. These low emissions of NH_3 could be attributed to the presence of an insoluble-N fraction, which presumably is mainly made up by tannin-protein complexes. Previous studies with sheep have shown that this fraction was increased upon feeding tannins and polyphenolic compounds (Powell et al., 1994; Waghorn, 2008). Powell et al. (2009) also reported a higher concentration of neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) in faeces of cows fed birdsfoot trefoil (*Lotus corniculatus)* silage containing 1.5% - 1.7% tannins (in DM) compared to faeces of cows fed alfalfa silage and quinone-containing red clover. Hence it could be postulated that higher excretion of insoluble N fractions might slow down the mineralization rate of manure-N and over time increase the availability of N to crops (Powell et al., 2009; Eckard et al., 2010).

Slowly decomposable carbohydrates are also required to stabilize soil organic matter in the long term under irrigated conditions in arid and semiarid climates, and to slow down N release (Hassink, 1994; Kyvsgaard et al., 2000; Van Kessel et al., 2000; Siegfried et al., 2013). Therefore when considering manure quality from animals fed CT, excretion of slowly decomposable carbohydrates along with slowly degradable N is an extra merit that might influence the turnover of N and C from faeces and the release of nutrients required for crop growth (Velthof et al., 2005). Indeed it is stated in literature that CT can also induce a depression in fibre digestion by inhibiting the growth of cellulolytic microorganisms and activities of fibrolytic enzymes, and by binding to dietary carbohydrates (Bae et al., 1993; Patra and Saxena, 2009; Patra and Saxena, 2011). Furthermore in the study of Powell et al. (2009) faeces produced upon ingestion of birdsfoot trefoil silage (1.5% - 1.7% tannin in DM) had also a higher concentration of

neutral detergent fibre (NDF). Hence inclusion of CT as feed additive might positively impact manure C and N contents and change the cycling of these two components in the soil. It has to be noted here that the binding of CT to carbohydrates varies with molecular weight, flexibility and water solubility of tannins (Seigler, 1998), and the extent of their influence on fibre digestibility depends on the availability of tannins after binding with dietary proteins (Barry and Manley, 1986).

Another aspect that might also affect the decomposition of manure and the cycling of N and C in soil upon feeding CT to livestock is their influence on microbial outflow from the rumen due to lower ruminal protein degradation and/or fiber digestion (Makkar, 2003; Waghorn, 2008) and on the fate of CT along the lower digestive tract, which will affect further fermentation and digestion processes. The effect of CT on rumen microbial outflow is a very important criterion to be considered as the rate and extent of protein degradation and/or alteration of the rumen fermentation system will influence microbial growth in the rumen (Patra and Saxena, 2011). The fate of CT in the lower gastrointestinal tract will further affect metabolism of nutrients and microbial turnover in the hindgut, which in turn will affect the chemical composition and microbial community of the faeces (Van Vliet et al., 2007; Ley et al., 2008). Previous reports suggested that CT are subjected to modification and/or disappearance in the gastrointestinal tract of ruminants (Terrill et al., 1994; Perez-Maldonado and Norton, 1996), or to degradation by microorganisms resulting in simple phenolic compounds and phenols that can be further metabolized into non-aromatic compounds (e.g. short-chain fatty acids, lactate, succinate, ethanol), CO_2 and H_2 (Selma et al., 2009; Moco et al., 2012) which all together will affect microbial turnover and digestion in the hindgut. It was also proposed that CT in the lower digestive tract might again bind to N (undigested dietary N, undigested bacterial N, endogenous N; Patra and Saxena, 2011) leading to changes in the availability of N to hindgut microorganisms. This might also induce a higher excretion of undigested dietary N, undigested bacterial N and endogenous N. Interference of CT with the fermentation of carbohydrates in the hindgut might also affect the availability of energy for microbial growth. Therefore the interdependencies between the fate of CT along the digestive tract and the microbial turnover in the rumen and hindgut, and the resulting fractionation of N (i.e. bacterial and endogenous N, water-soluble N, undigested dietary N) in the excreta is crucial for determining manure quality in view of providing slowly decomposable C and N, and for the cycling of these two constituents in soil due to changes in faecal microbial biomass.

In comparison to sheep and cattle, goats are able to ingest forages high in secondary plant compounds because their saliva that contains higher amounts of tannin-binding salivary

proteins (histatins and proline-rich proteins), provides a primary mechanism by which adverse effects of CT are lowered through the precipitation of these proteins (Mueller-Harvey, 2006; Waghorn, 2008). Moreover, goats have larger parotid glands than sheep, which produce more parotid saliva (Vaithiyanathan et al., 2001; Marsh et al., 2006), especially upon ingestion of CT (Salem et al., 2013).

1.2.2 Feeding charcoal or activated charcoal (AC), and combining CT and charcoal feeding

Activated carbons (charcoal) are carbonaceous compounds subjected to high temperature treatments in order to increase their surface area for adsorption or chemical reactions (Girgis et al., 2002). Physical activation of activated carbons involve reduction of the volatile components from the carbonized material at 800 - 1100°C in the presence of oxidizing agents such as O_2 or CO_2 (Yalcin and Arol, 2002). Fixation 'carbonization' of the carbonaceous materials at lower temperatures (400 - 700°C) is also possible chemically, by adding dehydrating agents such as H_2SO_4, $ZnCl_2$, KOH, and H_3PO_4 (Girgis et al., 2002).

A change in the chemistry and microbial community of faecal excreta is assumed to occur upon feeding charcoal due to changes in the microbial community in rumen and hindgut, and subsequent changes in fermentation and digestion of nutrients along the digestive tract, when considering the beneficial effects of this compound as a non-digestible adsorbent for poisons, drugs, toxins, fat, gases and noxious substances (Van et al., 2006 ; Thu et al., 2010; Chu et al., 2013). It has been reported that feeding charcoal resulted in an increase in feed intake, body weight gain and feed utilization in chicken (Kutlu et al., 2001), pigs (Thu et al., 2010; Chu et al., 2013), and goats (Van et al., 2006) and this has been attributed to the ability of this compound to improve digestibility upon eliminating toxins, gasses, anti-nutrients, and impurities from the digestive tract (Struhsaker et al., 1997; Banner et al., 2000; Poage et al., 2000; Rao and Chopra, 2001; Van et al., 2006; Thu et al., 2010). The excretion of the indigestible fraction of carbonized C in faeces might help to stabilize SOC (Glaser et al., 2002; Dempster et al., 2012; Lentz and Ippolito, 2012) or provide similar effects as the biochar, which is produced by thermal degradation of organic materials under low temperatures and limited oxygen supply (Lehmann and Joseph, 2009). In cropping systems biochar applied to soil can reduce emissions of CO_2, N_2O, CH_4 (Lehmann, 2007; Renner, 2007; Yanai et al., 2007; Major et al., 2009) and leaching of NH_4^+ and NO_3^- (Singh et al., 2010; Knowles et al., 2011), and influence the composition of the microbial community, whereby the charcoal serves as a microbial habitat (Lehmann et al., 2011). Data from feeding bamboo

charcoal to fattening pigs showed that the emissions of ammonia, methane, amines and hydrogen sulphide from animals' faeces was reduced (Chu et al., 2013). These observations suggest that emissions of the mentioned gases is reduced because N is utilized for microbial growth, hence, increasing the recycling of N in soil. Therefore, considering both the positive effects of charcoal feeding on livestock performance and on the recycling of C and N from livestock excreta to soil, it seems topical to investigate in more detail the effects of AC feeding on the chemical composition and microbial community of faecal excreta.

Van et al. (2006) reported that feeding 1 gram of bamboo charcoal per kilogram live weight of goats on a tannin-rich *Acacia mangium* (58 g CT kg^{-1} DM) diet increased N digestibility and N retention, and reduced urinary N excretion. Thus by combining the positive effects of charcoal on manure quality and its neutralizing impact on the negative effects of tannins (Struhsaker et al., 1997; Banner et al., 2000; Poage et al., 2000; Rao and Chopra, 2001; Van et al., 2006), a stronger enhancement in manure quality is plausible. Inclusion of CT might foster the concentration of slowly degradable faecal N, while the digestibility and retention of N will be improved upon the addition of AC. The excreted indigestible fraction of carbonized C will further improve the cycling of C and N in soil.

1.3. Effects of condensed tannins and activated charcoal on digesta passage

The efficiency of digestion which will determine the quality of faecal excreta is influenced by the retention time of feed particles and the content of digesta in the various compartments of the digestive tract (Van Soest, 1982). Moreover it has been suggested that there is a correlation between dry matter digestibility and the concentration of N and NDF in the ingested diet as well as particle passage through the mixing compartment, particle half time and total tract mean retention time (Schlecht et al., 2007). Hence understanding the mechanisms by which CT and AC influence digestion and particulate passage through the digestive tract is crucial to interpret their effects on excreta quality. In literature there are few studies investigated the effect of CT on digesta passage in ruminants and their results are inconsistent. This can be attributed to the differences in CT effects on protein and fibre digestion and/or digestive enzymes and epithelial lining (Mueller-Harvey, 2006; Waghorn, 2008; Patra and Saxena, 2011). Silanikove et al. (2001) reported a delay in the passage of fluid and particulate matter along the digestive tract of goats fed carob leaves (*Ceratonia siliqua*; 0.7% CT). The tannins in carob leaves caused a depression of fluid and particulate content in the rumen and accelerated the passage of fluid from the abomasum, while the passage of digesta through the small and

large intestine was delayed. In sheep it was found that feeding up to 50% mountain mahogany leaves (*Cercocarpus montanus*; 11.2% CT in DM) also caused a depression in ruminal fluid volume but didn't change fluid dilution rates, outflow rates, and fluid turnover time (Nunez-Hernandez et al., 1991). Yet, feeding *Lotus pedunculatus* (55 g CT kg^{-1} DM) to sheep had no influence on rumen digesta pool but caused a depression in dilution rate (Waghorn et al., 1994). Even though no studies have been found on the effects of AC on digesta passage, it is expected that, as AC can support intestinal functions through eliminating poisons and impurities, it may also influence the rate of digesta passage.

1.4. Research hypotheses, study objectives and thesis structure

Considering the above findings on the effects of condensed tannins and/or (activated) charcoal on digestion of nutrients and excreta quality, it was hypothesized that feeding protein-binding CT and/or AC will result in livestock manure that meets the specific requirements of irrigated organic crop farming under arid sub-tropical conditions (Figure 1) due to: (i) increased faecal N and/or C concentrations; (ii) provision of long-lived C species to be sequestered in the soil; (iii) effects on the metabolism of rumen and hindgut microbial communities which will affect the composition of the microbial biomass in the faeces.

To this end this research project aimed at evaluating the effects of adding different concentration of a commercial source of CT (i.e. quebracho tannin extract, QTE) and/or AC, on intake of feed and nutrients and digestibility, passage rate of digesta, N balance and partitioning of N excretion between urine and faeces, rumen microbial protein synthesis and faecal microbial biomass. Data presented in this thesis was collected from two different experiments.

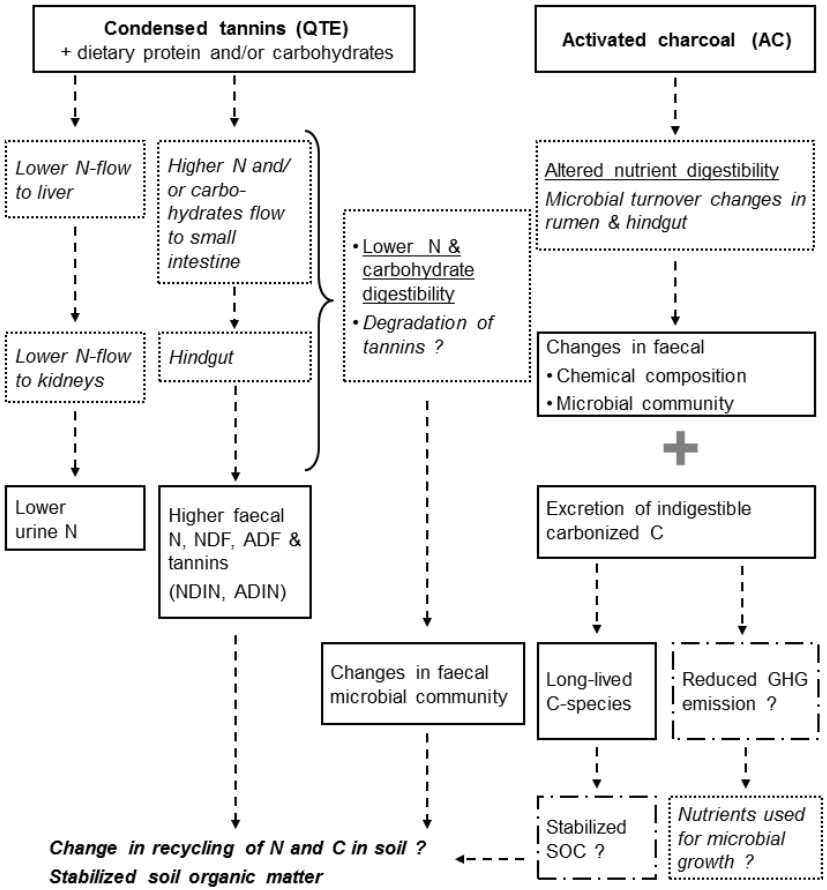

Figure 1: Flow chart illustrating the hypothesized effects of QTE and AC feeding to goats on manure quality. Bold boxes indicate parameters studied in this thesis. Italic writing in dotted boxes indicates assumed effects, while regular writing and underline in dotted boxes indicates measured effects. Dashed boxes with regular writing indicate effects studied in RTG 1397-F1. ADIN= acid detergent insoluble N; GHG= greenhouse gas emissions; NDIN= neutral detergent insoluble N; SOC= soil organic carbon.

1.4.1. Digestibility and passage rate of digesta when feeding CT and AC

This trial, which is detailed in Chapter 2, was conducted in Oman (February - April 2011) as a completely randomised design of three subsequent periods in which 21 days of adaption were followed by 7 sampling days, and the parameters illustrated in Figure 2 were determined.

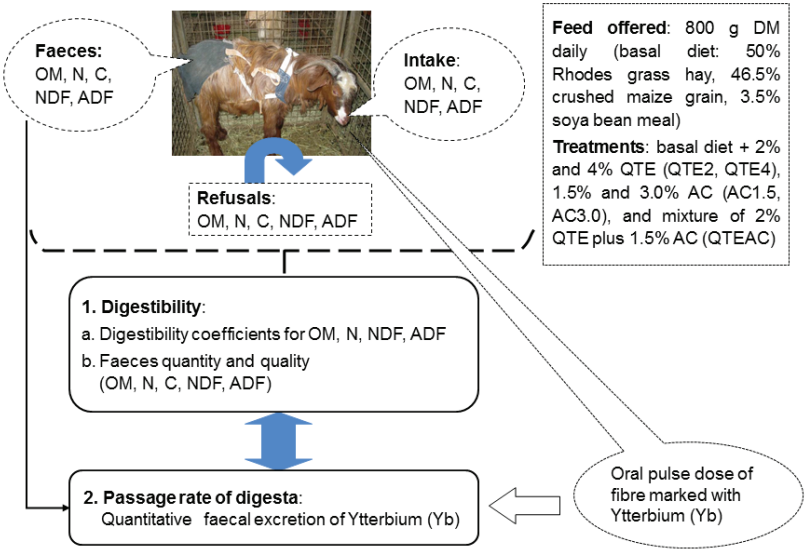

Figure 2: Flow chart illustrating data collection for the effects of feeding QTE and AC to goats on feed and nutrients intake, digestibility, faecal excretion, and passage rate of digesta (Chapter 2).

1.4.2. Nitrogen balance and microbial turnover in rumen and hindgut

A completely randomised N-balance trial was conducted over three subsequent periods (September - November 2011), in which again 21 days of adaptation were followed by six sampling days. In this trial the parameters illustrated in Figure 3 were studied; the results are presented in Chapters 3 and 4.

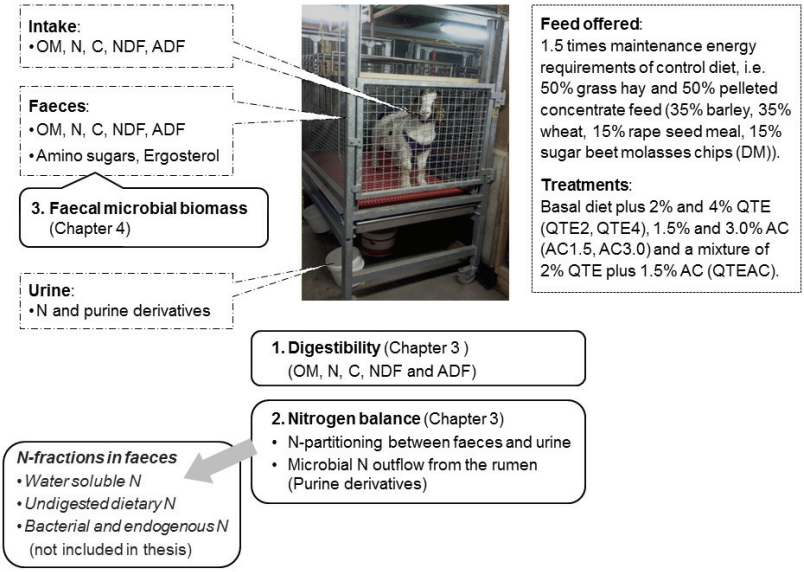

Figure 3: Flow chart illustrating the collection of data to determine the effects of QTE and AC feeding to goats on nutrients intake, excretion and digestibility, N balance and microbial biomass in faeces (Chapters 3 and 4).

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2. Influence of quebracho tannin extract (*Schinopsis balansae*) and activated charcoal on nutrient intake, digestibility, digesta passage and quality of faecal excreta in goats

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Abstract

Maintaining soil organic matter under irrigated arid sub-tropical conditions is challenging due to high carbon (C) turnover, along with increased nitrogen (N) losses via leaching and emissions. Supplementing ruminants' diet with activated charcoal (AC) might enrich their manure with long-lived C species to be sequestered in soil, and the faecal fraction of slowly decomposable N could be increased by feeding condensed tannins (CT) that reduce ruminal protein degradation and urinary N excretion. We therefore investigated the effects of feeding AC and quebracho tannin extract (QTE, a source of CT) on feed and nutrients intake, digestibility and rate of digesta passage in goats, and on the resulting quality of faeces.

A completely randomised feeding trial was conducted over three subsequent periods, with 24 male goats assigned to five treatments and a control. Goats were offered a basal diet (control) which consisted of 50% Rhodes grass hay, 46.5% crushed maize and 3.5% soybean meal. Treatment diets contained 2% and 4% QTE (QTE2, QTE4), 1.5% and 3.0% AC (AC1.5, AC3.0) and a mixture of 2% QTE plus 1.5% AC (QTEAC). Samples of feed, refusals and faeces were analysed for proximate constituents following standard protocols. Digesta passage was computed with a one-compartment age-dependent Gamma-2 model based on a pulse dose application of Ytterbium-mordanted fibre and quantitative collection of faeces during 7 days thereafter.

There were no significant effects of QTE and AC on the intake of feed and nutrients. The digestibility of dry and organic matter as well as N were reduced by QTE2 and QTE4 ($P<0.001$), and the digestibility of neutral and acid detergent fibre (NDF, ADF) decreased in QTE4, QTEAC and the two AC treatments ($P<0.001$). Ruminal particle passage ranged from 0.051 h^{-1} (QTEAC) to 0.056 h^{-1} (control; $P>0.05$). Except for the correlation between total tract mean retention time and faecal C/N ratio ($r= -0.35$, $P<0.05$) there was no influence of digesta passage parameters on the quality of faeces. While the faecal N concentration was not altered by AC and QTE ($P>0.05$), AC treatments enhanced the faecal concentration of NDF, ADF and C ($P<0.001$). Including up to 3% of AC in goats' diets might thus help to build up soil organic matter when using the resulting manure in irrigated sub-tropical environments.

2.1. Introduction

In the arid sub-tropical conditions of Oman maintaining soil organic carbon (C) is a challenge due to year-round high temperatures and flood irrigation, which in combination induce high C turnover and subsequently CO_2 emissions (Siegfried et al., 2013). In the

long term this might also lead to increased nitrogen (N) losses via leaching or emission as any built-up of soil organic matter is not expected (Siegfried et al., 2013). High application of manure is one attempt to maintain soil organic matter in such conditions (Buerkert et al., 2010). However, previous studies in dairy cattle have shown that the type of forage consumed influences faecal N and fibre concentrations and the relative partitioning of N into faecal fibre fractions (Powell et al., 2006, 2009) and thus affects manure-related C and N cycling in soils (Somda and Powell, 1998; Powell et al., 2006).

2.1.1 Effects of dietary tannins on manure quality

Feeding moderate concentrations (<5%) of condensed tannins (CT) to ruminants has been shown to shift N excretion from urine to faeces by lowering dietary protein degradation in the rumen (Mueller-Harvey, 2006; Waghorn, 2008; Patra and Saxena, 2011). Yet, the main aim of such a feeding strategy is to increase the flow of non-ammonia N to the abomasum and lower digestive tract in order to increase the absorption of amino acids in the small intestine (Komolong et al., 2001; Frutos et al., 2002; Min et al., 2003; Mueller-Harvey, 2006). Nitrogen excreted via urine in the form of urea is very volatile and easily nitrified and leached to groundwater or emitted as nitrous oxide (Misselbrook et al., 2005; Eckard et al., 2010). Steinfeld et al. (2006) estimated that ruminants' urine accounts for 65% of global anthropogenic nitrous oxide emissions, and Makkar et al. (2007) reported that 70% of urinary N is lost as environmental pollutant under tropical conditions. The higher faecal N excretion associated with feeding tannins has been related to the incomplete dissociation of tannin-protein complexes in the abomasum and the lower digestive tract, which to some extent might negatively affect the apparent digestibility of N without affecting N retention (Komolong et al., 2001; Beauchemin et al., 2007; Grainger et al., 2009; Al-Dobaib, 2009; Chapter 3). It has been suggested that the presence of tannin-protein complexes in faeces might lead to slower N release from manures when applied to crop fields (Makkar, 2003, 2007; Waghorn, 2008; Grainger et al., 2009). Powell et al. (2011) reported that the excreta of lactating Holstein cows fed up to 18.0 g kg^{-1} of feed DM of a mixture of dietary tannin extract that consisted of red quebracho (*Schinopsis lorentzii*) and chestnut (*Castanea sativa*) showed reduced ammonia emission when applied to soil-containing lab-scale chambers. The same excreta also showed a reduced NH_3 emission when applied to lab-scale ventilated chambers with concrete floors; moreover, direct application of the tannin extract to the barn floor inhibited urease activity and decreased ammonia emission by 20% (Powell et al., 2011). Studies with sheep also reported that the insoluble-N fraction in faeces increased due to the consumption of tannins and polyphenolic compounds (Powell et al., 1994; Waghorn, 2008). Powell et al. (2009) also reported a higher concentration of neutral detergent

insoluble N (NDIN) and acid detergent insoluble N (ADIN) in faeces of cows fed birdsfoot trefoil (*Lotus corniculatus)* silage containing 1.5% - 1.7% tannins (DM) than those fed alfalfa silage (*Medicago sativa*) and quinone-containing red clover (*Trifolium pratense*). Higher faecal excretion of undigested and insoluble N fractions might slow down the mineralization rate and increase the availability of N to crops (Sørensen et al., 1994; Powell et al., 1999; Powell et al., 2009).

In ruminants consumption of CT has also been related to a depression in fibre digestion (Patra and Saxena, 2011), however, reports on effects of tannins on fibre digestibility are inconsistent. This is due to the fact that binding of CT to carbohydrates is pH independent and varies with molecular weight, flexibility and water solubility of tannins (Seigler, 1998). It is also influenced by the availability of tannins after binding with dietary proteins (Barry and Manley, 1986). Inhibiting cellulolytic microorganisms and reducing the activity of fibrolytic enzymes are mechanisms by which CT can affect fibre digestion (Bae et al., 1993; Patra and Saxena, 2009). Patra et al. (2011) observed that tannins present in seed pulp of *Terminalia chebuala* and in *Allium sativum* bulbs enhanced the digestibility of nutrients and fibre fractions when fed to sheep at 10 g kg^{-1} of total DM intake, and attributed this to an increased number of fibrolytic bacteria in the rumen.

In contrast, feeding *Calliandra calothyrsus* to sheep at 30% of the total diet reduced fibre-degrading bacteria although the efficiency of microbial protein synthesis was not affected (McSweeney et al., 2001). McAllister et al. (2005) reported that CT from a total of nine different legume forages and varieties, respectively, had different inhibitory effects on *Fibrobacter succinogenes*. Beauchemin et al. (2007) found no effect of quebracho tannin extract (QTE) on DM, NDF and ADF digestibility when fed to cattle at up to 2%. Al-Dobaib (2009), on the other hand, observed that adding QTE to alfalfa hay decreased fibre digestibility in sheep at an inclusion of 22.5 g kg^{-1} DM but had no effect at an inclusion of 7.5 and 15 g kg^{-1} DM. Bhatta et al. (2007) even reported an increase in ADF and cellulose digestibility when *Prosopis cineraria* leaves (91 mg CT kg^{-1} DM) were fed to lambs at 25%, 50% and 75% of the complete diet.

Retention time and content of digesta in the various compartments of the digestive tract are crucial for determining the efficiency of digestion (Van Soest, 1982). Silanikove et al. (2001) studied the effects of tannins in carob leaves (*Ceratonia siliqua*; 0.7% CT) with and without polyethylene glycol (PEG; tannin binding agent) on rumen volume and kinetics, and on the retention time of fluid and particulate components of the digesta along the gastrointestinal tract of goats. They reported a delay in the passage of fluid and particulate matter along the digestive tract due to the interaction between tannins and the

digestive enzymes and epithelial lining. Feeding up to 50% mountain mahogany leaves (*Cercocarpus montanus*; 11.2 % CT in DM) reduced ruminal fluid volume in sheep but didn't change fluid dilution rate, outflow rate, and fluid turnover time (Nunez-Hernandez et al., 1991). In contrast, feeding *Lotus pedunculatus* (55 g CT kg^{-1} DM) to sheep had no influence on the pool of rumen digesta but caused a depression in dilution rate (Waghorn et al., 1994). The inconsistency in the results obtained from these few studies suggests that CT exhibit different effects on protein and fibre digestion and/or digestive enzymes and epithelial lining.

However, considering the quality of manure that is obtained from animals fed CT, the excretion of slowly decomposable carbohydrates along with slowly degradable N might influence the turnover of N and C from faeces and the release of nutrients required for crop growth (Velthof et al., 2005). Slowly decomposable carbohydrates are required for long-term stabilization of soil organic matter and slowed-down N release under irrigated conditions in arid and semiarid climates (Hassink, 1994; Kyvsgaard et al., 2000; Van Kessel et al., 2000; Siegfried et al., 2013).

2.1.2 Effects of dietary carbon on manure quality

The terms 'activated carbon' or 'activated charcoal' depict carbonaceous compounds that are physically or chemically activated to increase their surface area for adsorption or chemical reactions (Girgis et al., 2002). Physical activation is usually performed on carbonized materials in which the volatile components of the raw material are reduced by heating at 800 - 1100°C in the presence of oxidizing agents such as O_2 or CO_2 (Yalcin and Arol, 2002). Chemical activation involves fixing 'carbonization' of the carbonaceous materials by dehydrating agents such as H_2SO_4, $ZnCl_2$, KOH, and H_3PO_4 at lower temperatures (400-700°C; Girgis et al., 2002).

The use of activated charcoal (AC) for medical and veterinary purposes as a universal poison antidote is reported in peer-reviewed international literature since about 40 years, but the beneficial effects of charcoal as an indigestible adsorbent for poisons, drugs, toxins, fat, gases and noxious substances have been known for hundreds of years (Van et al., 2006; Thu et al., 2010; Chu et al., 2013). Today charcoal is used as feed additive to improve the intake of plants high in secondary plant compounds (such as terpenoids, phenols and alkaloids), and to activate intestinal functions through eliminating poisons and impurities from the digestive tract (Struhsaker et al., 1997; Banner et al., 2000; Poage et al., 2000; Rao and Chopra, 2001; Van et al., 2006). The latter effect of charcoal and AC has been found to increase feed intake, body weight and feed utilization in chicken (Kutlu et al., 2001) and pigs (Thu et al., 2010; Chu et al., 2013). Van et al. (2006) also

demonstrated that feeding 1 gram of bamboo charcoal per kilogram live weight of goats on a tannin-rich *Acacia mangium* diet increased N digestibility and N retention, and reduced urinary N excretion. Considering these beneficial effects of charcoal together with recently published data on the ability of bamboo charcoal to decrease noxious gas emissions of ammonia, methane, amine and hydrogen sulfide from faeces of fattening pigs (Chu et al., 2013), and the use of biochars as soil conditioners and carbon sequesters (Lehmann and Joseph, 2009), it seems topical to also evaluate the effects of feeding AC on manure quality. The ability of AC to neutralize anti-nutrients and products of digestion and fermentation in the gastrointestinal tract and to improve nutrient digestibility (Van et al., 2006; Thu et al., 2010) could ultimately influence excreta chemistry. Faecal excretion of indigestible carbonized C might help to improve the stability of soil organic matter over time, which is crucial for improving overall soil fertility (Blackwell et al., 2009; Verheijen et al., 2009), reducing nutrient leaching losses and emission of greenhouse gases (Yanai et al., 2007; Major et al., 2009).

In view of the above considerations it can be hypothesized that including condensed tannins and activated charcoal, either separately or in combination, into ruminants' diets should increase faecal N and/or C concentrations by affecting digesta passage and digestibility of proximate diet constituents along the gastrointestinal tract. The resulting manure might help stabilizing soil organic matter and reduce N and C losses in irrigated organic cropping systems of arid (sub-)tropical regions (Siegfried et al., 2013). Therefore the present study tested how feeding different concentrations of condensed tannins and activated charcoal to goats affects their intake of feed and nutrients, diet digestibility and digesta passage as well as quantity and quality of faeces.

2.2. Materials and methods

2.2.1 Experimental design

Adopting a completely randomized design, a digestibility trial was conducted on an organic farm near Sohar (Northern Oman, mean annual temperature: 27°C, mean annual precipitation 102 mm). During three subsequent periods of 21 days of adaption followed by 7 sampling days each, 24 male Jabal Akhdar goats (37.4 kg ± 3.1) were randomly and equally assigned to five treatments and one control group. The animals were kept in individual pens of 1.5 m x 1.5 m in a roofed but otherwise open and well aerated stable. The goats were weighed every 9 days, to coincide with the start of each adaption and end of each sampling period. The three sampling periods corresponded to the first week of February and March and second week of April 2011, respectively, when average maximum temperature was 22.2°C, 22.9°C and 28.7°C, average minimum temperature

was 17.5°C, 18.5°C, and 22.5°C, and relative humidity averaged 48.3%, 47.5%, and 70.1%, respectively. To avoid bias in determining the effects of QTE and AC on intake and digestibility, passage rate of digesta and faecal quality, all goats were offered the same quantity of feed, namely 800 g DM per day of a basal diet (control) which, on DM basis, consisted of 50% Rhodes grass hay (*Chloris gayana* Kunth), 46.5% crushed maize and 3.5% soybean meal. In addition to these ingredients, the experimental diets contained 2% (QTE2) and 4% (QTE4) quebracho (*Schinopsis balansae* Engl.) tannin extract (QTE; purchased from Otto Dille®, Norderstedt, Germany), 1.5% (AC1.5) and 3% (AC3.0) coconut-shell derived activated charcoal (AC; AquaSorb® CP1; Jacobi Carbons GmbH Frankfurt/Main, Germany) as well as a combination (QTEAC) of 2% QTE and 1.5% AC. The required amounts of QTE and AC powder, respectively, were mixed with the crushed maize and soybean meal, moistened (1 liter of water for each 10 kg of dry mixture) and pelleted thereafter using a mechanical press. The chemical composition of the hay and the six different types of pellets is shown in Table 1. The daily ration was divided into two equal portions, which were offered daily at 7:30 a.m. and 4:30 p.m. The concentrate pellets and the hay (chopped into 15-20 cm long pieces) were fed separately, starting with the pellets that were usually ingested within 10-15 minutes. Eventual refusals of pellets and hay were collected prior to each subsequent feeding portion. Mineral licking blocks were provided throughout the experiment period and water was offered *ad libitum*.

2.2.2 Determination of diet digestibility

During the sampling period, representative samples of hay and pellets offered were collected daily and pooled at the end of each sampling week. After quantification of an animal's feed refusals, the leftovers of days 1 to 3 and days 4 to 7, respectively, were pooled, homogenized and sub-sampled for analysis. Faeces were quantitatively collected into fabric bags that were attached to the goats by harnesses and emptied twice daily. Faeces of days 1 to 3 and days 4 to 7, respectively, were pooled, homogenized and sub-sampled. Except for the samples of hay on offer that were stored air-dry, all other samples were kept at -20°C until analysis.

2.2.3 Determination of digesta passage

The passage rate of digesta particles through the gastrointestinal tract (GIT) was determined after sampling periods 2 and 3, respectively, by quantifying the faecal excretion of Ytterbium (Yb). To this end, all goats received a single oral pulse dose of fibre marked with Yb at morning feeding of day 1 of each of the two periods.

Table 1: Proximate composition of hay and concentrate pellets offered to goats at a 0.5/0.5 dry weight ratio during three periods of an experiment set up as a completely randomized design. Values are means of 2 samples per component and period.

Diet	Period	DM	OM	CP	C	NDF	ADF
		(g kg^{-1} FM)		(g kg^{-1} DM)		(g kg^{-1} OM)	
	1	929	876	118	421	660	327
Hay	2	979	900	99	432	621	312
	3	924	901	75	429	666	339
Pellets (Concentrate + additive)							
Control	1	928	981	111	446	72	29
	2	963	981	113	442	91	46
	3	912	980	124	448	75	31
QTE2	1	928	982	114	468	135	84
	2	965	980	107	438	85	39
	3	913	979	111	448	80	29
QTE4	1	928	977	109	465	117	70
	2	964	977	102	447	68	33
	3	914	975	109	454	71	44
AC1.5	1	934	977	112	464	104	65
	2	965	981	109	445	98	75
	3	914	978	102	454	98	40
AC3.0	1	933	970	113	458	104	53
	2	968	975	114	482	173	122
	3	913	981	107	463	106	32
QTEAC	1	919	977	113	446	63	29
	2	965	976	110	463	118	73
	3	914	976	106	462	104	51

Pellets (all on dry matter basis): control=46.5% crushed maize and 3.5% soybean meal; QTE2: 2% Quebracho tannin extract in overall diet (4% in pellet); QTE4: 4% QTE in overall diet (8% in pellet); AC1.5: 1.5% activated charcoal in overall diet (3% in pellet); AC3.0: 3% AC in overall diet (6% in pellet); QTEAC: 2% QTE and 1.5% AC in overall diet.

DM: dry matter; FM: fresh matter; OM: organic matter; CP: crude protein; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre.

To prepare the marked fibre, Rhodes grass hay was milled to 5 mm particle size. The particles were boiled in EDTA-free neutral detergent solution for one hour and afterwards repeatedly rinsed with tap water until all foam was removed. After oven drying (70°C) the particles were soaked for 24 hours in 12.4 mmol l^{-1} aqueous solution of $Yb(CH_3COO)_3 \bullet 4H_2O$ (Teeter et al., 1984; Villalobos et al., 1997) and again thoroughly rinsed with tap water. To remove any remaining unbound Yb, particles were then soaked in 100 mmol l^{-1} solution of acetic acid for 6 hours. Afterwards they were rinsed again with tap water and dried (70°C). A sub-sample of marked fiber was kept to determine the Yb concentration.

On day 1 of the passage rate trial, each goat was offered marked fibre particles corresponding to 5.6 mg Yb kg^{-1} live weight (LW). In cases where goats refused to consume the marked fibre immediately, it was mixed with 40 g of crushed soybean meal. The starting time (t_0) of marker passage was individually defined as the time when a goat had completely ingested the marked fibre. In the few cases where the ingestion of marked fibre took longer than 30 minutes, t_0 was considered as the half time of marker consumption.

Faeces were quantitatively collected every four hours on days 1 and 2, every eight hours on days 3 and 4 and every twelve hours on days 5 to 7; each sample was dried at 60°C to weight constancy, milled to 1 mm particle size and analysed for the concentration of Yb.

2.2.4 Chemical analysis of samples

Prior to analysis, all samples of feeds, refusals and faeces were dried to constant weight in a hot-air drying oven at 60°C and then ground to 1 mm particle size. The DM and organic matter (OM) contents were determined according to Naumann and Bassler (1976). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions were measured using a semi-automated Ankom 220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) following the method of Naumann and Bassler (1997), not including decalin and sodium sulphite. Residual ash was excluded from the NDF and ADF values. The nitrogen (N) and carbon (C) contents were quantified in a C/N–TCD analyser (Elementar Analysensysteme GmbH, Hanau, Germany); crude protein (CP) was calculated by multiplying N concentrations with the factor 6.25.

To determine Yb concentration, 200 mg of air-dried marked fibre and faeces samples, respectively, were mixed with 3 ml of 65% (v/v) nitric acid and digested at 190°C for 10 hours in closed Teflon® vessels (Heinrichs et al., 1986). The residues were filtered into 50 ml flasks and filled up with distilled water after washing the filter paper several times. The Yb concentration was determined as the average of three independent readings by an

inductively coupled plasma mass spectrometer (ICP-MS; Optimass800, GBC Scientific Equipment Australia).

2.2.5 Data analysis

Particle passage through the GIT was computed based on the cumulative quantitative outflow of Yb (marker concentration in faeces times faecal mass) at time t_i , using the models published by Richter and Schlecht (2006). Leaching of Yb due to disassociation from the fibre particles was only observed in one case, to which the disassociation model ('Type-D model') was applied; in all other cases the normal model ('Type-N model') was used (Richter and Schlecht, 2006). SAS® 9.3 (SAS Institute Inc., Cary, NC, USA) was used for these model calculations (PROC NLIN method=dud), from which the following parameters resulted: Time of first marker appearance in faeces (TT), rate of passage of fibre-bound marker through the mixing compartment (λ, Gamma-2 parameter), half time of marker in the mixing compartment (T_{50}: $0.8392 \times 2\lambda^{-1}$), particle mean retention time in the mixing compartment (CMRT: $2\lambda^{-1}$) and particle mean retention time in the total tract (TMRT: $TT + 2\lambda^{-1}$).

The mixed model procedure in SAS was used to conduct ANOVA. Periods and treatments were tabulated as fixed effects while animals were considered as random effect. The model used was:

$$y_{ij} = \beta_1 x_{1ij} + \beta_2 x_{2ij} + b_{i1} z_{1ij} + \varepsilon_{ij} \qquad \text{[Eq. 1]}$$

where y_{ij} is the value of the outcome variable for a particular ij case, β_1 and β_2 are the fixed effect coefficients for treatment and period, respectively, x_{1ij} and x_{2ij} are the fixed effect variables for observation j in group i, b_{i1} is the random effect coefficient, z_{1ij} is the random effect variable (animal), and ε_{ij} is the error for case j in group i.

The interactions between periods and treatments were tested. Least squares means were used to compare results with significant F-values. Significance was declared at $P \leq 0.05$. Spearman correlation statistics and probabilities were computed using the CORR procedure.

2.3. Results

2.3.1 Effects of QTE and AC on intake and digestibility

Across the three periods, there was no significant effect of QTE and AC on dry matter and organic matter intake (Table 2). Crude protein intake was significantly reduced in QTE4 by 2.8% and in AC3.0 by 2.6%. However, the reduction in CP intake was even more pronounced in AC1.5 (-4.2%) and QTEAC (-4.3%). Whereas the intake of total C

was significantly higher in QTE4 (+2.5%) than in the control, there was no significant effect of QTE2 and of the two AC treatments on total C intake. Furthermore, neither QTE nor AC inclusion affected NDF and ADF intake (P>0.05). Intake of DM, OM, C, NDF, and ADF was significantly higher in period 2 than in periods 1 and 3, while CP intake was highest in period 1 (Table 2).

The addition of QTE and AC had no significant effect on the OM concentration of the ingesta (Table 3). However, there was a significant effect of QTE and AC on the CP concentration of ingesta, which was reduced by 1.6% to 3.7% across the five treatments. In comparison to the control, all QTE and AC treatments slightly, but nevertheless significantly, increased the C concentration of ingesta by 0.6% to 2.4% across the five treatments. Ingesta NDF concentration was significantly reduced in QTE4 (-3.0%) while it was increased by 1.5% in QTE2 and by 2.5% in AC3.0. Whereas QTE4 had no significant effect on the ADF concentration of ingesta, it increased by 1.6% to 4.9% across the other four treatments, with the highest increase realized in AC3.0.

The digestibility of DM and OM was significantly reduced by QTE addition as compared to the control (-3% in QTE2 and QTEAC, -6% in QTE4; Table 4). Similarly, QTE significantly reduced the digestibility of CP, especially at QTE4 (-12.0%), but also at QTE2 (-5.2%) and QTEAC (-4.8%). Both AC and QTE reduced the digestibility of NDF by 2% to 3% (treatments AC1.5, AC3.0 and QTEAC) and even 4% (QTE4). The digestibility of ADF was reduced by 11% in treatment QTE4 and by 5% to 6% in treatments AC1.5, AC3.0 and QTEAC. Across the entire experiment, DM, OM, CP, NDF, and ADF digestibility decreased from period 1 to 3.

2.3.2 Effects of QTE and AC on digesta passage

At 15.6 h and 21.0 h, laminar flow (TT) of the fibre particles through the non-mixing GIT segments (Table 5) was lowest for QTE4 and highest for AC3.0 ($P \leq 0.05$). For all other parameters of particle passage, no significant differences manifested between the control and any test diet. At 0.056 h^{-1}, the outflow of particles from the mixing compartment (λ) was numerically highest for the control diet and lowest for diet QTE2 (0.050 h^{-1}). In consequence, the average residence time of particles in the mixing compartment (CMRT) was lowest for the control (36 h) and highest for QTE2 (41 h), whereas the shortest and the longest total tract mean retention time (TMRT) was obtained for treatments QTE4 (53 h) and QTE2 (59 h), respectively.

The nonparametric Spearman's rank□correlation analysis indicated that both TT and TMRT (which is a derived variable of TT and □, see 2.2.5) were significantly influenced by the animals' live weight (r=0.43; P<0.01 in both cases).

Table 2: Intake (g $kg^{-0.75}$ d^{-1}) of proximate diet constituents by goats (12 per treatment) offered five experimental and one control diet during three 7-day periods of an experiment set up as a completely randomised design.

Component	Treatment								Period				
	Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	SEM[1]	P ≤	1	2	3	SEM	P ≤
DM	49.1^{ab}	49.4^{a}	49.5^{a}	48.2^{b}	48.4^{ab}	48.1^{b}	0.59	0.037	49.2^{a}	51.0^{b}	46.2^{c}	0.51	0.001
OM	45.7^{ab}	46.1^{a}	46.1^{a}	45.0^{b}	45.2^{ab}	44.8^{b}	0.55	0.028	45.4^{a}	47.7^{b}	43.3^{c}	0.48	0.001
CP	4.85^{a}	4.77^{ac}	4.71^{bc}	4.65^{b}	4.73^{bc}	4.64^{b}	0.057	0.001	5.29^{a}	4.94^{b}	3.95^{c}	0.050	0.001
C	19.86^{a}	20.19^{ab}	20.37^{b}	19.84^{a}	20.20^{ab}	19.77^{a}	0.240	0.047	19.95^{a}	21.04^{b}	19.13^{c}	0.210	0.001
NDF[2]	17.65^{ab}	18.04^{b}	17.34^{a}	17.39^{a}	17.99^{b}	17.38^{a}	0.259	0.043	17.61^{a}	18.23^{b}	17.05^{c}	0.216	0.001
ADF[3]	8.80^{a}	9.05^{ab}	8.85^{a}	8.93^{ab}	9.19^{b}	8.79^{a}	0.132	0.070	8.84^{a}	9.41^{b}	8.56^{c}	0.109	0.001

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

DM: dry matter; OM: organic matter; CP: crude protein; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre.

Within rows, means with different superscripts differ at $P \leq 0.05$; P-values in columns indicate the overall effect of treatment and period, respectively.

[1] Standard error of the mean.

[2] NDF: Treatment x period interaction, $P = 0.0164$.

[3] ADF: Treatment x period interaction, $P \leq 0.001$.

Table 3: Proximate composition of the ingesta of goats (12 per treatment) offered five experimental and one control diet during three 7-day periods of an experiment set up as a completely randomised design.

Component		Treatment								Period				
		Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	SEM[1]	P≤	1	2	3	SEM	P≤
OM	(g kg^{-1} DM)	932	932	933	933	933	932	1.8	0.383	924^{a}	936^{b}	938^{c}	1.7	0.001
CP[2]	(g kg^{-1} OM)	106^{a}	104^{b}	102^{c}	103^{d}	104^{e}	103^{d}	0.2	0.001	116^{a}	104^{b}	91^{c}	0.1	0.001
C[2]	- " -	436^{a}	439^{b}	441^{cfd}	441^{d}	447^{e}	442^{f}	0.3	0.001	439^{a}	442^{b}	442^{b}	0.2	0.001
NDF[2]	- " -	388^{a}	394^{b}	376^{c}	387^{a}	397^{b}	389^{a}	2.3	0.001	388^{a}	383^{b}	394^{c}	1.8	0.001
ADF[2]	- " -	193^{a}	198^{b}	192^{a}	199^{b}	203^{c}	196^{b}	1.1	0.001	195^{a}	198^{b}	198^{b}	0.9	0.001
C/N[2]	(g g^{-1})	25.9^{a}	26.7^{b}	27.3^{c}	27.2^{d}	27.1^{d}	27.1^{d}	0.03	0.001	23.6^{a}	26.7^{b}	30.3^{c}	0.02	0.001
NDF/N[2]	- " -	23.1^{a}	23.9^{b}	23.2^{a}	23.9^{b}	24.1^{b}	23.9^{b}	0.16	0.001	20.8^{a}	23.2^{b}	27.0^{c}	0.13	0.001
ADF/N[2]	- " -	11.5^{a}	12.0^{bd}	11.9^{b}	12.3^{c}	12.3^{c}	12.1^{d}	0.08	0.001	10.5^{a}	12.0^{b}	13.6^{c}	0.06	0.001

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

OM: organic matter; CP: crude protein; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre, N nitrogen (CP / 6.25).

Within rows, means with different superscripts differ at $P \leq 0.05$; P-values in columns indicate the overall effect of treatment and period, respectively.

[1] Standard error of the mean. [2] CP; C; NDF; ADF: Treatment x period interactions; $P \leq 0.001$.

Furthermore, TT was related to the quantitative intake (g $kg^{-0.75}$ LW) of DM and OM (r = -0.39 and r = -0.38; $P \leq 0.05$), NDF and C (r = -0.36, $P \leq 0.05$ in both cases). TMRT on the other hand was related to the intake of DM, OM and C at r = -0.46 ($P \leq 0.01$ in all cases), and to the intake of CP and NDF at r = -0.36 ($P \leq 0.05$) and r = -0.41 ($P \leq 0.01$), respectively. In contrast to this, neither ingesta quality nor the digestibility of any proximate constituents had a significant impact on any of the particle passage parameters.

2.3.3 Effects of QTE and AC on quantity and quality of faeces

The quantitative excretion of DM, OM and N was higher in treatments QTE and QTEAC than in the control (Table 6), which is consistent with the effect of QTE on DM, OM, and CP digestibility. There was a significant increase in total C excretion in QTE2, QTE4, AC3.0 and QTEAC by 22%, 44%, 13%, and 24%, respectively. NDF and ADF excretion increased by 30% and 44% with QTE4, and to a lesser extent (NDF: 10% to 13%; ADF: 18% to 27%) with treatments QTE2, AC1.5, AC3.0 and QTEAC. The steady increase in DM, OM, N, C, NDF and ADF excretion across the three periods (Table 6) was consistent with the gradually declining apparent digestibility of these constituents (Table 4).

Compared to the control, faecal OM concentration was increased by 2% to 3% with QTE addition (P>0.05), and faecal N concentration was insignificantly altered with QTE and AC addition (Table 7). Whereas sole QTE addition only slightly increased faecal C concentration (1% to 2%), AC1.5 and QTEAC increased faecal C concentration by 6% and AC3.0 by 13%. In addition, there was a significant increase in faecal NDF (10% to 14%) and ADF (19% to 28%) concentration associated with AC inclusion. As a consequence of higher C, NDF, ADF concentrations, the C/N, NDF/N and ADF/N ratios were higher in faeces of goats on treatments AC1.5, AC3.0 and QTEAC. The continuous increase in the ratio of C/N, NDF/N and ADF/N in faeces across the three periods (Table 7) was consistent with concomitant increase in the C/N, NDF/N and ADF/N ratio of the ingesta (Table 3). From the nonparametric Spearman's rank□correlation analysis it appeared that among the parameters of particle passage only TMRT was related at r = −0.35 (P≤0.05) to the C/N ratio in faeces. Yet, the proximate composition of faeces was correlated to the quantitative intake of feed and specific nutrients as well as to ingesta quality and digestibility (Table 8).

Table 4: *In vivo* digestibility (g kg^{-1}) of proximate diet constituents determined in goats (12 per treatment) offered five experimental and one control diet during three 7-day periods of an experiment set up as a completely randomised design.

Component	Treatment								Period				
	Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	SEM[1]	P≤	1	2	3	SEM	P≤
DM	840[a]	815[b]	787[c]	837[a]	840[a]	815[b]	4.3	0.001	857[a]	816[b]	793[c]	3.1	0.001
OM	850[a]	822[b]	793[c]	845[a]	848[a]	822[b]	4.6	0.001	861[a]	823[b]	806[c]	3.4	0.001
CP	778[a]	738[b]	686[c]	769[a]	787[a]	741[b]	9.1	0.001	799[a]	743[b]	707[c]	7.5	0.001
NDF[2]	836[a]	821[ab]	784[c]	814[b]	816[b]	811[b]	6.3	0.001	846[a]	803[b]	792[b]	4.6	0.001
ADF[3]	800[a]	781[ad]	709[c]	760[bd]	754[b]	751[b]	8.6	0.001	818[a]	751[b]	709[c]	6.1	0.001

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre.

Within rows, means with different superscripts differ at $P<0.05$; P-values in columns indicate the overall effect of treatment and period, respectively.

[1] Standard error of the mean.

[2] NDF: Treatment x period interaction, P=0.004.

[3] ADF: Treatment x period interaction, P=0.003.

Table 5: Parameters (mean values) of gastrointestinal passage of feed particles in goats (7 per treatment) offered five experimental and one control diet in periods two and three of an experiment set up as a completely randomised design.

Treatment	TT (h)	λ (h^{-1})	T_{50} (h)	CMRT (h)	TMRT (h)
Control	20.0^{ac}	0.056	30.4	36.2	56.2
QTE2	18.4^{abc}	0.050	34.2	40.7	59.4
QTE4#	15.6^{b}	0.055	31.0	36.9	53.3
AC1.5	17.3^{ab}	0.052	32.3	38.5	55.4
AC3.0#	21.0^{c}	0.054	31.4	37.4	57.6
QTEAC#	18.6^{abc}	0.051	33.3	39.7	58.7
*SEM	1.24	0.0024	1.27	1.52	1.70
P–Value	0.047	0.273	0.259	0.259	0.128

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

TT: time of first marker appearance in faeces; λ: rate of particle passage through the mixing compartment; T50: halftime of the marker in the mixing compartment; CMRT: particle mean retention time in the mixing compartment; TMRT: particle mean retention time in the total gastrointestinal tract.

Within columns, means with different superscripts differ at $P<0.05$; P-values in the last row indicate the overall effect of treatment.

6 animals per treatment.

* Standard error of the mean.

Table 6: Quantitative excretion (g $kg^{-0.75}$ d^{-1}) of proximate constituents by goats (12 per treatment) offered five experimental and one control diet during three 7-day periods of an experiment set up as a completely randomised design.

Component	Treatment								Period				
	Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	SEM[1]	P≤	1	2	3	SEM	P≤
DM	7.85^{a}	9.14^{b}	10.60^{c}	7.79^{a}	7.72^{a}	8.91^{b}	0.246	0.001	7.03^{a}	9.40^{b}	9.58^{b}	0.189	0.001
OM	6.86^{a}	8.21^{b}	9.61^{c}	6.90^{a}	6.86^{a}	7.70^{b}	0.243	0.001	6.34^{a}	8.44^{b}	8.43^{b}	0.188	0.001
N	0.17^{a}	0.20^{b}	0.23^{c}	0.17^{a}	0.16^{a}	0.19^{b}	0.008	0.001	0.17^{a}	0.20^{b}	0.19^{c}	0.007	0.001
C	3.08^{a}	3.74^{bd}	4.42^{c}	3.28^{ad}	3.49^{d}	3.82^{b}	0.113	0.001	2.99^{a}	4.00^{b}	3.93^{b}	0.088	0.001
NDF[2]	2.90^{a}	3.20^{ab}	3.78^{c}	3.21^{b}	3.30^{b}	3.29^{b}	0.117	0.001	2.71^{a}	3.59^{b}	3.54^{b}	0.087	0.001
ADF	1.78^{a}	1.96^{ad}	2.56^{c}	2.10^{bd}	2.26^{b}	2.19^{bd}	0.090	0.001	1.60^{a}	2.34^{b}	2.48^{b}	0.065	0.001

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

DM: dry matter; OM: organic matter; N: nitrogen; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre.

Within rows, means with different superscripts differ at $P<0.05$; P-values in columns indicate the overall effect of treatment and period, respectively.

[1] Standard error of the mean.

[2] NDF: Treatment x period interaction, P=0.04.

Table 7: Proximate composition of faeces excreted by goats (12 per treatment) offered five experimental and one control diet during three 7-day periods of an experiment set up as a completely randomised design.

Component		Treatment								Period				
		Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	SEM[1]	P≤	1	2	3	SEM	P≤
OM[2]	(g kg^{-1} DM)	876^{a}	898^{b}	905^{b}	879^{a}	885^{a}	895^{b}	3.60	0.001	894^{a}	897^{a}	878^{b}	2.70	0.001
N	- " -	25.6	24.6	24.2	24.5	23.2	23.9	0.73	0.228	26.9^{a}	24.0^{b}	22.1^{c}	0.60	0.001
C	- " -	450^{a}	456^{b}	460^{b}	476^{c}	508^{d}	479^{c}	2.00	0.001	473^{a}	475^{a}	467^{b}	1.40	0.001
NDF[2]	(g kg^{-1} OM)	423^{a}	398^{a}	391^{a}	465^{b}	482^{b}	418^{a}	11.20	0.001	433	430	426	8.00	0.808
ADF	- " -	257^{ab}	238^{a}	264^{ab}	306^{c}	328^{c}	277^{b}	9.10	0.001	257^{a}	281^{b}	297^{b}	6.50	0.001
C/N	(g g^{-1})	17.9^{a}	18.8^{ab}	19.0^{abc}	19.5^{bc}	22.9^{d}	20.4^{c}	0.53	0.001	17.9^{a}	20.0^{b}	21.4^{c}	0.38	0.001
NDF/N[3]	- " -	17.0^{ab}	16.4^{a}	16.4^{a}	19.1^{b}	21.9^{c}	17.8^{ab}	0.79	0.001	16.5^{a}	18.2^{b}	19.6^{b}	0.56	0.001
ADF/N	- " -	10.3^{ab}	9.9^{a}	11.1^{abc}	12.7^{c}	14.9^{d}	11.8^{bc}	0.57	0.001	9.8^{a}	11.9^{b}	13.6^{c}	0.40	0.001

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

OM: organic matter; N: nitrogen; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre.

Within rows, means with different superscripts differ at P<0.05; P-values in columns indicate the overall effect of treatment and period, respectively.

[1] Standard error of the mean.

[2] OM and NDF: Treatment x period interaction, P=0.004.

[3] NDF/N: Treatment x period interaction, P=0.008.

Table 8: Spearman correlation coefficients (r_s) and significance levels[†] of the individual relationships between diet quality, quantitative intake and digestibility of diet constituents[‡] with quality of faecal excreta of goats offered six different diets. All coefficients are based on 39 observations per variable.

Variable / Dependent / Independent	Quality of faeces						
	N	C	NDF	ADF	C/N	NDF/N	ADF/N
			(g kg^{-1} OM)			ratio (g g^{-1})	
Diet quality (g kg^{-1} OM)							
N	0.33*						-0.32*
C			0.35*	0.45**	0.52***	0.39*	0.45**
NDF						0.33*	
ADF				0.33*	0.33*	0.33*	0.33*
C/N ratio	-0.37*			0.35*	0.38*		0.43**
NDF/N ratio	-0.41**			0.35*	0.44**	0.33*	0.46**
ADF/N ratio	-0.34*			0.46**	0.42**	0.33*	0.52***
Intake (g kg$^{-0.75}$)							
DM	0.35*				-0.32*		
OM	0.31*						
N	0.40*				-0.36*		
C	0.34*	0.65***					
NDF	0.32*						
Digestibility(g kg^{-1})							
DM		0.38*	0.55***				
OM			0.51***			0.32*	
N			0.55***			0.52***	-0.35*
NDF	0.33*				-0.35*	-0.32*	-0.54***
ADF	0.34*			-0.47**	-0.39*		

[†] Significance levels: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; empty cells depict insignificant relationships between variables.

[‡] ADF: acid detergent fibre; C: carbon; DM: dry matter; N: nitrogen; NDF: neutral detergent fibre; OM: organic matter.

2.4. Discussion

2.4.1 Effects of QTE and AC on feed intake, digesta passage and digestibility

There was no significant effect of QTE and AC inclusion on feed intake when compared to the control diet; however the intake of OM and DM was relatively lower in AC1.5, AC3.0 and QTEAC treatments in comparison to QTE2 and QTE4. For QTE this supports the suggestion to limit the concentration of CT in ruminants' diets to 5% (50 g kg^{-1} DM) of the total ration (Min et al., 2003), since at higher concentrations CT can negatively affect feed intake due to changes in palatability, conditioned aversion and reduced rate of feed digestion in the rumen (Mueller-Harvey, 2006; Waghorn, 2008). However, the extent of CT effects on feed intake depends on the physical and chemical properties of tannins, and the diet / tannin interactions (Min et al., 2003; Patra and Saxena, 2011). Vasta et al. (2009) reported that feeding QTE at 4% of diet DM to lambs reduced DM intake when these lambs were fed fresh vetch (*Vicia sativa*), while it had no effect when the lambs were fed concentrate. Athanasiadou et al. (2001) even reported an improved feed intake in sheep when QTE was included in a high protein diet at 6% (on fresh weight basis). The current results are consistent with those of Beauchemin et al. (2007) and Al-Dobaib (2009) who reported that the inclusion of QTE up to 2% and 3%, respectively, did not alter the DM intake of cattle and sheep on roughage-dominated rations.

The amount of AC included in the diet (AC1.5: 0.26 g kg^{-1} LW; AC3.0: 0.52 g kg^{-1} LW) was lower than the recommended 2 g kg^{-1} LW (Prasad et al., 2000). The missing effect of AC on DM and OM intake is consistent with the results obtained by Van et al. (2006), who up to an inclusion of 1.5 g kg^{-1} LW determined no effect of bamboo charcoal on the DM intake of goats fed *Acacia mangium* plus a supplemental diet which consisted of concentrate and para grass.

The higher feed intake observed in period 2 may be explained by the lower average temperature and humidity, especially in comparison to period 3, even though in the latter a higher feed intake of the constantly growing male goats would have been expected (Mary and David, 2009). The higher CP intake in period 1 than in periods 2 and 3 has to be attributed to better hay quality in period 1 (Table 1).

The digestibility of DM, OM, and CP was negatively affected by the addition of QTE. Al-Dobaib (2009) demonstrated an *in vitro* reduction of OM degradation at increasing concentrations of QTE, which was attributed to the reactivity of tannins and their formation of complexes with proteins, fibre constituents, microbial cell walls and

enzymes (Makkar et al., 1989; McSweeney et al., 2001). Al-Dobaib (2009) also reported a reduction of the OM digestibility by 3% to 13% when QTE was included at 2% and 3% in an alfalfa-based ration of sheep. The more pronounced effect observed by Al-Dobaib (2009) at 3% QTE inclusion may be attributed to his grinding of alfalfa hay to 4 mm prior mixing it with QTE, followed by mechanical pelleting of the mixture at 85°C, which might have led to complex-formation of fibre constitutes with QTE. This assumption seems to be confirmed by the reported reduction of NDF and ADF digestibility by 22.4% and 21.8%, respectively (Al-Dobaib, 2009). Furthermore, Al-Dobaib (2009) observed a strongly negative effect of CT on CP digestibility (-11.3% and -18.3% at 2% and 3% QTE, respectively), which might have been be due to an increased solubility of plant protein and reaction with CT in the rumen after the fine grinding of the alfalfa hay. Similar effects have been reported in cattle where QTE reduced CP digestibility by 5% and 15% when included at 1% and 2% of dietary DM, respectively, while the concomitant reduction in ADF digestibility was 5.5% and 10%, respectively (Beauchemin et al., 2007). The lower influence of QTE on DM, OM, CP, NDF, and ADF digestibility observed in our goats could have been due to the lower susceptibility of goats to CT effects since their larger parotid glands produce more saliva compared to sheep (Vaithiyanathan et al., 2001; Marsh et al., 2006), especially upon ingestion of CT (Salem et al., 2013). Histatins and proline-rich saliva proteins which are precipitated by dietary CT are considered to be the primary mechanisms by which adverse effects of CT are lowered (Mueller-Harvey, 2006; Waghorn, 2008). Tannin-binding salivary proteins are contained in saliva of goats in contrast to saliva of sheep and cattle which contains lower or no tannin-binding salivary proteins (Mueller-Harvey, 2006).

From the results obtained in the present study and by Al-Dobaib (2009), it can be assumed that the effect of QTE at 2% of dietary DM was primarily due to binding dietary proteins and lowering their ruminal degradation. Dietary CT complex with dietary proteins after mastication, and the remaining unbound CT might react with lignocellulose leading to decreased microbial digestion of fibre (Barry and Forss, 1983; Barry and Manley, 1986).

The currently observed effects of QTE on protein digestion are consistent with previous studies in ruminants (McNeill et al., 1998; Barry and McNabb, 1999; Komolong et al., 2001; Puchala et al., 2005; Beauchemin et al., 2007). Puchala et al. (2005) reported an increased intestinal amino acid absorption in goats fed CT-containing forage, due to decreased ruminal protein degradation that was associated with lower concentrations of $N\text{-}NH_3$ in the rumen and urea-N in plasma. Similar observations were made in ewes grazing *Lotus corniculatus* in comparison to ewes grazing CT-free forage (Min et al.,

1998). Beauchemin et al. (2007) demonstrated the ability of QTE to decrease protein degradation in the rumen and hence ruminal NH_3 production in cattle, which was associated with a reduction in apparent CP digestibility. Al-Dobaib (2009) reported lower urinary N excretion upon feeding QTE to sheep, which was correlated with a reduction of whole tract N digestibility and increased linearly with increasing QTE concentrations in the diet. The shift in N excretion from urine to faeces due to the lower ruminal N degradation and ammonia loss upon QTE ingestion (Dawson et al., 1999; Foley et al., 1999) and formation of tannin-protein complexes in faeces is beneficial for the environment (Makkar, 2003; Patra and Saxena, 2011). Nitrogen excreted in the faeces mainly occurs organically bound and is not easily prone to gaseous emission as are ammonia and nitrous oxide, or to leaching as is nitrate (Misselbrook et al., 2005; Steinfeld et al., 2006; Eckard et al., 2010). Moreover faeces that contain tannin-protein complexes decompose slowly in soil, leading to slower mineralization of organic N (Eckard et al., 2010).

The higher quantitative excretion of faecal N associated with feeding CT can also be related to lower post-ruminal digestibility of tannin-bound digesta protein (Cortes et al., 2009), decreased activity of intestinal enzymes (Patra and Saxena, 2011), impaired intestinal function (Mbatha et al., 2002) and increased secretion of endogenous proteins (Butter et al., 1999). The ability of QTE to protect dietary proteins from excessive ruminal degradation and to shift N excretion from urine to faeces without affecting N retention was confirmed in growing male goats fed up to 4% QTE (Chapter 3).

As far as the effects of AC are concerned, Van et al. (2006) reported an increase in DM digestibility by 11% and 14% and an increase in OM digestibility by 11% and 12% when bamboo wood charcoal was added at 0.5 g kg^{-1} LW and 1 g kg^{-1} LW, respectively, whereas in the current study AC had no effect on DM and OM digestibility. Prasad et al. (2000) recommended feeding 2 g kg^{-1} LW of AC to cattle - the amounts of AC used in the present study were 0.26 and 0.52 g kg^{-1} LW (AC1.5, AC3.0). The more pronounced effect of bamboo charcoal observed by Van et al. (2006) might also be attributed to the lower feed intake of their goats which was 640 and 646 g DM day^{-1} in comparison to 742 and 748 g DM day^{-1} (AC1.5; AC3.0) in the present study. Furthermore, Van et al. (2006) also observed an increase in CP, NDF and ADF digestibility when bamboo charcoal was fed at up to 1.0 g kg^{-1} LW. This is in contrast to the results obtained in the current study where AC had no effect on CP digestibility and NDF and ADF digestibility were significantly reduced as compared to the control diet. This might be attributed to the higher adsorptive capacity of the coconut-shell-derived, steam activated AC used in our study, with a presumably increased surface area, tamped density and micro-porosity in

comparison to the burned and manually ground bamboo charcoal (2 mm particle size) used by Van et al. (2006). Inactivation of the *Acacia* tannins by the charcoal in the latter study might be another explanation for the improved digestibility of DM, OM, CP, NDF and ADF reported by Van et al. (2006). In the present study AC had no effect on QTE since in the combined treatment the digestibility of these proximate constituents was reduced in a similar way as in the QTE2 treatment.

The steady decline in the apparent digestibility of all proximate constituents from period 1 to 3 can be ascribed to the increased concentration of fibre fractions and the continued reduction in the CP concentration of feed offered throughout the three experimental periods (Al-Asfoor et al., 2012). This might have caused a reduction in rumen ammonia release required for the growth of microbial mass including fibrolytic bacteria (Russell et al., 1992; Sultan et al., 2009).

All parameters of particle passage determined by pulse-dose application of Yb-mordanted fibre were very similar to values reported from studies with goats in other semi-arid sub-tropical and tropical regions on green feeds, local hays and straws (Lechner-Doll et al., 1990; Silanikove et al., 1993; Schlecht et al., 2007). Surprisingly, QTE and AC inclusion did not affect the outflow of feed particles from the rumen (λ) and particle mean retention time in the mixing compartment, that is the rumen, but only accelerated (partly significantly, but mostly by trend) the laminar flow of feed particles through the postruminal GIT (TT), except for the AC3.0 treatment. The latter observation is of little surprise, as activated charcoal is habitually used in human and veterinary medicine to adsorb diarrhoea-causing pathogens and toxins and slow digesta passage through the intestinal tract (Chu et al., 2013). Yet, as this phenomenon only occurred at comparatively higher ingestion of AC and seemingly only affected the post-ruminal passage rate, it is of little surprise that parameters of particulate passage did not directly correlate with the digestibility of proximate diet constituents and the quality of faeces. Since all goats, throughout the three experimental periods, were offered 800 g DM of feed per day, their slow but continued growth lead to a reduction in feed and nutrient intake when related to metabolic mass.

In consequence, the declining intake of DM, OM, CP, NDF and ADF that resulted from the unchanged feed offer coincided with an increase in the volume of the various GIT compartments, in particular rumen and large intestine, of the growing animals. This explains the negative correlation between (decreasing) intake of proximate constituents per kilogram of metabolic mass and the numerically unaltered parameters TT and TMRT depicted in section 2.3.2.

2.4.2 Effects of QTE and AC on faecal excretion and quality

The increase in faecal OM concentration and in total DM and OM excretion of goats fed QTE was associated with a lower digestibility of DM and OM in QTE2, QTE4, and QTEAC. Despite the lower apparent digestibility of CP associated with feeding QTE, the concentration of N in faeces was not significantly different from control, AC1.5, and AC3.0 treatments. Hence it can be assumed that few tannin-protein complexes were excreted in faeces, which might have been due to a higher dissociation of such complexes in the abomasum and small intestine (Patra and Saxena, 2011). This contrasts the observation by Beauchemin et al. (2007) who reported a reduction in the digestibility of acid detergent insoluble nitrogen when cattle were fed up to 2% QTE, indicating that tannin-protein complexes were not completely disassociated in the abomasum. Powell et al. (2009) also reported a higher concentration of neutral detergent insoluble N and acid detergent insoluble N in faeces of cows fed *Lotus corniculatus* silage containing 1.5% to 1.7% tannins (in DM) compared to those fed alfalfa silage and quinone-containing red clover.

There was a significant increase in the faecal C, NDF and ADF concentration of goats fed AC1.5 and AC3.0, but only in faecal C concentration of goats fed QTEAC. For the pure AC treatments, this in consequence resulted in a higher faecal C/N, NDF/N, and ADF/N ratio, which was however at least partly also affected by the higher C/N, NDF/N, and ADF/N ratios in the ingesta (Table 3). Especially for the faecal concentration of nitrogen, but also of NDF and ADF as well as for the C/N, NDF/N and ADF/N ratios in faeces, a low to moderate, mostly positive correlation was obtained with parameters of diet quality as well as with some variables of quantitative intake and digestibility. Even though the individual results depicted in Table 8 are not unexpected *per se*, positive r_s values >0.5 for the effects of DM, OM and N digestibility on faecal NDF concentration again point to an enhancement of the faecal concentration of more slowly degradable C factions by dietary AC incorporation (where DM, OM and N digestibility remained unchanged as compared to the control), but probably also to interferences of AC (as well as QTE) particles contained in faeces with the fibre analysis; this is discussed in more detail in Chapter 3. To another part, however, the higher faecal C and fibre concentrations that were observed in faeces derived from AC-supplemented goats can certainly be ascribed to the indigestibility of the activated charcoal.

Slowly decomposable manure C is required under irrigated conditions in arid and semiarid climates to reduce the high carbon turnover provoked by high ambient temperatures and flood irrigation (Siegfried et al., 2013). The mineralization process of nutrients in organic manure is determined by the C/N ratio (Kyvsgaard et al., 2000; Van

Kessel et al., 2000) as well as by the concentration of slowly decomposable C which is needed for a long-term effect of manure on soil organic matter and N stabilization (Hassink, 1994). Although the C/N ratios obtained in the current study are lower than the threshold value of 20 to 25 below which organic fertilizer is subjected to fast mineralization of N (Senesi, 1989; Myers et al., 1994), several studies showed that the organic N fraction in ruminant faeces is not easily decomposed by soil microorganisms (Bosshard et al., 2009; Jost et al., 2013).

2.5. Conclusions

Feeding up to 4% QTE to goats reduced the apparent digestibility of CP but did not increase faecal N concentration. On the other hand, dietary inclusion of up to 3.0% AC increased the faecal concentration of NDF, ADF and C. This might help to stabilize soil organic matter in arid sub-tropical environments where year-round irrigation and high ambient temperatures lead to a rapid depletion of soil C stock. As the addition of up to 3% AC to goats' diet did not negatively affect quantitative feed intake and only moderately reduced the digestibility of proximate diet constituents, such diet amendment seems promising in view of enhancing manure stability without negatively affecting the animals' physiology.

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3. Effects of quebracho tannin extract (*Schinopsis balansae*) and activated charcoal on nitrogen balance and microbial protein synthesis in the rumen of growing Boer goats

Abstract

Under irrigated arid sub-tropical conditions, organic fertilizer rich in slowly decomposable nitrogen (N) and carbon (C) is needed for soil fertility maintenance. Feeding ruminants with condensed tannins (CT) will lower ruminal protein degradation, reduce urinary N excretion and might increase the faecal fraction of slowly decomposable N. Supplementing the diet with activated charcoal (AC) might enrich the animals' manure with long-lived C species to be sequestered in the soil. The current study investigated the effects of feeding AC and quebracho tannin extract (QTE, a source of CT), on goats' N balance, the microbial protein synthesis in their rumen and the quality of their faeces.

The complete randomised feeding trial comprised three subsequent periods, with twelve male Boer goats (28 kg ±3.9) assigned to five treatments and a control (50% grass hay, 50% concentrate pellets). Treatment diets contained 2% and 4% QTE (QTE2, QTE4), 1.5% and 3.0% AC (AC1.5, AC3.0) and a mixture of 2% QTE plus 1.5% AC (QTEAC). All diets were offered at 1.5 times maintenance requirements. In addition to the determination of the N balance following standard protocols, microbial nitrogen supply (MNS) from the rumen was calculated from the daily excretion of purine derivatives (PD).

There was no significant effect of QTE and AC on organic matter (OM), N and metabolizable energy intake, but digestibility of OM, neutral detergent fibre and acid detergent fibre were reduced ($P<0.05$). QTE induced a shift in N excretion from urine to faeces ($P<0.001$) without altering N retention in spite of reduced N digestibility ($P<0.01$) as compared to the control and AC treatments. MNS and efficiency of microbial protein synthesis were also not influenced by feeding QTE and AC, but both treatments induced a higher faecal excretion of C in the form of slowly decomposable carbohydrates. The results demonstrate that QTE can be included up to 4% into the diet of goats without affecting N utilization while increasing slowly decomposable N and C fractions in the manure.

3.1. Introduction

The turnover of nitrogen (N) and carbon (C) from organic manure is determined by its C/N ratio and fibre content which in turn affects the release of nutrients required for crop growth (Velthof et al., 2005). In arid sub-tropical Oman, N and C losses from organic manures through leaching and gaseous emissions are high due to elevated temperatures and frequent wet-dry cycles associated with flood irrigation (Siegfried et al., 2013).

Similar observations exist for cropping systems in Australia and oases systems of northern Oman, Iran, and Uzbekistan (Dalal et al., 2003; Hans et al., 2005; Jalali, 2005; Scheer et al., 2009). Under such conditions, organic fertilizers should therefore contain slowly degradable N as well as slowly decomposable carbohydrates with a long-term stabilizing effect on soil organic matter (Hassink, 1994; Janssen, 1996; Handayanto et al., 1997; Kyvsgaard et al., 2000; Van Kessel et al., 2000). Since manure quality depends on the quality of feed ingested by farm animals (Powell et al., 1999; Petersen et al., 2007; Van Vliet et al., 2007; Al-Asfoor et al., 2012), the interdependencies of feeding, animal performance and manure quality have to be considered simultaneously (Petersen et al., 2007).

Excessive protein degradation in the rumen entails high urea N losses due to enhanced production of ammonia that is not completely utilized by rumen microbes and which to some extent might lower the flow of non-ammonia N to the duodenum and absorption from the intestine (Broderick, 1995; Van Horn et al., 1996; Min et al., 2000; Patra and Saxena, 2011). Lowering ruminal protein degradation by feeding diets with moderate concentrations (<5%) of condensed tannins (CT) is one attempt to increase the flow of non-ammonia N to the abomasum and lower digestive tract, and thus potentially increase absorption of amino acids in the small intestine (Komolong et al., 2001; Frutos et al., 2002; Min et al., 2003; Mueller-Harvey, 2006). Since complexes between CT and proteins are stable and insoluble at pH 3.5-7.0, the degradation of proteins in the rumen is reduced (Patra and Saxena, 2011). The reversibility of tannin-protein complexes post-ruminally is influenced by the interaction that occurs at rumen pH, which is determined by the chemical structure and molecular weight of both tannins and proteins, and by post-ruminal pH (Patra and Saxena, 2011). Incomplete dissociation of tannin-protein complexes in the abomasum and the small intestine, disturbance of the digestive enzymes and the epithelial lining of the digestive tract by CT may lead to reduced absorption of amino acids in the small intestine and lower apparent digestibility of N (Silanikove et al., 2001; Mbatha et al., 2002; Beauchemin et al., 2007; Grainger et al., 2009). For example, Komolong et al. (2001) have demonstrated that increased amounts of quebracho (*Schinopsis balansae*) tannin extract (QTE) gradually reduced the absorption of amino acids in the small intestine of sheep fed an alfalfa-based diet. In consequence, less N is excreted as urea (Min et al., 2000; Makkar, 2003; Patra and Saxena, 2011), and at the same time N release from manures in cropping systems is reduced when tannin-protein complexes are present (Grabber et al., 2001; Makkar, 2007; Grainger et al., 2009).

Lower ruminal protein degradation when feeding CT might also impair microbial protein outflow from this compartment. However, reports on the effects of CT on microbial

protein synthesis are inconsistent, since the latter depends on rate and extent of feed protein degradation and/or alteration in fermentation processes, which is also determined by the availability of other nutrients required for microbial growth. Min et al. (2003) reported a positive relationship between dietary concentrations of CT and the flow of non-ammonia N to the duodenum, while the microbial protein outflow was decreased. In contrast, an enhanced microbial protein outflow was reported from sheep fed alfalfa-based diets mixed with QTE (10 and 20 g kg^{-1} DM; Al-Dobaib, 2009). *In vitro* studies also showed an improved microbial protein synthesis when incubating tamarind (*Tamarindus indica*) seed husk containing 145 - 155 g CT per kg DM (Bhatta et al., 2001). Makkar (2003) stated that although tannins decrease nutrient availability, those nutrients still available are directed towards microbial mass synthesis rather than production of short chain fatty acids. Yet, feeding dried *Leucaena* leaves (116 g CT kg^{-1} DM) to sheep had no effect on microbial protein synthesis (McNeill et al., 2000). Lower protozoal counts and a higher proportion of propionate were induced by feeding tannins (Makkar, 2003). An increased efficiency of microbial protein synthesis might explain the beneficial effects of tannins found at low dietary concentrations, such as improved wool growth and live weight gain, milk production and fertility (Makkar, 2003; Bhatta et al., 2007). Even though it reduced the population of fibre-degrading bacteria *(Fibrobacter succinogenes* and *Ruminococcus* spp.*)*, feeding *Calliandra calothyrsus* at 30% of the diet did not affect the efficiency of microbial protein synthesis in sheep (McSweeney et al., 2001).

Physically or chemically activated carbonaceous compounds have an increased surface area for adsorption or chemical reactions (Girgis et al., 2002). Physical activation is usually performed on carbonised materials that are heated to 800 - 1100°C in the presence of oxidizing agents such as O_2 or CO_2 (Yalcin and Arol, 2002). Chemical activation involves fixing "carbonization'' of the carbonaceous materials by dehydrating agents such as H_2SO_4, $ZnCl_2$, KOH, and H_3PO_4 at lower temperatures (400 - 700°C; Girgis et al., 2002).

The prominent ability of charcoal to adsorb secondary plant compounds, that is terpenoids, phenols and N-containing compounds, make it a potential feed additive to improve the intake of plants high in such substances (Struhsaker et al., 1997; Banner et al., 2000; Poage et al., 2000; Rao and Chopra, 2001; Van et al., 2006). Van et al. (2006) reported that the inclusion of 1 g kg^{-1} live weight of bamboo charcoal increased N digestibility and N retention, and reduced urinary N excretion in goats fed tannin-rich *Acacia* fodder. These authors suggested considering charcoal as a potential feed additive for improving N utilization. Apart from this report, however, there is lack of information

on the effects of feeding (activated) charcoal on the N metabolism of ruminants. Nevertheless, the remaining stable fractions of charcoal C that are excreted in faeces can provide long-lived C species that are sequestered in the soil and will stabilize soil organic matter. In crop production, charcoal has been used as soil conditioner and carbon sequester for over 2000 years, playing an important role in reducing the release of greenhouse gases, nutrient leaching and in improving soil productivity (Yanai et al., 2007; Lehmann and Joseph, 2009; Blackwell et al., 2009; Major et al., 2009; Verheijen et al., 2009).

Summarizing the above findings on the effects of tannin as well as charcoal feeding, the addition of protein-binding condensed tannins (CT) and/or activated charcoal (AC) to ruminants' diets might (i) enhance the efficiency of rumen microbial protein synthesis and N retention of the animal, (ii) increase faecal N and/or C concentrations, (iii) slow down N release from organic manure, and (iv) provide long-lived C species to be sequestered in the soil. Such livestock manures would perfectly meet the specific requirements of irrigated crop farming under arid sub-tropical conditions (Siegfried et al., 2013). Therefore the current study examined the effects of feeding different concentrations of a commercial source of CT (that is, QTE) or/and AC, on feed and nutrients intake and digestibility, N balance, rumen microbial protein synthesis and faeces quality of growing male goats.

3.2. Materials and methods

3.2.1 Experimental design

At the premises of Göttingen University, a completely randomised N-balance trial was conducted over three subsequent periods (from September 14 to November 21, 2011), where each period consisted of 21 adaptation days followed by two times six sampling days. Twelve male, non-castrated Boer goats (28 kg ±3.9) were paired according to their weight and age and assigned to two groups. In each period one of 6 diets was offered to one goat per group; each diet was tested only once on the same goat. The two goats that were assigned to the same treatment, but belonged to different groups, were housed together in a fenced cage throughout the adaptation period. The separation into two subgroups was necessary since only 6 metabolic cages were available, and in each experimental period (of 2 times 6 days) the two goats per treatment were housed in a cage successively. Before each adaptation period and after the last sampling period, goats were fasted overnight and weighed on two consecutive mornings; the average weight served to adjust the amount of feed offered to 1.5 times maintenance energy requirements (GfE, 2003). The control diet consisted of 50% grass hay (*Lolium perenne)* and 50%

pelleted concentrate feed (35% barley, 35% wheat, 15% rape seed meal, 15% sugar beet molasses chips; all on dry matter (DM) basis). Two test diets additionally contained powdered quebracho tannin extract (*Schinopsis balansae,* purchased from Otto Dille®, Norderstedt, Germany). According to our analysis (see below) the murrey-colored powder contained 75% total phenols; it was added to the diet at levels of 2% (QTE2) and 4% (QTE4) of dry matter (DM). A powder of activated charcoal (AC), generated by steam activation from coconut shells (AquaSorb® CP1; Jacobi Carbons GmbH Frankfurt/Main) was added at levels of 1.5% (AC1.5) and 3.0% (AC3.0) of the total ration (on DM basis), and the fifth test diet contained a mixture of QTE and AC (QTEAC: 2% QTE and 1.5% AC). The required amounts of QTE and AC powder, respectively, were mixed with the concentrate feed, moistened (1 litre of water for each 10 kg of dry mixture) and pelleted thereafter using a mechanical press. The chemical composition of the hay and the six different types of pelleted concentrate is presented in Table 1. Feed was offered twice daily in two equal portions, at 7:30 a.m. and 4:30 p.m. The concentrate feed was offered first and was totally consumed within 15 - 20 minutes. Afterwards grass hay was offered and occurring refusals were collected prior to the next feeding.

3.2.2 Sampling procedures

Representative sub-samples of feed offered were taken from each lot of pelleted concentrate feed and each 80 - 100 kg bale of hay; sub-samples were pooled at the end of each period. Feed refusals were quantified daily and pooled per animal for each 6 days experimental period; from the homogenised pool a representative sub-sample was stored at −20°C until analysis. Faeces were quantitatively collected into fabric bags that were attached to the goats by harnesses and emptied twice daily. Faeces were pooled for two consecutive days each and then were thoroughly homogenised; afterwards two representative sub-samples were frozen at −20°C until analysis. Urine was gathered from the metabolic cages directly into acidified containers (10 ml of 10% sulphuric acid) and adjusted to a pH below 3 to avoid bacterial degradation of purine derivatives (PD).

Table 1: Chemical composition of grass hay and concentrate pellets offered to goats at 50% of the ration each during three periods of an experiment set up as Youden square.

Component	DM	OM	N	C	NDF	ADF	ME
	(g kg^{-1} FM)		(g kg^{-1} DM)		(g kg^{-1} OM)		(MJ kg^{-1}DM)
Hay	929	934	16.6	443	600	348	8.3
Pellets							
Control	908	963	26.1	448	158	99	11.0
QTE2	906	963	25.4	458	171	94	10.8
QTE4	908	961	24.0	470	194	85	10.5
AC1.5	914	964	24.8	446	189	106	10.9
AC3.0	915	965	23.9	461	215	119	10.7
QTEAC	910	962	24.3	450	194	117	10.6

Pellets: control =35% barley, 35% wheat, 15% rape seed meal, 15% sugar beet molasses chips; all on dry matter (DM) basis; QTE2: 2% Quebracho tannin extract in overall diet (4% in pellet, on dry matter basis); QTE4: 4% QTE in overall diet; AC1.5: 1.5% activated charcoal in overall diet (3% in pellet, on dry matter basis); AC3.0: 3% AC in overall diet; QTEAC: 2% QTE and 1.5% AC in overall diet.

FM: fresh matter; DM: dry matter; OM: organic matter; N: nitrogen; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre; ME: metabolizable energy derived from *in vitro* gas production (Menke and Steingass, 1987).

Urine was quantitatively collected twice daily, before feeding. Urine samples of two consecutive days were pooled and a sub-sample of 500 ml was frozen at −20°C for total N determination. For the analysis of purine derivatives, a sub-sample of 50 ml of each 2-days pool of urine was taken, passed through filter paper (MN 615 ¼, Macherey-Nagel GmbH & Co.KG Germany) and diluted with distilled water (1:5). From this, three tubes of 10 ml were frozen at −20°C prior analysis.

3.2.3 Chemical analysis

Samples of feed offered, refusals and fresh faeces were dried in a hot-air drying oven at 60°C to constant weight and then ground to 1 mm particle size prior to analysis. DM and organic matter (OM) contents were determined according to standard procedures (Naumann and Bassler, 1976). The concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using a semi-automated Ankom 220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) following Naumann and Bassler (1997) without using decalin and sodium sulphite. Residual ash was excluded from the

NDF and ADF values. The Hohenheim gas test was used to determine *in vitro* metabolizable energy content in each lot of pelleted concentrate and in one representative sample of hay from each of the three periods, following Menke and Steingass (1987). Nitrogen (N) and carbon (C) contents were determined by means of a C/N–TCD analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). N content of urine samples was measured using the micro Kjeldahl method (AOAC, 2000). The daily urinary excretion of purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) was measured using reversed-phase-HPLC (JASCO® HSS-1500, Groß-Umstadt, Germany). Allantoin was analysed following the method of Chen et al. (1993), whereas uric acid, xanthine and hypoxanthine were analysed after Balcells et al. (1992). They were determined in triplicate for each 2-days pool (see 3.2.3) by peak integration as follows: Allantoin at 360 nm, uric acid at 286 nm, hypoxanthine at 248 nm and xanthine at 268 nm. Microbial nitrogen supply (MNS) was calculated from the daily excretion of PD following Chen and Gomes (1995). The efficiency of the microbial protein synthesis (EMPS) was estimated in g microbial N per kg OM apparently digested in the rumen (DOMR), whereby DOMR equals 0.65 times digestible OM intake (Santoso et al., 2007).

3.2.4 Statistical analysis

An ANOVA was conducted by means of the mixed model procedure in SAS 9.3 (SAS Institute Inc., Cary, NC, USA), with treatments and periods as fixed effects and animals considered as random effect. The model used was:

$$y_{ij} = \beta_1 x_{1ij} + \beta_2 x_{2ij} + b_{i1} z_{1ij} + \varepsilon_{ij} \qquad \text{[Eq. 1]}$$

where y_{ij} is the value of the outcome variable for a particular ij case, β_1 and β_2 are the fixed effect coefficients for treatment and period, respectively, x_{1ij} and x_{2ij} are the fixed effect variables for observation j in group i, b_{i1} is the random effect coefficient, z_{1ij} is the random effect variable (animal), and ε_{ij} is the error for case j in group i.

Interactions between periods and treatments were tested in advance; since no interactions were found these were excluded from the model. Least squares means were used to compare results with significant F-values. Significance was declared at $P<0.05$.

3.3. Results

3.3.1 Effects of QTE and AC on intake and digestibility

There was no significant effect of QTE and AC on OM, NDF, ADF, and ME intake of goats. However, the intake of OM, ME, NDF and ADF (Table 2) and N (Table 3) was higher in period 2 than in period 1 and 3. In comparison to the control, the OM digestibility was reduced by 6.7% with QTE2 and by 9.9% with QTE4 (Table 2). Feeding AC also reduced OM digestibility by 4.3% (AC1.5) and 3.8% (AC3.0) as compared to the control. With diet QTEAC the reduction in OM digestibility was 6.6%. NDF digestibility was reduced by 13.6%, 13.2% and 10.4% with QTE2, QTE4 and QTEAC, whereas ADF digestibility was reduced by 27.5%, 35.5% and 24.0% with the respective treatments (Table 2). AC1.5% reduced the digestibility of NDF and ADF by 11.3% and 17.5%, while the respective reduction with AC3.0 was only 6.8% and 13.7% and not different from the control. With respect to the experimental period, the digestibility of proximate diet constituents was higher in period 3 than in periods 1 and 2 (Table 2).

3.3.2 Effects of QTE and AC on N balance and microbial protein synthesis

QTE and AC did not significantly alter N intake (Table 3). However, N excretion was significantly reduced with QTE2 (-10.3%), QTE4 (-12.5%) and QTEAC (-10.0%). Although AC1.5 and AC3.0 also reduced total N excretion as compared to the control (-1.3% and -3.8%) this was not significant. In consequence, a significantly higher amount of digested N was determined with treatments AC than with treatments QTE, but not with QTEAC (Table 3). Total and faecal N excretion was lowest in period 3, whereas the quantity of digested N was highest in this period (Table 3).

Although N retention was not significantly affected by QTE and AC (Table 3), values increased as compared to the control with QTE2 (+18.4%), QTE4 (+7.7%) and QTEAC (+10.9%), and decreased with AC1.5 (-16.6%) and AC3.0 (-6.9%). In consequence, the ratio of retained to digested N was higher with QTE2 (+27.2%), QTE4 (+33.5%) and QTEAC (+25.2%) as compared to the control, while the ratio was similar to the control with AC3.0 and significantly lower with AC1.5 (Table 3).

Compared to the control, a quantitative increase in N excretion with faeces was observed with QTE2 (+30.5%), QTE4 (+48.7%) and QTEAC (+25.8%). Concomitantly, quantitative urine N excretion was reduced by 26.7%, 37.3% and 23.1%, respectively (Table 3). This was consistent with the tendency for lower urinary PD excretion and

Table 2: Intake, digestibility and faecal excretion of diet constituents by goats offered five experimental and one control diet during three periods of an experiment set up as Youden square.

	Treatment								Period				
Variable	Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	*S.E.M.	P≤	1	2	3	*S.E.M.	P≤
Intake per day													
OM (g/kg$^{0.75}$)	54.3	53.2	52.1	51.1	53.5	54.1	2.12	0.923	51.1^{a}	55.9^{b}	52.6^{ab}	1.60	0.039
ME (kJ/kg$^{0.75}$)	553	541	523	532	539	543	18.9	0.863	519^{a}	562^{b}	533^{a}	14.4	0.038
NDF (g/kg$^{0.75}$)	20.1	19.5	19.2	19.3	20.9	20.6	1.28	0.799	18.6^{a}	21.5^{b}	19.7^{ab}	0.97	0.043
ADF (g/kg$^{0.75}$)	11.9	11.2	10.2	11.0	12.0	12.0	0.75	0.336	10.6^{a}	12.3^{b}	11.2^{ab}	0.56	0.045
Digestibility (coefficient)													
OM	0.821^{a}	0.766^{bc}	0.740^{c}	0.786^{b}	0.790^{b}	0.767^{b}	0.009	0.001	0.762^{a}	0.767^{a}	0.806^{b}	0.006	0.001
NDF	0.767^{a}	0.663^{b}	0.666^{b}	0.680^{b}	0.715^{ab}	0.687^{b}	0.023	0.035	0.657^{a}	0.690^{a}	0.741^{b}	0.016	0.006
ADF	0.684^{a}	0.496^{bcd}	0.441^{d}	0.564^{bc}	0.590^{ac}	0.520^{bcd}	0.037	0.003	0.506^{a}	0.542^{ab}	0.600^{b}	0.027	0.045
Faecal excretion (g/kg$^{0.75}$/d)													
OM	9.9^{a}	12.3^{bcd}	13.5^{d}	10.9^{ac}	11.2^{abc}	12.8^{bd}	0.60	0.002	12.0^{a}	13.1^{a}	10.2^{b}	0.44	0.001
NDF	4.9^{a}	6.1^{b}	6.4^{b}	5.8^{ab}	6.0^{b}	6.5^{b}	0.38	0.032	6.1^{a}	6.7^{a}	5.1^{b}	0.28	0.001
ADF	3.8^{a}	5.3^{bc}	5.6^{c}	4.6^{ab}	4.9^{bc}	5.7^{c}	0.32	0.001	5.0^{a}	5.6^{b}	4.4^{a}	0.23	0.002
C	4.3^{a}	5.6^{bc}	6.2^{c}	5.3^{b}	5.6^{bc}	6.2^{c}	0.28	0.001	5.7^{a}	6.1^{a}	4.9^{b}	0.21	0.001

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

OM: organic matter; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre; ME: metabolizable energy; the latter was calculated from *in vitro* gas production (Menke and Steingass, 1987). Treatment means with different superscripts within rows differ at P≤0.05; P-values in columns indicate the overall effect of treatment and period, respectively, in the model [Eq. 1]. * Standard error of the mean.

Table 3: Nitrogen balance, urinary excretion of purine derivatives and calculated ruminal microbial protein synthesis by goats offered five experimental and one control diet during three periods of an experiment set up as Youden square.

	Treatment								Period				
Variable	Control	QTE2	QTE4	AC1.5	AC3	QTEAC	*S.E.M.	P≤	1	2	3	*S.E.M.	P≤
Nitrogen balance													
Intake (g/kg$^{0.75}$/d)	1.166	1.134	1.078	1.096	1.096	1.125	0.0376	0.517	1.076^{a}	1.164^{b}	1.107^{ab}	0.0284	0.037
Excretion (g/kg$^{0.75}$/d)	0.823^{a}	0.738^{bc}	0.720^{c}	0.812^{a}	0.791^{ab}	0.740^{bc}	0.0237	0.022	0.800^{a}	0.780^{ab}	0.732^{b}	0.0168	0.028
Faeces	0.230^{a}	0.300^{b}	0.342^{c}	0.240^{a}	0.234^{a}	0.290^{b}	0.0137	0.001	0.290^{a}	0.295^{a}	0.234^{b}	0.0101	0.001
Urine	0.595^{a}	0.437^{bc}	0.373^{c}	0.575^{a}	0.551^{a}	0.458^{b}	0.0243	0.001	0.510	0.486	0.498	0.0176	0.553
Digestion (g/kg$^{0.75}$/d)	0.936^{a}	0.832^{b}	0.738^{c}	0.852^{ab}	0.864^{ab}	0.833^{b}	0.0310	0.004	0.786^{a}	0.869^{b}	0.873^{b}	0.0231	0.007
Retention (g/kg$^{0.75}$/d)	0.337	0.399	0.363	0.281	0.314	0.374	0.0407	0.265	0.276^{a}	0.384^{b}	0.375^{b}	0.0312	0.008
N Retained/ N Digested	0.359^{ac}	0.457^{ab}	0.480^{b}	0.320^{c}	0.359^{ac}	0.450^{ab}	0.0405	0.027	0.346^{a}	0.443^{b}	0.424^{b}	0.0306	0.026
Faecal N / Urinary N	0.397^{a}	0.694^{b}	0.949^{c}	0.417^{a}	0.431^{a}	0.643^{b}	0.0485	0.001	0.608^{a}	0.674^{a}	0.484^{b}	0.0352	0.002
Microbial Protein Synthesis													
PD excreted (mmol/d)	12.58	11.51	9.43	11.18	11.90	9.47	1.057	0.191	10.66	11.00	11.37	0.751	0.785
PD absorbed (mmol/d)	14.91	13.61	11.05	13.21	14.10	11.11	1.292	0.188	12.58	12.99	13.43	0.918	0.793
$^{\&}$MNS (g/d)	10.84	9.90	8.04	9.61	10.25	8.08	0.939	0.188	9.14	9.45	9.76	0.667	0.793
$^{\#}$EMPS	29.36	27.10	24.00	30.03	30.73	22.48	2.498	0.120	27.35	27.07	27.43	1.769	0.988

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet. Treatment means with different superscripts within rows differ at P≤0.05; P-values in columns indicate the overall effect of treatment and period, respectively, in the model [Eq. 1].

* Standard error of the mean. $^{\&}$ Microbial N supply (from rumen to duodenum).

$^{\#}$ Efficiency of microbial protein synthesis = g of microbial N per kg of digestible OM apparently fermented in the rumen (DOMR); DOMR=0.65 x digestible OM.

lower MNS from the rumen, which decreased by 8.7%, 25.9% and 25.5% with QTE2, QTE4 and QTEAC (Table 3), whereas feeding AC had no effect on PD excretion and MNS. Compared to the control, the efficiency of microbial protein synthesis was reduced with QTE2 (-7.7%), QTE4 (-18.3%) and QTEAC (-23.4%) and enhanced with AC1.5 (+2.3%) and AC3.0 (+ 4.7%), but effects were not significant (Table 3).

3.3.3 Effects of QTE and AC on faeces quality

The concentration of OM in faecal dry matter increased ($P<0.05$) with treatments QTE2 (+4.4%) and QTE4 (+5.2%) as compared to the control (Table 4), and the same was observed for treatments AC1.5 (+2.6%), AC3.0 (2.3%) and QTEAC (3.3%). Concomitantly, faecal C excretion increased in all QTE and AC treatments. While faecal NDF concentration tended to decrease in the QTE treatments ($P>0.05$) and increased significantly (Table 4) in the AC treatments as well as in QTEAC, faecal ADF concentration was increased by all treatments, though not always significantly. A significant increase in faecal N concentration was only observed for QTE4 (+8.6%), while the two AC treatments significantly reduced faecal N concentration by 6.6% (AC1.5) and 10.8% (AC3.0) as compared to the control diet.

Table 4: Chemical composition of faeces excreted by goats offered five experimental and one control diet in experiment set up as Youden square. Treatment means across the three experimental periods.

	DM	OM	N	C	NDF	ADF
Treatment	(g kg^{-1} FM)		(g kg^{-1} DM)		(g kg^{-1} OM)	
Control	933^{ab}	844^{a}	23.5^{af}	439^{a}	496^{abd}	391^{a}
QTE2	928^{cd}	881^{cd}	24.2^{ab}	458^{b}	494^{ad}	436^{bc}
QTE4	924^{d}	888^{d}	25.5^{bc}	459^{b}	467^{ab}	409^{ab}
AC1.5	935^{a}	866^{b}	22.0^{de}	487^{c}	535^{c}	423^{abc}
AC3.0	937^{a}	863^{b}	21.0^{e}	501^{d}	530^{c}	436^{bc}
QTEAC	930^{bc}	873^{bc}	22.6^{df}	484^{c}	509^{cd}	454^{c}
*SEM	1.71	4.27	0.49	4.33	10.99	14.90
P≤	0.001	0.001	0.001	0.001	0.001	0.058

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.
FM: fresh matter; DM: dry matter; OM: organic matter; N: nitrogen; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre. Treatment means with different superscripts within columns differ at $P\leq0.05$; P-values in the last row indicate the overall treatment effect in the model [Eq. 1]. * Standard error of the mean.

3.4. Discussion

3.4.1 Effects of QTE and AC on intake and digestibility

A high concentration of tannins in feed can alter intake due to changes in palatability and resulting conditioned aversion or reduced rate of digestion in the rumen (Mueller-Harvey, 2006; Waghorn, 2008). Effects depend on physical and chemical properties of the specific tannin and the interactions between the tannin and diet constituents (Min et al., 2003; Patra and Saxena, 2011). In the current study the added concentrations of 2 % and 4% QTE were below 50 g CT kg^{-1} DM, which is the upper threshold concentration considered beneficial for improving dietary protein utilization in ruminants (Min et al., 2003). Up to a concentration of 4% in total diet DM, QTE had no significant effect on OM intake in the present experiment. This agrees with findings of Al-Dobaib (2009) who reported that up to a concentration of 22.5 g kg^{-1} DM of QTE (the latter containing 75% CT) the DM intake of alfalfa hay by sheep was not affected. Similarly, Beauchemin et al. (2007) observed that up to 18.2 g kg^{-1} DM the addition of QTE (the latter containing 91% CT) had no significant effect on feed intake in cattle. The negative effect of QTE on OM digestibility observed in the present study can be ascribed to formation of tannin complexes with proteins, fibre constituents, microbial cell walls and enzymes (Makkar et al., 1989; McSweeney et al., 2001). Whereas Al-Dobaib (2009) reported a 12.6% reduction in OM digestibility at 2.25% QTE, and a reduction in NDF and ADF digestibility of 22.4% and 21.8%, the reduction in OM and NDF digestibility upon QTE feeding was less pronounced in our study. Probably these differences are due to the fact that in the experiment of Al-Dobaib (2009) the ground alfalfa hay (4 mm) was mixed with QTE powder and afterwards pelleted at 85°C using steam, whereas in our study the QTE powder was mixed with the ground concentrate and a bit of water and mechanically pelleted. The pre-treatment with moisture and high temperature (Al-Dobaib, 2009) might have reduced the protein-binding ability of CT at temperatures > 40°C (Makkar and Becker, 1996; Makkar, 2003), but might also have increased the binding of CT to temporarily solubilized hemicelluloses. Al-Dobaib (2009) also reported an *in vitro* reduction of OM degradation as the level of QTE increased, which is consistent with an *in vitro* study done by Getachew et al. (2008) and an *in sacco* study by Hervas et al. (2003). However, Beauchemin et al. (2007) found no effect of QTE on DM, NDF and ADF digestibility when fed to cattle at up to 1.82%, even though their QTE had a higher concentration of CT than the extracts used by Al-Dobaib (2009) and in the present study. Bhatta et al. (2007) even reported an increase in ADF and cellulose digestibility when *Prosopis cineraria* leaves (91 mg CT kg^{-1} DM) were fed to lambs at 25%, 50% and 75%

of the complete diet. The binding of CT to carbohydrates is pH independent but varies with molecular weight, flexibility and water solubility of tannins (Seigler, 1998); it is also influenced by the availability of tannins after binding with dietary proteins (Barry and Manley, 1986). Inhibiting cellulolytic microorganisms and activities of fibrolytic enzymes are further mechanism by which CT can affect fibre digestion (Bae et al., 1993; Patra and Saxena, 2009). However, Patra et al. (2011) observed that tannins present in seed pulp of *Terminalia chebuala* and in *Allium sativum* bulbs enhanced the digestibility of nutrients and fibre fractions when fed to sheep at 10 g kg^{-1} of total DM intake, and attributed this to an increased number of fibrolytic bacteria in the rumen. When *Calliandra calothyrsus* was fed to sheep at 30% of the diet, fibre-degrading bacteria were reduced but the efficiency of microbial protein synthesis was not affected (McSweeney et al., 2001). McAllister et al. (2005) reported that CT from different legume forages, namely *Lespedeza cuneata*, crown vetch (*Coronilla varia*), and sainfoin (*Onobrychis viciifolia*), from stems of *Hedysarum alpinum*, alfalfa seeds, whole plants of birdsfoot trefoil (*Lotus corniculatus* var. *corniculatus*) and three varieties of big trefoil (*Lotus pedunculatus*) had different inhibitory effects on *Fibrobacter succinogenes*. In the present study the effect of QTE was more pronounced for the digestibility of ADF than of NDF. This contradicts the suggestion of Bae et al. (1993) that CT are able to bind to and inhibit more efficiently the activity of extracellular than of cell-associated enzymes, making hemicellulose more sensitive to the inhibitory effect of CT in comparison to cellulose. Our results might therefore be explained by binding of QTE to cellulolytic microorganisms and fungi rather than to secreted enzymes; the former reaction ultimately inhibits microbial cell growth and synthesis of cell-associated enzymes.

The higher digestibility of proximate diet constituents in period 3 as compared to periods 1 and 2 may be explained by the changes in the metabolism of goats that were constantly gaining weight. This is supported by the results of the N balance which showed a gradual increase in the amounts of digested and retained N as the trial proceeded from period 1 to 3.

As far as feeding of charcoal or AC, respectively, is concerned, Prasad et al. (2000) recommended that this substrate can be fed at 2 g kg^{-1} of live weight (LW) to cattle. In the current study the amount of AC added to the diet was 0.41 g kg^{-1} LW (AC1.5) and 0.79 g kg^{-1} LW (AC3.0). The observation of Van et al. (2006) that the inclusion of up to 1.5 g kg^{-1} LW of bamboo charcoal in goats' diet did not affect DM intake of a mixed diet consisting of *Acacia mangium*, para grass plus a supplemental feed are consistent with the current results. Yet, in contrast to the results obtained in the present study, the inclusion of up to 1 g kg^{-1} LW of bamboo charcoal improved DM and OM digestibility

(Van et al., 2006). The negative effect of AC on OM digestibility even at 1.5% inclusion might be explained by differences in the adsorptive capacity of the AC used in the present trial as compared to the bamboo charcoal. Whereas Van et al. (2006) burned bamboo wood in iron containers for 6 hours and ground the remaining charcoal to 2 mm particle size, the steam activation of carbonaceous material from coconut shells (see 3.2.1) and grinding to powder has most probably increased the surface area, tamped density and micro-porosity of AC as compared to the bamboo-derived material (Kutlu et al., 2001; Miguel et al., 2003). Kutlu et al. (2001) reported that dietary wood charcoal can adsorb vitamins, fats and enzymes. Similarly, Van et al. (2006) suggested that some nutrients can be adsorbed by charcoal, along with phenolic compounds. Such inactivation of the *Acacia* tannins in the latter study might explain the improved digestibility of DM, OM, NDF and ADF reported by Van et al. (2006), whereas in the present experiment even the combined feeding of QTE and AC depressed the digestibility of these proximate constituents.

3.4.2 Nitrogen balance and microbial protein synthesis in the rumen

Beauchemin et al. (2007) demonstrated the ability of QTE to decrease ruminal protein degradation and NH_3 release in cattle, which was associated with a 5% reduction in apparent CP digestibility at 1% QTE and a 15% reduction at 2% QTE in the diet. Similarly, Al-Dobaib (2009) observed that the lower urine-N excretion associated with feeding increasing QTE concentrations was linearly correlated with a reduction of whole-tract N digestibility. In the present study, total N excretion was significantly reduced with QTE2, QTE4, and QTEAC, although less N was digested in these treatments. The latter did however not affect N retention across these treatments. These findings may be attributed to lower protein degradation in the rumen associated with CT, and to a higher availability of N in the abomasum and lower digestive tract (Min et al., 2003; Mueller-Harvey, 2006). The observed effects of QTE on the N metabolism of growing male goats are consistent with findings of previous studies (McNeill et al., 1998; Barry and McNabb, 1999; Komolong et al., 2001; Puchala et al., 2005; Mueller-Harvey, 2006; Beauchemin et al., 2007; Patra and Saxena, 2011). The absolute and relative shift in N excretion from urine to faeces with treatments QTE2, QTE4 and QTEAC supports the assumption of a lower protein degradation in the rumen (Patra and Saxena, 2011), but in combination with the slightly increased N retention an increased digestion of rumen-protected proteins in the small intestine seems plausible (Min et al., 2003; Mueller-Harvey, 2006; Patra and Saxena, 2011). Puchala et al. (2005) reported an increased intestinal amino acid absorption in goats fed S*ericea lespedeza* forage containing 17.7% CT as a result of decreased ruminal protein degradation; this was associated with lower rumen

concentrations of N-NH_3 and reduced plasma urea-N. Similar results were reported for ewes grazing *Lotus corniculatus* (28 g CT kg^{-1} DM) in comparison to ewes grazing CT-free forage (Min et al., 1998).

As far as the effects of charcoal feeding are concerned, no effects of AC1.5 and AC3.0 were observed with respect to the amounts of N digested, N retained and N excreted, and the partitioning of N excretion between urine and faeces was also unaltered. This contrasts with the results of Van et al. (2006) who, up to an offer of 1.5 g kg^{-1} LW of bamboo charcoal, observed an increase in CP digestibility, a significant improvement of N retention and a reduction of urinary N excretion, which, in this case, could not be attributed to the presence of *Acacia* tannins (58 g CT kg^{-1} DM) which were also ingested, in identical amounts, by goats fed the control diet, and was therefore interpreted as beneficial – detoxifying – effect of charcoal feeding (Van et al., 2006).

The present values obtained for MNS are similar to those of Andrade-Montemayor et al. (2004) who compared five different equations for sheep, cattle (Chen and Gomes, 1995) and goats (Belenguer et al., 2002) to predict a MNS of 8.99 to 11.85 g d^{-1} for goats offered a total mixed ration (either 50% alfalfa hay or barley straw, 50% concentrate; intake 40 g DM $kg^{-0.75}$ LW d^{-1}). Yet, our values for MNS and EMPS contrast the results for sheep reported by Al-Dobaib (2009), where up to 2% QTE in the diet increased MNS, and up to 3% QTE enhanced EMPS. This might have been due to the fact that in this study (Al-Dobaib, 2009), NDF and ADF digestibility were not reduced at 2% QTE, and at 3% QTE the reduction in ADF digestibility was lower than in our study and thus enough energy was supplied to support microbial growth in the rumen. Nevertheless, the present ratios of N retained to N digested (Table 3) were more favourable than the ratios of 0.31 to 0.37 at up to 3% QTE feeding reported by Al-Dobaib (2009), which might be due to the fact that his rams were mature while our male goats were still growing.

Feeding AC did not alter MNS and EMPS, probably due to the reduced digestibility of OM, NDF, and ADF and the subsequent discrepancy of rapid protein degradation and slow energy availability in the rumen, leading to the absorption of ammonia from the rumen, synthesis of urea and subsequent excretion with urine (Min et al., 2000; Makkar, 2003; Patra and Saxena, 2011).

The slightly increasing amounts of N digested and retained across all treatments from period 1 to 3 point to an increase in the digestive capacity and probably also to a change in the rumen microflora (Morand-Fehr et al., 1982; Mary and David, 2009) of the growing goats.

3.4.3 Quality of faeces and implications for manure use

Compared to the control, faecal carbon concentration was increased in all QTE and AC treatments, which can be interpreted as a consequence of the higher faecal NDF and ADF excretion and a concurrently reduced fibre digestibility. However, there was no increase in faecal NDF concentration in the QTE treatments, which may partly be due to the significant increase in the excreted quantity of faeces (Table 2). It also has to be noted that, compared to the control pellets, all treatment pellets were higher in NDF concentration and only the QTE2 and QTE4 pellets contained less ADF. Therefore, changes in the faecal NDF and ADF concentration upon feeding the QTE and AC diets should be interpreted prudently since interference between the used detergent fibre analysis method and tannins have been reported (Makkar et al., 1995) and might have affected both, the values for fibre concentration in the pellets and the faeces. Moreover, AC contained in the pellets might also have become part of the NDF and ADF residue during detergent fibre analysis, though reports on this issue are not available yet. On the other hand, the high faecal C concentration observed with AC diets was partly also due to the mere addition of indigestible and inert carbon to the undigested feed residue.

Lower ruminal N degradation and ammonia loss upon QTE ingestion is one explanation for the observed increase in the faecal N concentration (Dawson et al., 1999; Foley et al., 1999). However, it might also be due to a lower post-ruminal digestibility of tannin-bound digesta protein (Cortes et al., 2009), decreased activity of intestinal enzymes (Patra and Saxena, 2011), impaired intestinal function (Mbatha et al., 2002) and increased secretion of endogenous proteins (Butter et al., 1999). With activated charcoal, the partitioning of N excretion between urine and faeces was not altered in comparison to the control diet (see 3.4.2), which, in combination with the higher faecal OM and C concentration, respectively, reduced the faecal N concentration in the AC treatments.

Given the complexation of carbohydrates and proteins by QTE, which is supposed to be the main reason for the observed increase in faecal OM, C, NDF and N concentration, such faeces will most probably meet the specific requirements for manure in irrigated cropping systems under arid sub-tropical conditions such as in Oman (Siegfried et al., 2013) and similar regions. Slowly decomposable C (that is, NDF and ADF) is required for a long-term stabilizing effect of organic fertilizer on soil organic matter and for organic N accumulation in the soil (Hassink, 1994; Handayanto et al., 1997). Faecal N is mainly bound in organic form and is therefore less volatile than urinary N which is prone to leaching as nitrate or to gaseous emissions in the form of ammonia and nitrous oxide (Steinfeld et al., 2006; Eckard et al., 2010). In addition, tannin-bound faecal protein is

only slowly decomposing in soil, leading to a longer-term residual fertilization effect of respective manures (Somda et al., 1995; Powell et al., 2009; Eckard et al., 2010; Powell and Broderick, 2011).

3.5. Conclusions

The ability of QTE to protect dietary proteins from excessive ruminal degradation and to shift N excretion from urine to faeces without affecting N retention was confirmed in growing male goats. The reduced digestion of OM, NDF, ADF and N induced by QTE has apparently no effect on the efficiency of microbial protein synthesis in the rumen and supply of microbial protein to the small intestine. Since feeding QTE was also associated with higher excretion of slowly decomposable carbohydrates, including up to 4% QTE in growing goats' diets will be beneficial for both goats and field crop production, in the latter case through the increased faecal concentration of slowly decomposable N and C. Although feeding activated charcoal increases the faecal concentration of C and slowly decomposable carbohydrates, this feed additive cannot be recommended when aiming at improved N utilization in goats and reduced (urine-derived) emissions of ammonia and nitrous oxide to the environment.

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4. The effects of quebracho tannin extract (*Schinopsis balansae*) and activated charcoal on the microbial biomass in goat faeces

Abstract

In systems where intensive irrigated agriculture is practiced on sandy soils under arid sub-tropical and tropical conditions, organic fertilizers should contain slowly decomposable nitrogen and carbohydrate fractions with a stabilizing effect on soil organic matter. Faecal microbial biomass has a direct influence on the autochthonous soil microbial biomass and microbial residues, hence, on soil organic matter and subsequent cycling of carbon (C) and nitrogen (N) in soil. The current study investigated the effects of feeding activated charcoal (AC) and quebracho tannin extract (QTE, a source of condensed tannins), on feed and nutrients intake, digestibility, and excretion by growing male goats, and on faecal microbial biomass.

The complete randomised feeding trial comprised three subsequent periods with twelve male Boer goats (28 kg ±3.9) assigned to five treatments and a control (50% grass hay, 50% concentrate pellets). Experimental diets contained 2% and 4% QTE (QTE2, QTE4), 1.5% and 3.0% AC (AC1.5, AC3.0) and a mixture of 2% QTE plus 1.5% AC (QTEAC). All diets were offered at 1.5 times maintenance energy requirements. In addition to the proximate analysis performed on feed and faeces following standard protocols, faecal microbial biomass was determined by measuring the concentration of ergosterol and amino sugars as indices for fungal and bacterial communities.

There were no significant effects of QTE and AC on quantitative intake of feed and nutrients. Faecal concentration of slowly decomposable carbohydrates increased with QTE and AC feeding ($P<0.001$), due to a reduced digestibility of fibre fractions. QTE also reduced the digestibility of crude protein and increased faecal N concentration ($P<0.001$). Feeding QTE and AC did not alter total microbial C, however QTE shifted the microbial community structure towards fungi ($P<0.01$).

The results demonstrate that including up to 4% QTE in goats' diets enhances the concentration of slowly decomposable N and C fractions in the resulting manure, whereas feeding up to 3% AC increases the faecal concentration of slowly decomposable C fractions and increases the faecal C/N ratio.

4.1. Introduction

Continuous application of organic matter is very crucial to maintain soil organic carbon (SOC) under the arid sub-tropical conditions of Oman (Siegfried et al., 2011; Sradnick et al., 2014) and similarly hot environments where intensive irrigated agriculture is practiced on sandy soils. Under such circumstances carbon (C) is easily lost to the atmosphere due to high organic matter turnover rates; in the long-term this might also

lead to increased nitrogen (N) losses via leaching or emission since there is no substantial built-up of soil organic matter (Siegfried et al., 2013). Hence, organic fertilizers should contain slowly decomposable carbohydrates with a stabilizing effect on soil organic matter (Hassink, 1994; Janssen, 1996; Handayanto et al., 1997; Kyvsgaard et al., 2000; Van Kessel et al., 2000). Previous studies showed that feed ingested by ruminants plays a critical role for the chemical composition of manure and manure-related C and N cycling in soils, and hence the accumulation of SOC and the availability of nutrients for plant uptake (Somda and Powell, 1998; Powell et al., 2006, 2009; Petersen et al., 2007; Van Vliet et al., 2007; Al-Asfoor et al., 2012). The faecal concentration and diversity of microorganisms, which together with the macro- and mesofauna as well as microbial population of the soil affect the decomposition of faeces, is determined by the chemical composition of faeces (Chadwick et al., 2000; Van Vliet et al., 2007). In addition faecal microbial biomass has a direct influence on the autochthonous soil microbial biomass and microbial residues, hence, SOC. Therefore the interdependencies of livestock feeding, manure quality and manure decomposition in soil have to be considered simultaneously for an optimized utilization of nutrients and recycling of C and N in soil (Handayanto et al., 1997; Delve et al., 2001; Petersen et al., 2007; Jost et al., 2013).

Feeding moderate concentrations of up to 5% of condensed tannins (CT) to ruminants has been found to shift the excretion of N from urine to faeces. This is mediated by the formation of stable and insoluble tannin-protein complexes in the rumen, leading to a lower ruminal degradation of N, an increased flow to and absorption of amino acids in the small intestine and in consequence lower excretion of urine N (Min et al., 2000, 2003; Komolong et al., 2001; Frutos et al., 2002; Makkar, 2003; Mueller-Harvey, 2006; Patra and Saxena, 2011). The presence of tannin-protein complexes in faeces might lead to a slower N release from manures when applied to crop fields (Makkar, 2003; Makkar et al., 2007; Waghorn, 2008; Grainger et al., 2009). Moreover, CT have the ability to alter the microbial outflow from the rumen due to lower ruminal protein degradation (Makkar, 2003; Waghorn, 2008). However, data on the effects of CT on rumen microbial outflow are inconsistent, due to tannin and diet specific changes in the rate and extent of protein degradation and/or alteration of the rumen fermentation system (Patra and Saxena, 2011). Kok et al. (2013) studied the response of anaerobic cellulolytic rumen fungi in goats fed *Leucaena leucocephala* (6% CT in dry matter). They observed a fluctuation in the fungal population during 30 experimental days where in the first 10 days the fungal population decreased from 17.9 µg ml^{-1} rumen fluid to 8.6 µg ml^{-1}. After 15 days of feeding there was a significant increase (to 157.0 µg ml^{-1}), followed by another decrease to 50.6 µg ml^{-1}, which was not significantly different from the control (55.4 µg ml^{-1}).

Despite available information on the role of microbial diversity in the rumen and lower gastrointestinal tract, there is still lack of information on their diversity in the faeces in general, and their response to CT feeding in particular (Min et al., 2014). Only few studies tested the influence of CT on processes in the lower gastrointestinal tract, where free tannins might further affect nutrient metabolism and microbial turnover in the hindgut, which in turn will affect the chemical composition and microbial community of the faeces (Van Vliet et al., 2007; Ley et al., 2008). Feeding *Lotus corniculatus* (32 g CT per kg dry matter, DM) to sheep reduced the population of some specific proteolytic bacteria but did not affect total ruminal microbial protein synthesis and outflow to the abomasum (Min et al., 2002). The same group (Min et al., 2014) reported a selective alteration of bacteria and methanogenic archaea populations in the animals' hindgut and a reduction in their faecal concentrations when goats were fed pine bark (3.2% CT in DM). The reduction in faecal microbial population can partly be explained by the inhibition of bacterial growth and reduced fibre digestibility in the rumen (Min et al., 2005), whereas the prevalence of methanogenic archaea and other bacteria in the hindgut of animals other than swine is still not fully understood (Min et al., 2014).

Several studies have shown that CT are subjected to modification and/or disappearance in the gastrointestinal tract of ruminants (Terrill et al., 1994; Perez-Maldonado and Norton, 1996), yet the fate of CT along the digestive tract of ruminants still requires clarification. In the digestive system CT are subjected to microbial breakdown, resulting in simple phenolic compounds and phenols that can be further metabolized into non-aromatic compounds (e.g. short-chain fatty acids, lactate, succinate, ethanol), CO_2 and H_2 (Selma et al., 2009; Moco et al., 2012). There is still lack of knowledge on the fate of dietary CT in the lower digestive tract where free tannins might again bind to N (undigested dietary N, undigested bacterial N, endogenous N) leading to changes in the availability of N to the hindgut microorganisms, which will affect the composition and diversity of the latter. Moreover, the interdependencies between the microbial community and the production of enteric CH_4, H_2 and volatile fatty acids from feed and/or degradation of CT might play a critical role for the faecal microbial community (Min et al., 2014).

The use of activated charcoal as a universal poison antidote in human and veterinary medicine has been published internationally since 40 years (Chu et al., 2013), yet the beneficial effects of charcoal as a non-digestible adsorbent for poisons, drugs, toxins, fat, gases and noxious substances is known since hundreds of years (Van et al., 2006; Thu et al., 2010; Chu et al., 2013). In farm animals, activated charcoal (AC) or wood charcoal are used as feed additive to improve the intake of plants high in secondary compounds and to activate the intestinal function through eliminating poisons and impurities from the

digestive tract (Struhsaker et al., 1997; Banner et al., 2000; Poage et al., 2000; Rao and Chopra, 2001; Van et al., 2006). As a result of charcoal addition to the animals' diets, increase in feed intake, body weight and feed utilization have been reported for chicken (Kutlu et al., 2001), pigs (Thu et al., 2010; Chu et al., 2013) and goats (Van et al., 2006).

In cropping systems, biochar, which is produced by thermal degradation of organic materials under low temperatures and limited oxygen supply, is used as soil conditioner and carbon sequester (Lehmann and Joseph, 2009). It has been suggested that in soil biochar can reduce emissions of CO_2, N_2O, CH_4 (Lehmann, 2007; Renner, 2007; Yanai et al., 2007; Major et al., 2009) and leaching of NH_4^+ and NO_3^- (Singh et al., 2010; Knowles et al., 2011), and influence the microbial community composition (Lehmann et al., 2011). Chu et al. (2013) reported that feeding bamboo charcoal to fattening pigs decreased the emissions of ammonia, methane, amines and hydrogen sulphide from the animals' faeces. Considering both the positive effects of charcoal feeding on livestock performance and on emissions from livestock excreta, it seems indicated to investigate in more detail the effects of AC feeding on manure quality, since AC-related neutralisation of anti-nutrients along the gastrointestinal tract might also affect the microbial communities in the rumen and hindgut (Van et al., 2006; Thu et al., 2010; Chu et al., 2013) and thus the microbial community in the excreta. In addition, the properties of AC to serve as microbial habitat and to adsorb ammonia and other gases might enhance microbial growth in the digestive tract of ruminants and in soil (Lehmann et al., 2011). Moreover, the indigestible fraction of carbonized C in faeces might help to stabilize SOC over time if such manures are used in cropping systems (Glaser et al., 2002; Dempster et al., 2012; Lentz and Ippolito, 2012).

In soil science, amino sugars that are bound to soil organic matter are used to determine microbial mass (Amelung, 2001; Amelung et al., 2008). The determination of fungal glucosamine and bacterial muramic acid further helps to assess the contribution of these two main microbial groups to SOC (Appuhn and Joergensen, 2006; Joergensen and Wichern, 2008; Joergensen et al., 2010). However, glucosamine and muramic acid can also be used as indicators for microbial biomass in freshly excreted faeces (Rezaeian et al., 2004 a,b; Appuhn and Joergensen, 2006; Jost et al., 2011). Moreover glucosamine (chitin) has been used to evaluate fungal biomass in the rumen fluid of cattle (Sekhavati et al., 2009), and ergosterol, which is a fungal cell membrane component, has also been successfully extracted from soils, plant roots and livestock faeces as an indicator for fungal biomass (Appuhn and Joergensen, 2006; Joergensen and Wichern, 2008; Jost et al., 2011). In an experiment conducted by Jost et al. (2013), faeces dominated by fungal communities were characterized by lower gaseous emissions and a stronger short-term N

immobilization in the soil. While this induced lower immediate N uptake by plants, it might contribute to a longer-term soil N supply and especially to reduced gaseous N losses under conditions of frequent irrigation and high ambient temperatures.

Summarising the above mentioned effects of condensed tannins and/or (activated) charcoal on nutrient digestion and faeces quality, we aimed at investigating whether feeding CT and/or AC will result in livestock manure that meets the specific requirements of irrigated crop farming under arid sub-tropical conditions (Siegfried et al., 2013) due to: (i) increased faecal excretion of N and/or C and (ii) predominance of fungi over bacteria in the faeces.

4.2. Materials and methods

4.2.1. Experimental design

Faeces samples were taken from twelve intact male Boer goats (28 kg ±3.9) that were kept at the premises of the Section Animal Husbandry and Breeding at Göttingen University. These goats were taking part in a completely randomised N-balance trial, which was conducted over three subsequent periods (from September 14 to November 21, 2011), where each period consisted of 21 adaptation days followed by two times six sampling days. Animals were paired according to their weight and age and assigned to two groups. In each period one of 6 diets was offered to one goat per group; each diet was tested only once on the same goat. The two goats that were assigned to the same treatment, but belonged to different groups, were housed together in a holding pen throughout the adaptation period. The separation into two subgroups was necessary since only 6 metabolic cages were available, and in each experimental period (of 2 times 6 days) the two goats per treatment were housed in a cage successively. The control diet, which consisted of 50% ryegrass hay (*Lolium perenne* L.*)* and 50% concentrate feed (35% barley, 35% wheat, 15% rape seed meal, 15% sugar beet molasses chips; all on DM basis) was offered at 1.5 times maintenance energy requirements (GfE, 2003). The goats were weighed before each adaptation period and after the last sampling period; the average weight of two consecutive mornings was used to adjust the amount of feed offered.

Table 1: Chemical composition of ryegrass hay and concentrate pellets offered to goats at 50% of the ration each during three periods of an experiment set up as Youden square.

Component	DM (g kg^{-1} FM)	OM	CP	C	NDF	ADF	ME
		(g kg^{-1} DM)			(g kg^{-1} OM)		(MJ kg^{-1} DM)
Hay	929	934	103.8	443	600	348	8.3
Pellets							
Control	908	963	163.3	448	158	99	11.0
QTE2	906	963	158.7	458	171	94	10.8
QTE4	908	961	149.8	470	194	85	10.5
AC1.5	914	964	155.2	446	189	106	10.9
AC3.0	915	965	149.5	461	215	119	10.7
QTEAC	910	962	151.6	450	194	117	10.6

Pellets: control =35% barley, 35% wheat, 15% rape seed meal, 15% sugar beet molasses chips; all on dry matter (DM) basis; QTE2: 2% Quebracho tannin extract in overall diet (4% in pellet, on dry matter basis); QTE4: 4% QTE in overall diet; AC1.5: 1.5% activated charcoal in overall diet (3% in pellet, on dry matter basis); AC3.0: 3% AC in overall diet; QTEAC: 2% QTE and 1.5% AC in overall diet.

FM: fresh matter; DM: dry matter; OM: organic matter; CP: crude protein, calculated by multiplying nitrogen concentration by the factor 6.25; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre; ME: metabolizable energy derived from *in vitro* gas production (Menke and Steingass, 1987).

The experimental diets additionally contained powdered quebracho tannin extract (*Schinopsis balansae,* purchased from Otto Dille®, Norderstedt, Germany; 75% phenols according to our analysis) at levels of 2% (QTE2) and 4% (QTE4) of the total ration (on DM basis), or powder of activated charcoal (AC), generated by steam activation from coconut shells (AquaSorb® CP1; Jacobi Carbons GmbH Frankfurt/Main) at levels of 1.5% (AC1.5) and 3.0% (AC3.0) of the total ration (on DM basis), and a mixture of QTE and AC (QTEAC: 2% QTE and 1.5% AC). The required amounts of QTE and AC were thoroughly mixed with the finely ground concentrate feed and afterwards mechanically pressed into pellets after moistening the mixture with tap water (1 litre of water for each 10 kg of dry mixture). The chemical composition of the hay and the six different types of pelleted concentrate is presented in Table 1. Feed was offered twice daily in two equal portions, at 7:30 a.m. and 4:30 p.m. The concentrate feed was offered first and was

totally consumed within 15 - 20 minutes. Afterwards grass hay was offered and occurring refusals were collected prior to the next feeding. Drinking water was always available *ad libitum*.

4.2.2. Sampling procedures

Representative sub-samples of feed offered were taken from each lot of pelleted concentrate feed and each 80 - 100 kg bale of hay; sub-samples were pooled at the end of each period. Feed refusals were quantified daily and pooled per animal for each 6 day experimental period; from the homogenised pool a representative sub-sample was stored at -20°C until analysis. Faeces were quantitatively collected into fabric bags that were attached to the goats by harnesses and emptied twice daily during the sampling period. The collected faeces were pooled for two consecutive days each and then were thoroughly homogenised; afterwards two representative sub-samples equivalent to 250 g fresh matter (FM) each were taken and stored frozen at -20°C until analysis. Faecal samples used for the determination of microbial biomass were collected after gathering the overnight samples on day 2, 4 and 6 of the sampling period (i.e. mornings of day 3, 5 and 7). After emptying the fabric bags, goats were given one hour to excrete fresh faeces, of which about 30 g were immediately frozen at -20°C. In few cases where it took more than one hour to obtain 30 g of fresh faeces the fabric bags were checked every 20-30 minutes thereafter until the required amount was reached.

4.2.3 Chemical analysis

4.2.3.1 Proximate composition of feed and faeces

Samples of feed offered, feed refusals and fresh faeces were dried in a hot-air drying oven at 60°C to constant weight and then ground to 1 mm particle size prior to analysis. DM and organic matter (OM) concentrations were determined according to standard procedures (Naumann and Bassler, 1976). The concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using a semi-automated Ankom 220 Fibre Analyser (ANKOM Technology, Macedon, NY, USA) following the protocol of Naumann and Bassler (1997) without using decalin and sodium sulphite. Residual ash was excluded from the NDF and ADF values. The Hohenheim gas test was used to determine *in vitro* metabolizable energy content in each lot of pelleted concentrate and in one representative sample of hay from each of the three periods, following Menke and Steingass (1987). Nitrogen (N) and carbon (C) contents were determined by means of a C/N–TCD analyser (Elementar Analysensysteme GmbH, Hanau, Germany).

4.2.3.2 Ergosterol and amino sugars analysis

The analysis was performed separately on each of the three replicates (that is sample of day 2, 4, 6) of frozen faeces per goat (section 4.2.2). The fungal cell-membrane ergosterol was extracted from material equivalent to 0.5 g DM of freshly thawed faeces following the method of Zelles et al. (1987). The sample was transferred to a test tube, mixed with 10 ml methanol, 2.5 ml ethanol and 1 g KOH in a 30 ml test tube, after which it was saponified under reflux for 90 minutes at 70°C. The ergosterol was extracted after cooling in three steps by adding 35 ml petroleum ether. The supernatant (25 ml) was evaporated twice at 40°C in a vacuum rotary evaporator where at each time the non-polar fraction was dissolved in 5 ml methanol, which was then stored at 4°C until analysis. Ergosterol was determined at a wavelength of 282 nm according to Jost et al. (2011) by means of a reversed-phase HPLC with 100% methanol as the mobile phase.

The amino sugars muramic acid, glucosamine, galactosamine and mannosamine were determined by chromatographic separation using o-phthalaldehyd (OPA) reagent following Indorf et al. (2011). Thawed moist faeces (2 g) were first hydrolyzed with 10 ml 6 M HCl for 2 hours at 105°C. The hydrolysed samples were then filtered in a dust collector and separated in a test tube. The removal of HCl was performed on a 0.3 ml aliquot of the hydrolysates using a vacuum rotary evaporator at 40°C. The residues were dissolved in 1 ml distilled water and then centrifuged at 13000 r min^{-1} for 10 min prior to transferring them to vials that were stored at -18°C until analysis. A Phenomenex Hyperclone C18 column (Aschaffenburg, Germany; 125 mm length x 4 mm diameter) was used to perform the chromatographic separation at 35°C where the HPLC system consisted of a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS-3000TSL analytical autosampler with in-line split-loop injection and thermostat, and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. The temperature of the autosampler was 15°C. For the automated pre-column derivatisation a mixture of 50 µl OPA and 30 µl of sample were placed in the preparation vials and 15 µl of the indole derivates were injected after 120 s reaction time. In the mobile phase, which was delivered at a rate of 1.5 ml min^{-1}, there following eluents were used: Eluent A consisted of a mixture (97.8/0.7/1.5 v/v/v) of an aqueous phase (52 mmol sodium citrate and 4 mmol sodium acetate adjusted to pH 5.3 with HCl) to which methanol and tetrahydrofuran (THF) were added. Eluent B contained 50% water and 50% methanol (v/v). Fungal C was calculated by subtracting bacterial glucosamine from total glucosamine as an index for fungal residues, assuming that muramic acid and glucosamine occur at 1 to 2 molar ratio in bacterial cells (Engelking et al., 2007):

Fungal C (mg g^{-1} DM) = (glucosamine [mmol] - (muramic acid [mmol]) x 179.2 x 9

where 179.2 is the molecular weight of glucosamine (g mol^{-1}) and 9 is the conversion value of fungal glucosamine to fungal C (Appuhn and Joergensen, 2006). As an index for bacterial residues, bacterial C was estimated by multiplying the concentration of muramic acid by 45 (Appuhn and Joergensen, 2006). The sum of fungal C and bacterial C was considered as microbial residue C.

4.2.4 Statistical analysis

After testing all data for normality (Kolmogorov-Smirnov test), an ANOVA was conducted by means of the mixed model procedure in SAS 9.3 (SAS Institute Inc., Cary, NC, USA), with treatments and periods as fixed effects and animals considered as random effect. The model used was:

$$y_{ij} = \beta_1 x_{1ij} + \beta_2 x_{2ij} + b_{i1} z_{1ij} + \varepsilon_{ij} \qquad \text{[Eq. 1]}$$

where y_{ij} is the value of the outcome variable for a particular ij case, β_1 and β_2 are the fixed effect coefficients for treatment and period, respectively, x_{1ij} and x_{2ij} are the fixed effect variables for observation j in group i, b_{i1} is the random effect coefficient, z_{1ij} is the random effect variable (animal), and ε_{ij} is the error for case j in group i.

Interactions between periods and treatments were tested in advance but were only significant in a few cases for which they are reported. Despite this, the above-mentioned final model did not specifically account for interactions. Least squares means were used to compare results with significant F-values. Significance was declared at $P<0.05$.

4.3. Results

4.3.1 Effects of QTE and AC on feed intake and digestibility

There was no significant effect of QTE and AC on OM, CP, NDF, and ADF intake of goats (Table 2). However, the intake of OM, CP, NDF and ADF was higher in period 2 than in period 1 ($P<0.05$) and 3 ($P>0.05$; data not shown). The OM digestibility was reduced by 7% with QTE2 and by 10% with QTE4 (Table 2) in comparison to the control diet. The addition of AC also caused a reduction in OM digestibility by 4% (AC1.5 and AC3.0) when compared to the control, and in the mixed treatment (QTEAC) the OM digestibility was decreased by 7%. NDF digestibility was reduced by 14%, 13% and 10% with QTE2, QTE4 and QTEAC, whereas ADF digestibility was reduced by 28%, 36% and 24%, respectively (Table 2).

Table 2: Intake and digestibility of proximate diet constituents by goats offered five experimental and one control diet during three periods of an experiment set up as Youden square.

	Treatment							
Variable	Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	S.E.M.*	P≤
Intake per day (g $kg^{-0.75}$)								
OM	54.3	53.2	52.1	51.1	53.5	54.1	2.12	0.923
CP	7.29	7.09	6.74	6.85	6.85	7.03	0.23	0.517
NDF	20.1	19.5	19.2	19.3	20.9	20.6	1.28	0.799
ADF	11.9	11.2	10.2	11.0	12.0	12.0	0.75	0.336
Digestibility (coefficient)								
OM	0.821^{a}	0.766^{bc}	0.740^{c}	0.786^{b}	0.790^{b}	0.767^{b}	0.009	0.001
CP	0.806^{a}	0.731^{b}	0.681^{c}	0.778^{a}	0.786^{a}	0.745^{b}	0.010	0.001
NDF	0.767^{a}	0.663^{b}	0.666^{b}	0.680^{b}	0.715^{ab}	0.687^{b}	0.023	0.035
ADF	0.684^{a}	0.496^{bcd}	0.441^{d}	0.564^{bc}	0.590^{ac}	0.520^{bcd}	0.037	0.003

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.

OM: organic matter; CP: crude protein; NDF: ash-free neutral detergent fibre; ADF: ash-free acid detergent fibre.

Treatment means with different superscripts within rows differ at $P<0.05$; P-values in columns indicate the overall treatment effect in the model (section 4.2.4).

* Standard error of the mean.

AC inclusion at 1.5% of total diet significantly reduced the digestibility of NDF and ADF by 11% and 18%, while with an AC inclusion at 3.0% values were not significantly different from the control. The digestibility of crude protein was only influenced by the addition of QTE. In general, the digestibility of OM, CP and NDF was higher ($P<0.05$) in period 3 than in periods 1 and 2, while for the digestibility of ADF a significant difference only existed between period 3 and 1 (data not shown).

4.3.2 Effects of QTE and AC on faeces quality and faecal microbial biomass

The OM concentration in faecal dry matter increased with the addition of QTE and AC as compared to the control (Table 3), and simultaneously the faecal C concentration increased in all QTE and AC treatments. Whereas faecal ADF concentration increased in all treatments, faecal NDF concentration only increased with the two AC diets and the QTEAC treatment.

Table 3: Chemical composition of faeces excreted by goats offered five experimental and one control diet in an experiment set up as Youden square. Values are means across the three experimental periods.

	DM	OM	N	C	NDF	ADF
Treatment	(g kg^{-1} FM)		(g kg^{-1} DM)		(g kg^{-1} OM)	
Control	933^{ab}	844^{a}	23.5^{af}	439^{a}	496^{abd}	391^{a}
QTE2	928^{cd}	881^{cd}	24.2^{ab}	458^{b}	494^{ad}	436^{bc}
QTE4	924^{d}	888^{d}	25.5^{bc}	459^{b}	467^{ab}	409^{ab}
AC1.5	935^{a}	866^{b}	22.0^{de}	487^{c}	535^{c}	423^{abc}
AC3.0	937^{a}	863^{b}	21.0^{e}	501^{d}	530^{c}	436^{bc}
QTEAC	930^{bc}	873^{bc}	22.6^{df}	484^{c}	509^{cd}	454^{c}
SEM*	1.7	4.3	0.49	4.3	11.0	14.9
P≤	0.001	0.001	0.001	0.001	0.001	0.058

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet; QTEAC: 2% QTE and 1.5% AC in diet.
FM: fresh matter; DM: dry matter; OM: organic matter; N: nitrogen; C: carbon; NDF: neutral detergent fibre; ADF: acid detergent fibre.
Treatment means with different superscripts within columns differ at $P\leq0.05$; P-values in the last row indicate the overall treatment effect in the model (section 4.2.4).
* Standard error of the mean.

A significant increase in the faecal N concentration was only observed at QTE4 (+9% as compared to the control), while it was significantly reduced with the AC and QTEAC treatments.

There was a reduction (P<0.001) by 24% and 41% in faecal ergosterol concentration associated with feeding AC1.5 and AC3.0, respectively, as compared to the control (Table 4). Feeding QTE4 and QTEAC increased (P=0.071) faecal galactosamine concentration by 51% and 49% as compared to the control, whereas the increase induced by QTE2 (22%) was not significant. Concomitantly, QTE4 and QTEAC feeding increased (P<0.05) glucosamine by 77% and 50%, and fungal glucosamine by 98% and 82%. The increase in glucosamine and fungal glucosamine observed with feeding QTE2, AC1.5, and AC3.0 were not significantly different from the values obtained for the control.The total microbial C concentration in faeces increased by 12% with QTE2, by 32% with QTE4 and by 23% with QTEAC, while with AC1.5 and AC3.0 the increase was 11% and 8% as compared to the control (Table 4). The fungal C concentration increased (P<0.05) by 41%, 103%, and 76% over the control in faeces of goats on treatments QTE2, QTE4 and QTEAC, resulting in an increase in the fungal C to bacterial C ratio by 46%, 110%, and 82% in QTE2, QTE4, and QTEAC. In the AC treatments bacterial C increased by 12% (AC1.5) and 7% (AC3.0) as compared to the control, rendering changes in fungal C to bacterial C ratio insignificant (Table 4).

4.4. Discussion

4.4.1 Effects of QTE and AC on feed intake, digestibility and faeces quality

The inclusion of up to 4% QTE in the total diet DM had no effect on goats' OM intake. This is consistent with the findings of Al-Dobaib (2009) who reported that up to a concentration of 22.5 g kg^{-1} DM of QTE (containing 75% CT) had no effect on the DM intake of alfalfa hay by sheep. Beauchemin et al. (2007) also observed that up to a concentration of 18.2 g kg^{-1} DM, QTE (containing 91% CT) had no effect on feed intake in cattle. In the current study the concentrations of 2% and 4% QTE were within the concentration limit of up to 5% that is considered beneficial for improving dietary protein utilization in ruminants (Min et al., 2003). However it has been stated that high tannin concentrations can alter feed intake in ruminants due to changes in palatability, reduced rate of digestion in the rumen and conditioned aversion (Mueller-Harvey, 2006; Waghorn, 2008). The negative effect of QTE on OM digestibility observed in the present

Table 4: Concentration of cell membrane (ergosterol) and cell wall (amino sugars) components of fungi and bacteria, respectively, in faeces of goats offered five experimental and one control diet in an experiment set up as Youden square. Values are means across three experimental periods.

	Treatment							
Variable	Control	QTE2	QTE4	AC1.5	AC3.0	QTEAC	S.E.M.*	P≤
Ergosterol (μg g^{-1} DM)	178.4^{a}	149.7^{ab}	175.9^{a}	135.2^{bc}	106.2^{c}	164.1^{ab}	14.09	0.003
Amino sugars (μg g^{-1} DM)								
Muramic acid**	469.0	485.1	454.9	525.5	500.4	454.0	62.17	0.944
Mannosamine	229.0	282.6	233.8	291.2	180.9	220.2	66.06	0.841
Galactosamine	1281.4^{a}	1564.7^{ab}	1930.7^{b}	1361.9^{a}	1387.8^{a}	1902.7^{b}	192.35	0.071
Glucosamine	1759.5^{a}	2314.5^{ab}	3110.0^{c}	1961.4^{ab}	1954.3^{ab}	2630.5^{bc}	307.28	0.040
Fungal glucosamine	1194.8^{a}	1603.3^{ab}	2371.0^{c}	1256.7^{a}	1295.5^{a}	2170.1^{bc}	261.54	0.013
Microbial C (mg g^{-1} DM)								
Fungal C	10.48^{a}	14.82^{ab}	21.32^{c}	11.54^{a}	11.42^{a}	18.46^{bc}	2.340	0.016
Bacterial C***	21.11	20.46	20.47	23.65	22.52	20.43	2.798	0.929
Microbial C	31.59	35.28	41.78	35.19	33.94	38.90	5.286	0.563
Fungal C/ Bacterial C ratio	0.497^{a}	0.724^{a}	1.041^{b}	0.488^{a}	0.507^{a}	0.904^{b}	0.1101	0.005

Treatments: QTE2: 2% Quebracho tannin extract in diet; QTE4: 4% QTE in diet; AC1.5: 1.5% activated charcoal in diet; AC3.0: 3% AC in diet QTEAC: 2% QTE and 1.5% AC in diet. Treatment means with different superscripts within rows differ at P≤0.05; P-values in columns indicate the overall treatment effect in the model (section 4.2.4). * Standard error of the mean. ** Muramic acid: Treatment x period interaction, P=0.032. *** Bacterial C: Treatment x period interaction, P=0.026.

study can be explained by the formation of complexes between tannins and proteins, fibre constituents, microbial cell walls and enzymes (Makkar et al., 1989; McSweeney et al., 2001). Al-Dobaib (2009) reported a 13% reduction in OM digestibility at 2.25% QTE in the diet, and reduction in NDF and ADF digestibility of 22% each, while Beauchemin et al. (2007) found no effect of QTE on DM, NDF and ADF digestibility when fed to cattle at up to 1.8%, even though their QTE had a higher CT concentration than the extracts used by Al-Dobaib (2009) and in the present study.

Beauchemin et al. (2007) demonstrated the ability of QTE to decrease ruminal protein degradation and NH_3 release in cattle, which was associated with a 5% reduction in apparent CP digestibility at 1% QTE and a 15% reduction at 2% QTE in the diet. Al-Dobaib (2009) also observed that the lower urine-N excretion associated with feeding increasing QTE concentrations was linearly correlated with a reduction of whole-tract N digestibility. Lower ruminal N degradation and ammonia loss upon QTE ingestion is one explanation for the observed increase in the faecal N concentration (Dawson et al., 1999; Foley et al., 1999). However, it might also be due to a lower post-ruminal digestibility of tannin-bound digesta protein (Cortes et al., 2009), decreased activity of intestinal enzymes (Patra and Saxena, 2011), impaired intestinal function (Mbatha et al., 2002) and increased secretion of endogenous proteins (Butter et al., 1999).

Prasad et al. (2000) suggested that activated charcoal can be added to concentrate feed at up to 2 g kg^{-1} of live weight (LW) in cattle, whereas in the current study the amount of AC added to the diet was 0.41 g kg^{-1} LW (AC1.5) and 0.79 g kg^{-1} LW (AC3.0). Our results are consistent with those reported by Van et al. (2006) where the inclusion of up to 28.9 g d^{-1} (1.5 g kg^{-1} LW) of bamboo charcoal to goats' diet had no effect on DM intake of *Acacia* forage and total DM intake of a mixed diet consisting of *Acacia mangium*, para grass plus a supplemental feed. The negative effect of AC on OM digestibility even at 1.5% inclusion might be explained by the adsorptive capacity of the AC used in the present trial, which contradicts the results obtained by Van et al. (2006) where the inclusion of up to 1 g kg^{-1} LW of bamboo charcoal improved DM and OM digestibility. The steam activation of carbonaceous material from coconut shells (see 4.2.1) and grinding to powder increased the surface area, tamped density and micro-porosity of AC used in the present study as compared to the bamboo-derived material that was prepared by burning bamboo wood in iron containers for 6 hours and grinding the remaining charcoal to 2 mm particle size (Kutlu et al., 2001; Miguel et al., 2003, Van et al., 2006). The ability of AC to adsorb vitamins, fats, enzymes and nutrients along with phenolic compounds leads to a lower digestibility of feed constituents (Kutlu et al., 2001; Van et al., 2006), which seems to be confirmed by the only moderately reduced

digestibility of these proximate constituents in the combined QTEAC treatment as compared to the pure QTE2 treatment. Yet, since AC itself is indigestible and in the faeces is recovered in the OM fraction, the significantly reduced OM digestibility observed for the AC treatments must partly also be ascribed to this fact.

There was no effect of AC1.5 and AC3.0 feeding on CP digestibility, which contrasts with the results of Van et al. (2006) who observed an increased CP digestibility, a significant improvement of N retention and a reduction of urinary N excretion in goats when bamboo charcoal was fed at up to 1.5 g kg^{-1} LW, which was attributed to the beneficial – detoxifying – effect of the charcoal (Van et al., 2006).

An increased faecal C concentration was observed in all QTE and AC treatments. This increase can be attributed to the decrease in apparent NDF and ADF digestibility at least for the QTE treatments. Nevertheless, it is surprising that the NDF concentration in faeces did not increase in QTE treatments compared to the control (Table 3), demonstrating that mainly faeces quantity increased in these treatments. At this point it has to be mentioned that interferences between the used detergent fibre analysis and tannins have been observed (Makkar et al., 1995) and that results need to be interpreted with care. The increased faecal C concentration in AC treatments resulted mostly from the accumulation of inert AC in faeces. The observed increase in fibre concentrations, especially of NDF, in AC-derived faeces may also have added to the increased faecal C concentration, but was presumably an artefact of the detergent fibre analysis: From the results of the feed analysis (Table 1) it can be seen that the NDF concentration in AC-enriched pellets was much higher compared to the control feed, indicating that AC became part of the NDF residue during detergent fibre analysis. Therefore the results on NDF concentrations in faeces originating from AC treatments are difficult to interpret. Similar observations hold true for the ADF fraction in faeces, but differences to the control were less pronounced for this constituent.

4.4.2 Effects of QTE and AC on faecal microbial biomass

Direct microscopic methods and anaerobic cultivation methods used to study gastrointestinal microorganisms have been substituted in recent years by molecular methods as the former can underestimate microbial population (Denman and McSweeney, 2006; Joergensen and Wichern, 2008; Kok et al., 2013; Min et al., 2014). The use of molecular approaches also facilitates the analysis of the diversity and composition of microorganisms in the digestive tract and in faeces (Van Vliet et al., 2007; Sekhavati et al., 2009; Min et al., 2014). However, there is a limitation in using microbial DNA information, since this cannot be easily converted into microbial biomass

due to losses during extraction and to unknown or highly variable DNA concentrations among different species (Leckie et al., 2004; Joergensen and Emmerling, 2006; Kok et al., 2013). Therefore developing methods for assessing total microbial biomass in faeces is required to understand the dynamics of fermentation processes and microbial populations in the large intestine. Jost et al. (2011) suggested that ergosterol and amino sugars are suitable substances for determining microbial biomass in the faeces of cattle. To our knowledge this is the first study that uses ergosterol and amino sugars for the determination of microbial biomass in the faeces of goats.

The shift in microbial community structure towards fungal C in treatments QTE2, QTE4 and QTEAC can be attributed to the low availability of N in the rumen and/or hindgut due to the formation of tannin-protein complexes that fostered fungal growth as fungi prefer C-rich cell wall material and need less N than bacteria (Rezaeian et al., 2006; Jost et al., 2013). The fate of free CT in the lower gastrointestinal tract is still not well described. However, in the large intestine, where pH is 5.5 - 7.0, free tannins might form complexes with yet undigested dietary N and microbial N (Patra and Saxena, 2011), whereas in the rumen tannins form stable and insoluble complexes with proteins (Makkar, 2003). Another explanation for the observed shift in microbial community could be the selective inhibitory effects of CT on bacteria (Patra and Saxena, 2011). McSweeney et al. (2001) reported that when *Calliandra calothyrsus* (6% CT in DM) was fed to sheep at 30% of the diet, fibre-degrading bacteria were reduced while the efficiency of microbial protein synthesis and the outflow of protozoa, fungi and proteolytic bacteria from the rumen to the lower gastrointestinal tract were not affected. Furthermore, Min et al. (2014) showed that feeding pine bark (3.2% CT in DM) to goats reduced the faecal bacteria community. In contrast to QTE, the pure AC supplementation did not alter the microbial community structure as compared to the control, underlining the neutral nature of the used product. Despite the similarities in microbial C between goats' faeces (current study) and those of cattle (Jost et al., 2011; 33.0 mg microbial C g^{-1} DM), the average fungal C to bacterial C ratio was lower for goats on the control feed (0.50) than for heifers in the latter study (0.78), whereby fungal C represented 44% of total microbial C (Jost et al., 2011) as compared to 33% in our study. These differences might be due to the different types of feed offered to the heifers (a silage mix of grass, maize and sugar beet leaves; Jost et al., 2011) with a high concentration of structural carbohydrates such as ADF. The effect of diet on microbial C is further confirmed by comparing results from the current experiment to those obtained from dairy cattle fed maize silage and concentrate (Jost et al., 2013) with an overall microbial C concentration of 50.0 mg g^{-1} DM and an average fungal C to bacterial C ratio of 0.30. The differences

in total microbial C between the present study and Jost et al. (2013) are due to the higher CP content in their experimental diet, which was 540 g kg^{-1} DM (in comparison to 270 g kg^{-1} DM in the present study) and enhanced microbial growth and faecal microbial biomass (Van Vliet et al., 2007).

Rezaeian et al. (2004a) measured chitin, the polymer of glucosamine, as an indicator of fungi in sheep faeces. They reported a concentration of 10 mg g^{-1} DM, which is much higher than the values found in the present study. The higher chitin concentration in the study by Rezaeian et al. (2004a) might be related to an overestimation of the fungal population by their method. The colorimetric assay according to Chen and Johnson (1983) does not differentiate between fungal glucosamine, bacterial glucosamine and galactosamine. However, in faeces of heifers and cattle, the ergosterol concentration was 9 and 13 µg g^{-1} DM, respectively, (Jost et al., 2011; 2013). The differences in the results obtained by Jost et al. (2011; 2013) and in the current study might be related to the fact that not all faecal fungi contain ergosterol (Jost et al., 2011). The negative response in the ergosterol and fungal glucosamine concentrations to feeding QTE is also in accordance with this view. Yet, the results might also indicate that the fungal community in goat faeces contains more ergosterol in their cell membrane than that in cattle faeces. Further studies would be needed to investigate this. The concomitant increase of galactosamine and fungal glucosamine indicates that galactosamine was mainly of fungal origin in the present study. This contrasts the results obtained by Jost et al. (2011) who concluded that galactosamine was mostly of bacterial origin.

4.4.3 Potential benefits of QTE and AC feeding for manure use

The increased faecal concentration of OM, and C, and partly also of N, NDF and ADF associated with feeding QTE - and partly also with feeding AC - produced a manure that has the potential to meet the specific requirements of irrigated organic cropping systems under hot and arid conditions such as in Oman (Siegfried et al., 2013; Ingold et al., 2014). The mineralization process of nutrients from organic manure is determined by its C/N ratio (Kyvsgaard et al., 2000; Van Kessel et al., 2000) as well as by the content of slowly decomposable C, which is needed for a long-term effect of manure on soil organic matter and for N stabilization (Hassink, 1994). Despite the lower faecal N excretion associated with feeding AC and the resulting increase in the faecal C/N ratio, the respective values of 22 and 24 for AC1.5 and AC3.0 are still within the range of 20 - 25 in which organic manures are subjected to rapid N mineralization (Bosshard et al., 2009; Jost et al., 2013). Despite this, it has been shown that manure produced upon AC feeding is more slowly decomposed by soil microorganisms (Ingold et al., 2014) than unaltered manure, due to

the partly recalcitrant C. A by trend higher concentration of slowly decomposable faecal C (that is especially the ADF fraction) also resulted from QTE feeding. Yet, Ingold et al. (2014) showed that soil organic carbon originating from QTE manure is less stable than from AC manure. The former component increases the faecal concentration of mostly organic, tannin-bound nitrogen, which decomposes in soil more slowly than nitrogen from unaltered or AC-derived manure (Ingold et al., 2014), which leads to a longer-term residual fertilization effect (Powell et al., 2009; Eckard et al., 2010; Powell and Broderick, 2011). A third effect of QTE feeding is the shift in the faecal microbial community towards fungi. Faeces dominated by fungal communities were exhibiting lower gaseous N emissions and stronger N immobilization, and in consequence induced lower immediate N uptake by plants, but might contribute to a longer-term soil N supply (Jost et al., 2013).

4.5. Conclusions

Including up to 4% QTE in goats' diet yields manure that is potentially beneficial for crop production under irrigated arid sub-tropical conditions. This is due to an increased faecal N concentration and its slower mineralisation in soil; furthermore, there is a trend for an increased faecal concentration of slowly decomposable carbohydrates in the form of ADF. The shift in the faecal microbial community to fungi, which is also associated with QTE feeding, may be considered as an additional positive effect that in the short run might lead to lower gaseous N emission and stronger N immobilization but in the longer term to a continued steady N supply to plants. Activated charcoal can also be considered as a beneficial feed additive since it increases the faecal C concentration, particularly of slowly decomposable carbohydrates, as well as the faecal C/N ratio and thus has the potential to slow down the mineralisation of manure under the above-mentioned environmental conditions.

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5. General discussion

5.1 Effects of QTE and AC on nutrient intake and digestibility

In the present study the inclusion of up to 4% QTE to the total diet had no influence on palatability and intake of feed by goats in both trials. This is in agreement with previous studies conducted with sheep and cattle (Beauchemin et al., 2007; Al-Dobaib, 2009). Following the suggestion of Min et al. (2003), tannin concentrations < 5% (<50 g CT kg^{-1} DM) were included into the test diets to avoid changes in feed palatability, conditioned aversion and reduction in rumen fermentation rates which ultimately might affect feed intake (Mueller-Harvey, 2006; Waghorn, 2008), and to improve the utilization of dietary proteins in ruminants (Min et al., 2003). However it has to be noted that different tannins react differently with diet constituents (Min et al., 2003; Patra and Saxena, 2011), hence the specific impacts of tannins might vary with the offered diet (Athanasiadou et al., 2001; Vasta et al., 2009).

How a specific tannin or tannin mixture interacts with a specific diet will influence its impact on nutrient digestibility. This shows for example in the comparison of data from the current experiment (Chapters 2 and 3) with data obtained from sheep fed an alfalfa-based diet (Al-Dobaib, 2009) and cattle fed a forage-based diet (Beauchemin et al., 2007): The reduction in CP digestibility was higher in sheep and cattle when QTE was included at 3% and 2% of the total diet, respectively, than when included at 2% and 4% in the diet of goats (Chapters 2 and 3). One explanation for these different influences could be the higher content of CT in Al-Dobaib's (2009) study (75% CT in the extract used) as well as in the study of Beauchemin et al. (2007; 91% CT in the extract used) as compared to the QTE used in the trials in Oman and Germany (75% total phenols in the QTE purchased from Otto Dille®, Norderstedt, Germany). Secondly the different results could be due to the increased solubility of protein in the different diets, and/or the treatment of the feed with moisture and high temperature in the trial of Al-Dobaib (2009; alfalfa hay ground to 4 mm, then mixed with QTE and pelleted at 85°C using steam) might have influenced the extent of the interaction between CT and dietary proteins, whereas Beauchemin et al. (2007) first suspended QTE in warm water and then mixed it with feed by hand, and in the present trials QTE powder was mixed with finely ground concentrate and tap water (1 l per 10 kg of concentrate mixture) and was mechanically pelleted. Thirdly, differences could be related to the fact that goats are less susceptible to CT effects because of their relatively large parotid glands which produce more saliva (Vaithiyanathan et al., 2001; Marsh et al., 2006), especially upon CT ingestion (Salem et al., 2013), or to the existence of tannin-binding salivary proteins which are absent or much less concentrated in saliva of sheep and cattle (Mueller-Harvey, 2006; Waghorn, 2008).

At 3% QTE inclusion there was a 22% reduction in NDF and ADF digestibility in sheep (Al-Dobaib, 2009). In the current study there was also no significant effect of QTE on NDF and ADF digestibility at 2% in the first trial (Chapter 2), while the digestibilities were reduced by 14% when compared to the control in the second trial (Chapter 3). At 4% QTE in total diet there was a significant decrease in NDF digestibility by 6% in Jabal Akhdar goats and by 13% in Boer goats. The higher influence of QTE in sheep (Al-Dobaib, 2009) in comparison to the goats might be attributed to species differences and/or altered solubility of fibre fractions due to the already mentioned grinding of alfalfa and pelleting with CT at high moisture and temperatures, which might have increased the binding of CT to fibre. This also holds true for ADF digestibility where the impact of QTE at 4% (-11%) in Jabal Akhdar goats was lower than at 3% QTE in sheep (Al-Dobaib, 2009). However, in comparison to Boer goats (-28% ADF digestibility with QTE2, -36% ADF digestibility with QTE4, and -24% ADF digestibility with QTEAC), the reduction in ADF digestibility was lower in Jabal Akhdar goats and in sheep (Al-Dobaib, 2009). These differences between Jabal Akhdar and Boer goats might be a result of differences in age, breed, diet composition and in the interaction between the QTE and diet constituents during pellet preparation, even though the pelleting process was handled in an identical manner in Oman and in Germany. McSweeney et al. (2001) reported that feeding *Calliandra calothyrsus* at 30% of the diet did reduce the population of fibre-degrading bacteria *(Fibrobacter succinogenes* and *Ruminococcus* spp.*)*. Similar effects are also plausible for the growing male Boer goats (Chapter 3). It could also be postulated that these growing goats had not yet developed mechanisms to resist negative effects of CT. Previous studies have shown that ruminants adapt to condensed tannin-containing diets by shifts in the structure of the rumen microbial community towards tannin-resistant microorganisms (Smith and Mackie, 2004; Min et al., 2014). This proposition seems to be true as there was an improvement in digestibility coefficients along the three experimental periods in the second trial (Chapter 3). Moreover, the impact of QTE was much higher on ADF than on NDF digestibility, suggesting that the QTE used here mainly affected cellulolytic microorganisms and fungi directly rather than binding to secreted enzymes; the former mechanisms will ultimately inhibit microbial cell growth and synthesis of cell-associated enzymes. This finding is in contrast to the suggestion of Bae et al. (1993) who proposed that CT inactivate more efficiently extracellular enzymes than cell-associated enzymes. Differences between the two breeds of goats in terms of their microbial community structure in the rumen might be another explanation. A further factor might have been the structure of the pellets which were more firm and condensed in the trial with Boer goats, due to stronger pressing and finer

structure of the concentrate mixture in comparison to the ground soybean and maize used in the Oman trial.

Activated charcoal had no effect on feed intake, however the amounts added in the present study (AC1.5: 0.26 - 0.41 g kg^{-1} LW; AC3.0: 0.52 - 0.79 g kg^{-1} LW) were less than the recommended 2 g kg^{-1} LW (Prasad et al., 2000).

In the current study AC had a negative effect on OM, NDF, and ADF digestibility which contradicts the previous observation in chicken (Kutlu et al., 2001), pigs (Thu et al., 2010; Chu et al., 2013) and goats (Van et al., 2006) where an improvement in feed intake, body weight gain and feed utilization were recorded. This may have been related to the nature of the presently used finely powdered, steam-activated carbonaceous material from coconut shells that probably had an increased surface area, tamped density and micro-porosity as compared to the wood-derived material used in the other studies (Kutlu et al., 2001; Miguel et al., 2003; Van et al., 2006; Thu et al., 2010; Chu et al., 2013). This might have led to high adsorption of nutrients, fats, vitamins and enzymes (Kutlu et al. 2001; Van et al., 2006) and in consequence depressed the digestibility of the above-mentioned proximated constituents.

With respect to the QTEAC treatment, the results of the current study are in contrast to the findings of Van et al. (2006) who reported an improvement in DM, OM, NDF, ADF and CP digestibility in goats when bamboo charcoal was included at up to 1.5 g kg^{-1} LW to a diet consisting of *Acacia mangium* (58 g CT kg^{-1} DM), para grass plus a concentrate component. The presently observed negative effects of AC on OM, NDF, ADF and CP digestibility even at 1.5% inclusion and in combination with 2% QTE might be due to the already explained differences in the adsorptive capacity of the AC used in the present trial.

5.2. Effects of QTE and AC on nitrogen metabolism and microbial protein outflow from rumen

Including QTE at up to 4% in the total diet induced a shift in N excretion from urine to faeces, which was attributed to lower ruminal protein degradation and higher availability of N in the abomasums and lower digestive tract (Min et al., 2003; Mueller-Harvey, 2006). This assumption is confirmed by the lower total N excretion in QTE2, QTE4 and QTEAC despite the reduced CP digestibility, and by the slight increase in N retention (QTE2: +18.4%, QTE4: +7.7%, QTEAC: +10.9%), when compared to control and AC treatments. The observed effects of QTE on N metabolism were consistent with the results of studies in cattle (Beauchemin et al., 2007) and sheep (Al-Dobaib, 2009) where QTE caused a reduction in ruminal protein degradation and NH_3 release as well as in

urinary N excretion, which was correlated with a reduction of whole-tract N digestibility. Puchala et al. (2005) also reported an increase in the intestinal amino acid absorption in goats fed S*ericea lespedeza* forage containing 17.7% CT as a result of decreased ruminal protein degradation, which was indicated by lower rumen concentrations of $N\text{-}NH_3$ and reduced plasma urea-N. Similar results were reported for ewes grazing *Lotus corniculatus* (28 g CT kg^{-1} DM; Min et al., 1998).

The lower urinary excretion of N associated with feeding QTE was paralleled by an intrend lower urinary excretion of purine derivatives, which is indicating lower microbial protein outflow from the rumen. This further supports the proposition of an induced reduction in protein degradation upon QTE ingestion. The values obtained in the current study, however, are comparable to those obtained by Andrade-Montemayor et al. (2004) for goats fed a total mixed ration free of condensed tannins (either 50% alfalfa hay or barley straw, 50% concentrate; intake 40 g DM $kg^{-0.75}$ d^{-1}; microbial nitrogen flow 8.99 to 11.85 g N d^{-1}).

It has to be noted that despite a lower availability of nutrients associated with tannin feeding, those nutrients that are available are directed towards microbial mass synthesis rather than towards production of short chain fatty acids (Makkar, 2003). This might explain the positive effect of QTE on N metabolism, where despite the lower digestibility of OM, CP, NDF and ADF, the microbial protein synthesis in the rumen and its efficiency was not significantly altered but was lowered to an extent that is still beneficial for growing goats as can be concluded from the improved N retention (Chapter 3).

Feeding AC did not alter quantitative microbial protein synthesis and its efficiency, probably due to the low availability of volatile fatty acids associated with the reduced digestibility of OM, NDF, and ADF. In consequence, an excessive degradation of protein in the rumen without sufficiently available energy led to a higher excretion of N via urine and to a slight decrease in N retention (Min et al., 2000; Makkar, 2003; Patra and Saxena, 2011).

5.3. Effects of QTE and AC feeding on manure quality and faecal microbial community structure

The values of N concentration in faeces were comparable between the two trials, however in the first trial there was no significant difference between the six treatments whereas in the second trial there was a significant increase in N concentration in QTE4 and a tendency towards higher N concentration in QTE2 in comparison to the control, while N concentration was reduced in the AC and QTEAC treatments. These differences most probably are due to the stronger effect of QTE in Boer goats already outlined in

section 1 of this chapter. Despite this, manure produced upon QTE ingestion could provide longer-term residual fertilization effects since such manure decomposes slowly in soil due to a slower mineralization of the tannin-protein complexes (Powell et al., 2009; Eckard et al., 2010; Powell and Broderick 2011; Ingold et al., 2014).

The concentration of C was increased in all treatments, which was more pronounced in AC and QTEAC treatments in both trials. It is suggested that the inclusion of the indigestible and inert carbon via AC has contributed to this effect. The higher concentration of slowly decomposable carbohydrate fractions (NDF and ADF) might have to some extent also played a role in elevating the concentration of C in the excreta. However, considering the high concentration of NDF and ADF constituents in the pellets of the AC treatments, the interpretation of such data should be done with care, since it is possible that AC became part of the NDF and ADF residue during detergent fibre analysis (Chapter 3). However, there is no available information on this aspect in literature, and a systematic testing of the interference of AC powder with pelleted concentrate feeds as well as with faeces post digestion seems strongly advisable. Despite this, Ingold et al. (2014) reported that goat manure produced upon AC feeding was decomposed more slowly by soil microorganisms than unaltered goat manure, due to the partly recalcitrant C.

The increase in faecal C concentration in QTE treatments could be partly related to an increase in the OM concentration of faeces, even though there was no constant and clear trend of increasing NDF and ADF concentrations in faeces of these treatments. In the first trial the NDF fraction was slightly but not significantly reduced compared to the control, and the ADF concentration was slightly lowered in QTE2 and slightly increased in QTE4. In the second trial there was a slight but insignificant decrease in NDF in QTE4, whereas ADF significantly increased in QTE2. Interference between the used detergent fibre analysis method and tannins has been reported (Makkar et al., 1995), which might have affected the determination of fibre concentrations in pellets and faeces and influenced the study's results and their interpretation.

One distinct outcome of feeding QTE (QTE2, QTE4, and QTEAC) is the shift in microbial community towards fungi. Jost et al. (2013) reported that faeces dominated by fungal communities showed lower gaseous emissions and stronger N immobilization, suggesting that the induced lower immediate N uptake by plants might contribute to a longer-term N supply in the soil. This shift in microbial biomass towards fungi could to some extent be explained by the lower availability of N, since fungi needs less N than bacteria (Rezaeian et al., 2006; Jost et al., 2013). As stated in section 2 of this chapter, QTE in the current study reduced the total N excretion and enhanced N retention,

indicating that nitrogenous feed compounds have been utilized by goats in the small intestine and were protected from excessive degradation in the rumen (Makkar, 2003). There is still no information available on the fate of CT in the hindgut, however it could be postulated that CT contained in QTE rebound again with undigested dietary protein and/or microbial protein in the lower digestive tract (Patra and Saxena, 2011) making N unavailable for bacterial growth in the hindgut. Previous studies have also shown that CT can selectively inhibit growth of bacteria: McSweeney et al. (2001) reported that feeding *Calliandra calothyrsus* (6% CT in DM) to sheep reduced the number of fibre-degrading bacteria, while the efficiency of microbial protein synthesis and the outflow of protozoa, fungi and proteolytic bacteria from their rumen to the lower gastrointestinal tract was not affected. On the other hand, Min et al. (2014) showed that feeding pine bark (3.2% CT in DM) to goats reduced the faecal community of bacteria which is consistent with our results. However, the dietary inclusion of AC had no influence on total microbial biomass or the structure of the microbial community in faeces, which points to the neutral behavior of this product with respect to the habitual microbial communities along the digestive tract.

Despite the reduced TT and TMRT (which is derived from TT and lambda) associated with feeding AC, the outflow of particles from the rumen was unaltered by AC and QTE inclusion in the diet, and the parameters of passage rate measured in the current experiment had no influence on faeces quality.

5.4. Testing the initial hypotheses

Before concluding the general discussion of the results obtained by supplementing goats' diets with QTE and AC, the initially postulated hypotheses are revisited and checked for validity in the following table:

Table 1: Test of the initial study hypotheses against the obtained results

Hypothesis	Verification
(i) Inclusion of QTE and/or AC will increase faecal N and/or C concentrations	This hypothesis is accepted as QTE increased N excretion at least at 4% (Chapter 3). It also increased faecal C concentration, which was partly associated with increased quantitative excretion and excretion of slowly decomposable C, at least in the form of ADF. AC did only increase faecal C concentration due to the addition of indigestible and recalcitrant C and the excretion of slowly decomposable C in the form of NDF and ADF.
(ii) There is provision of long-lived C species to be sequestered in the soil upon feeding AC.	This hypothesis is accepted as AC lead to a higher C concentration in both trials.
(iii) Effects of both QTE and AC on the metabolism of rumen and hindgut microbial communities will affect the composition of the microbial biomass in the faeces.	For QTE this hypothesis is accepted as there was a shift in faecal microbial community towards fungi and a slight decrease in the outflow of microbial protein from the rumen (indicated by the measurement of purine derivatives), although it was not significant. AC effects were neutral with respect to the faecal microbial community, since microbial biomass in faeces was not altered in comparison to the control.

5.5. Conclusions and recommendations

Including up to 4% QTE into goats' diet offers the potential to shift N excretion from urine to faeces and enhance the performance of goats. This measure also induces faecal excretion of protein-tannin complexes which potentially mineralize slowly in soil. Furthermore, QTE feeding in tendency also leads to an increased faecal concentration of slowly decomposable carbohydrates in the form of ADF. The induced shift in the faecal microbial community towards fungi can be considered as an additional merit associated with QTE feeding, which might secure plant N-supply on a longer-term basis. Hence, manure derived from QTE supplemented goats should meet the specific requirements for crop production under the irrigated arid sub-tropical conditions of Oman and in similar systems. However, this has to be verified in field cropping experiments.

The inclusion of up to 3.0% AC into the diet of goats increased the faecal concentration of NDF, ADF and C. Field trials demonstrated that manure produced upon AC feeding is more slowly decomposed by soil microorganisms due to the partly recalcitrant C, and perhaps also to slowly decomposable carbohydrates. This could ultimately stabilize soil organic matter in environments where year-round high temperatures and flood irrigation of, for example vegetables, lead to rapid depletion of the soil C stock. In the longer term this might also decrease N losses via leaching and gaseous emissions due to the build-up of soil organic matter. Since mineralization of manure depends not only on the concentration of slowly decomposable C but also on the C/N ratio, feeding AC to goats reaps an additional benefit which is the increased manure C/N ratio.

Feeding QTE and AC together at 2% and 1.5%, respectively, resulted in a combination of the beneficial effects of both QTE and AC. In this treatment AC had low or neutral effect on QTE, and the latter induced an improvement in N retention, a shift in N excretion towards faeces, and a shift in the faecal microbial community towards fungi. The QTEAC treatment also produced manure with a high concentration of NDF, ADF and C that resulted from the AC component. Hence such combination is highly recommended when aiming at improving N utilization in goats and reducing (urine-derived) emissions of ammonia and nitrous oxide to the environment, while at the same time fostering soil organic matter build-up.

Yet, with respect to the increased concentrations of NDF and ADF in faeces from QTE and AC supplemented goats it is highly recommended to test, in a systematic approach, if and to what extent feed and faeces containing QTE and AC particles or their post-digestive derivatives interfere with standard (ANKOM-based) detergent fibre analyses, in order to avoid conclusions that are based on analytical flaws.

5.6. References

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