Effects of Different Feeding Regimes on the Digestibility and Faecal Excretion of Nitrogen, Soluble Carbohydrates and Fibre Fractions in Water Buffaloes kept under Subtropical Conditions




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Dedication

To
My Mother Al-Kishri, F
My wife Al-Kishri, N
The memory of my late grandmother Al-Mosawi, S.
And my late father Al-Asfoor, H.
may they rest in peace

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Summary

In the course of the ‘Livestock Revolution’, extension and intensification of, among others, ruminant livestock production systems are current phenomena, with all their positive and negative side effects. Manure, one of the inevitable secondary products of livestock rearing, is a valuable source of plant nutrients and its skillful recycling to the soil-plant interface is essential for soil fertility, nutrient - and especially phosphorus - uses efficiency and the preservation or re-establishment of environmentally sustainable farming systems, for which organic farming systems are exemplarily.

Against this background, the PhD research project presented here, which was embedded in the DFG-funded Research Training Group 1397 ‘Regulation of soil organic matter and nutrient turnover in organic agriculture[1]’ investigated possibilities to manipulate the diets of water buffalo (*Bubalus bubalis* L.) so as to produce manure of desired quality for organic vegetable production, without affecting the productivity of the animals used.

Consisting of two major parts, the first study (chapter 2) tested the effects of diets differing in their ratios of carbon (C) to nitrogen (N) and of structural to non-structural carbohydrates on the quality of buffalo manure under subtropical conditions in Sohar, Sultanate of Oman. To this end, two trials were conducted with twelve water buffalo heifers each, using a full Latin Square design. One control and four tests diets were examined during three subsequent 7 day experimental periods preceded each by 21 days adaptation. Diets consisted of varying proportions of Rhodes grass hay, soybean meal, wheat bran, maize, dates, and a commercial concentrate to achieve a (1) high C/N and high NDF (neutral detergent fibre)/SC (soluble carbohydrate) ratio (HH), (2) low C/N and low NDF/SC ratio (LL); (3) high C/N and low NDF/SC ratio (HL) and (4) low C/N and high NDF/SC (LH) ratio. Effects of these diets, which were offered at 1.45 times maintenance requirements of metabolizable energy, and of individual diet characteristics, respectively, on the amount and quality of faeces excreted were determined and statistically analysed. The faeces produced from diets HH and LL were further tested in a companion PhD study (Mr. K. Siegfried) concerning their nutrient release in field experiments with radish and cabbage.

The second study (chapter 3) focused on the effects of the above-described experimental diets on the rate of passage of feed particles through the gastrointestinal tract of four randomly chosen animals per treatment. To this end, an oral pulse dose of 683 mg fibre particles per kg live weight marked with Ytterbium (Yb; 14.5 mg Yb g^{-1} organic matter) was dosed at the start of the 7 day experimental period which followed 21 days of adaptation. During the first two days a sample for Yb determination was kept from each faecal excretion, during days 3 – 7 faecal samples were kept from the first morning and the first evening

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[1] http://www.uni-kassel.de/fb11/dec/research-training-group-1397_en.html

defecation only. Particle passage was modelled using a one-compartment age-dependent Gamma-2 model. In both studies individual feed intake and faecal excretion were quantified throughout the experimental periods and representative samples of feeds and faeces were subjected to proximate analysis following standard protocols.

In the first study the organic matter (OM) intake and excretion of LL and LH buffaloes were significantly lower than of HH and HL animals, respectively. Digestibility of N was highest in LH (88%) and lowest in HH (74%). While NDF digestibility was also highest in LH (85%) it was lowest in LL (78%). Faecal N concentration was positively correlated ($P \leq 0.001$) with N intake, and was significantly higher in faeces excreted by LL than by HH animals. Concentrations of fibre and starch in faecal OM were positively affected by the respective dietary concentrations, with NDF being highest in HH (77%) and lowest in LL (63%). The faecal C/N ratio was positively related ($P \leq 0.001$) to NDF intake; C/N ratios were 12 and 7 for HH and LL ($P \leq 0.001$), while values for HL and LH were 11.5 and 10.6 ($P > 0.05$).

The results from the second study showed that dietary N concentration was positively affecting faecal N concentration ($P \leq 0.001$), while there was a negative correlation with the faecal concentration of NDF ($P \leq 0.05$) and the faecal ratios of NDF/N and C/N ($P \leq 0.001$). Particle passage through the mixing compartment (λ) was lower ($P \leq 0.05$) for HL (0.033 h^{-1}) than for LL (0.043 h^{-1}) animals, while values of 0.034 h^{-1} and 0.038 h^{-1} were obtained for groups LH and HH. At 55.4 h, total tract mean retention time was significantly ($P \leq 0.05$) lower in group LL that in all other groups where these values varied between 71 h (HH) and 79 h (HL); this was probably due to the high dietary N concentration of diet LL which was negatively correlated with time of first marker appearance in faeces (r= -0.84, $P \leq 0.001$), while the dietary C concentration was negatively correlated with λ (r= -0.57, $P \leq 0.05$).

The results suggest that manure quality of river buffalo heifers can be considerably influenced by diet composition. Despite the reportedly high fibre digestion capacity of buffalo, digestive processes did not suppress the expression of diet characteristics in the faeces. This is important when aiming at producing a specific manure quality for fertilization purposes in (organic) crop cultivation. Although there was a strong correlation between the ingestion and the faecal excretion of nitrogen, the correlation between diet and faecal C/N ratio was weak. To impact on manure mineralization, the dietary NDF and N concentrations seem to be the key control points, but modulating effects are achieved by the inclusion of starch into the diet. Within the boundaries defined by the animals' metabolic and (re)productive requirements for energy and nutrients, diet formulation may thus take into account the abiotically and biotically determined manure turnover processes in the soil and the nutrient requirements of the crops to which the manure is applied, so as to increase nutrient use efficiency along the continuum of the feed, the animal, the soil and the crop in (organic) farming systems.

Zusammenfassung

Im Zuge der „Livestock Revolution“ sind unter anderem die Ausweitung und Intensivierung der auf Wiederkäuer basierenden Viehhaltungssysteme gegenwärtige Phänomene, mit allen ihren positiven und negativen Nebenwirkungen. Tierischer Dung, ein unvermeidliches Nebenprodukt der Viehhaltung, ist eine wertvolle Quelle pflanzlicher Nährstoffe, und seine wohlüberlegte Rückführung zum System Boden/Pflanze ist essentiell für die Bodenfruchtbarkeit, die Nährstoff- und besonders Phosphor-Nutzungseffizienz und die Erhaltung oder Einrichtung umweltverträglicher Landbewirtschaftungssysteme, für welche die Ökologische Landwirtschaft ein Paradebeispiel darstellt.

Die vorliegende Dissertation, die im Rahmen des von der Deutschen Forschungsgemeinschaft finanzierten Graduiertenkollegs 1397 „*Steuerung des Humus- und Nährstoffhaushalts in der Ökologischen Landwirtschaft*[2]“ durchgeführt wurde, untersuchte daher Möglichkeiten, die Rationsgestaltung von Wasserbüffeln (*Bubalus bubalis* L.) derart zu beeinflussen, dass Dung einer für den ökologischen Gemüseanbau wertvollen Qualität erzeugt wird ohne die Produktivität der Tiere negativ zu beeinflussen.

Die unter subtropischen Bedingungen in Sohar, Sultanat von Oman, durchgeführte Forschungsarbeit beinhaltet zwei Aspekte: die erste Studie (Kapitel 2) prüft den Einfluss von Rationen, die in ihrem Kohlenstoff- (C) / Stickstoff- (N) Verhältnis und in ihrem Verhältnis von strukturellen zu nichtstrukturellen Kohlenhydraten variieren, auf die Qualität des Büffeldungs. Zu diesem Zweck wurden zwei Versuche mit je zwölf Wasserbüffelfärsen durchgeführt; jeder Versuch war als vollständiges Lateinisches Quadrat angelegt. Eine Kontroll- und vier Testrationen wurden während drei aufeinander folgender Versuchsperioden von je 28 Tagen Dauer überprüft; einer siebentägigen experimentellen Phase ging dabei jeweils eine 21-tägige Adaptationsperiode voraus. Die Rationen bestanden aus wechselnden Anteilen an Rhodes-Gras Heu, Sojaschrot, Weizenkleie, Mais (gemahlen), Datteln und einem handelsüblichen Konzentratfuttermittel; diese Futtermittel wurden derart kombiniert, dass die Testrationen sich auszeichneten durch ein: (1) hohes C/N und hohes NDF (Neutrale Detergenzienfaser) /SC (lösliche Kohlenhydrate; engl.: soluble carbohydrates) Verhältnis (HH); (2) niedriges C/N und niedriges NDF/SC Verhältnis (LL); (3) hohes C/N und niedriges NDF/SC Verhältnis (HL), (4) niedriges C/N und hohes NDF/SC Verhältnis (LH). Der Einfluss dieser Rationen, die in einer dem individuellen 1.45-fachen Erhaltungsbedarf an umsetzbarer Energie entsprechenden Menge angeboten wurden, auf die Menge und die Qualität des ausgeschiedenen Kotes wurde bestimmt und statistisch analysiert. Der anhand der Rationen HH und LL produzierte Kot wurde in einer Begleitstudie (Dissertation Herr K. Siegfried) auf seine Nährstofffreisetzung in Anbauversuchen mit Rettich und Kohl geprüft.

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[2] http://www.uni-kassel.de/fb11/dec/graduiertenkolleg-1397.html

Die zweite Studie (Kapitel 3) konzentriert sich auf die Auswirkung der oben beschriebenen experimentellen Rationen auf die Passagerate der Futterpartikel durch den Magen-Darm-Trakt von vier zufällig ausgewählten Tieren pro Behandlung. Zu diesem Zweck wurde zu Beginn einer siebentägigen Experimentalphase eine einmalige orale Gabe von 683 mg (pro Kilogramm Lebendgewicht) mit Ytterbium (Yb; 14.5 mg Yb g^{-1} Organische Substanz) markierter Faserpartikel verabreicht. Während der ersten beiden Tage nach Markergabe wurde von jeder Kotausscheidung eine Probe für die Bestimmung der Yb-Konzentration einbehalten, vom dritten bis zum siebten Tag wurde jeweils eine Probe von der ersten Abkotung morgens und abends einbehalten. Die Passage der Futterpartikel durch den Gastrointestinaltrakt wurde unter Verwendung eines einfachen altersabhängigen Gamma-2 Modells beschrieben. In beiden Studien wurden die individuelle Futteraufnahme und Kotausscheidung während der experimentellen Perioden quantitativ erfasst und entsprechende repräsentative Proben der qualitativen Analyse zugeführt.

In der ersten Studie waren die Aufnahme und Ausscheidung an organischer Substanz (OS) der Grupen LL und LH erheblich niedriger als die der Gruppen HH und HL. Die Stickstoff-Verdaulichkeit war in Gruppe LH am höchsten (88%) und in Gruppe HH am niedrigsten (74%). Während die NDF-Verdaulichkeit ebenfalls in Gruppe LH am höchsten war (85%), war sie in Gruppe LL am geringsten (78%). Die Kot-N Konzentration war positiv mit der N-Aufnahme korreliert ($P \leq 0.001$), und war in Gruppe LL signifikant höher als in Gruppe HH. Die Konzentrationen von Zellwandbestandteilen und Stärke in der fäkalen OS wurden durch die jeweiligen Konzentrationen der Rationen positiv beeinflußt, wobei der Kot-NDF Gehalt in Gruppe HH am höchsten (77%) und in Gruppe LL am niedrigsten (63%) war. Das C/N Verhältnis des Kotes war positiv mit der NDF-Aufnahme korreliert ($P \leq 0.001$); es betrug 12 für Gruppe HH und 7 für Gruppe LL ($P \leq 0.001$), während die Mittelwerte für die Gruppen HL und LH bei 11.5 und 10.6 lagen ($P > 0.05$).

Die Ergebnisse der zweiten Studie zeigten erneut, dass die N-Konzentration der Ration die Kot-N Konzentration positiv beeinflußt ($P \leq 0.001$), während eine negative Wechselbeziehung mit der Kot-NDF Konzentration ($P \leq 0.05$) und dem NDF/N und C/N Verhältnis im Kot festgestellt wurde ($P \leq 0.001$). In Gruppe HL war die Passagerate (λ) von Futterpartikeln durch den Reticulo-Rumen mit 0.033 h^{-1} kürzer ($P \leq 0.05$) als in Gruppe LL (0.043 h^{-1}); die Werte für die Gruppen LH und HH betrugen 0.034 h^{-1} und 0.038 h^{-1}. Mit 55.4 Stunden Dauer war die Gesamtretentionszeit für Faser in Gruppe LL erheblich kürzer als die der anderen Gruppen ($P \leq 0.05$), für welche dieser Wert zwischen 71 h (HH) und 79 h (HL) variierte. Dies lag vermutlich an der hohen N-Konzentration der Ration LL, welche mit dem Zeitverzug zwischen Markergabe und Erstaustritt des Markers im Kot negativ korreliert war (r=0.84, $P \leq 0.001$). Demgegenüber war die C-Konzentration der Ration negativ mit λ korreliert (r=0.57, $P \leq 0.05$).

Die Ergebnisse lassen den Schluß zu, dass die Qualität des Dungs von Wasserbüffelfärsen durch die Rationsgestaltung beträchtlich beeinflußt werden kann. Trotz der in der Literatur betonten hohen Kapazität des Büffels für die Faserverdauung konnten Verdauungsprozesse den deutlichen Einfluß unterschiedlicher Rationen auf die Kotqualität nicht nivellieren. Dies ist ein wichtiges Faktum wenn für den (ökologischen) Pflanzenbau Dung einer spezifischen Qualität erzeugt werden soll. Trotz der starken Wechselbeziehung zwischen Aufnahme und Kotausscheidung von Stickstoff war die Wechselbeziehung zwischen dem C/N Verhältnis der Ration und des Kotes schwach. Hinsichtlich der Beeinflussung der Mineralisierung des Dungs scheinen den NDF- und N-Konzentrationen der Ration Schlüsselfunktionen zu zukommnen, wobei modulierende Effekte durch die Einbeziehung von Stärke in die Ration erzielt werden. Innerhalb der Grenzen, die durch die metabolischen und (re)produktiven Anforderungen der Tiere an Energie- und Nährstoffversorgung definiert werden, kann die Rationsgestaltung daher die abiotisch und biotisch bedingten Dung-Umsetzungsprozesse im Boden und die Nährstoffansprüche der angebauten Nutzpflanzen mit berücksichtigen, um die Nährstoff Nutzungseffizienz entlang des Kontinuums Futtermittel, Tier, Boden und Pflanze in der (ökologischen) Landwirtschaft zu erhöhen.

List of abbreviations

ADF: Acid detergent fibre

CP: Crude protein

CWC: Cell wall constituents

DM: Dry matter

GE: Gross energy

GIT: Gastrointestinal tract

GWP: Global warming potential

ME: Metabolizable energy

MFE: Mineral fertilizer equivalent

MN: Microbial nitrogen

NDF: Neutral detergent fibre

OM: Organic matter

RDP: Rumen degradable protein

RUD: Rumen undegradable protein

SC: Soluble carbohydrates

SOM: Soil organic matter

VFA: Volatile fatty acids

Chapter 1

General Introduction and Research Objectives

1.1 Introduction

One essential concern in organic agriculture is to control soil organic matter and nutrient budgets, since the availability of organic matter and nutrients in the soil are key to improve soil productivity. 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 Forschungsgemeinschaft (German Research Foundation). This research project is sub-divided into twelve sub-projects, which focus on possibilities to regulate soil organic matter (SOM) and nutrient budgets by soil management, crop rotation, and manure quality through adapted feeding of animals. Further focal points were the quality of crop residues, the turnover of litter and SOM, especially carbon, nitrogen and other relevant nutrients, losses of carbon and nutrients trough gaseous emissions (CO_2, CH_4, N_2O, NH_3) and liquid (NO_3, dissolved organic carbon, cations) outflows, and the coupling of certain of these aspects by modelling.

The current research project (RTG 1397-D2) focused on possibilities to manipulate the diets of water buffalo (*Bubalus bubalis*) so as to produce manure of desired quality. The latter should meet the nutrient requirements - in time and amount - of high value organic vegetables grown under subtropical conditions. Therefore, the manure produced in this project was handed over to the partner vegetable production project (RTG 1397-F1) for field testing in experiments with radish, carrots and cauliflower.

1.2. Literature review and study objectives

1.2.1. Nutrient fluxes in livestock and crop production systems

An enlarged demand for high-quality food products of animal origin together with an increasing number of people around the globe has raised the necessity to increase the volume of animal production. On the other hand, the obligation to protect the planet's environment has also risen in the last decades. As far as agriculture is concerned, major tasks are preventing the accumulation of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), ammonia (NH_3), and nitrous oxide (N_2O) in the atmosphere, and managing soil and water contaminants such as phosphorus (P), nitrogen (N) and potassium (K) (Tamminga 1996). Especially livestock production is considered a major contributor to greenhouse gases emissions and global warming (Powell et al. 2009), as well as to the pollution of aquifers through nutrient-rich leachates from manures and slurries (Petersen et al. 2007).

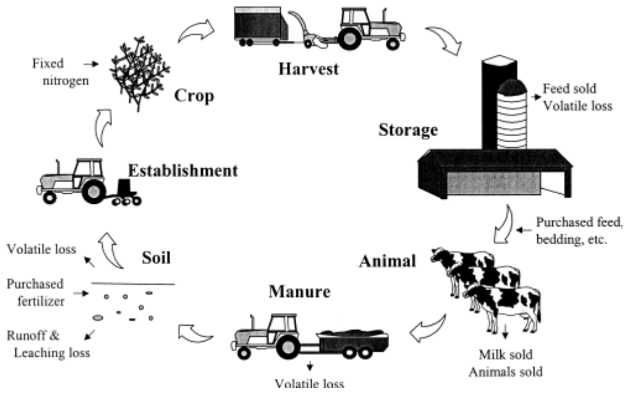

Figure 1: Dairy forage system model of material and nutrient flows, indicating pathways of nutrient losses (taken from Rotz et al. 1999)

One essential challenge for livestock managers is to regulate the flow of feed nutrients (Satter et al. 2002). Dietary adjustment is considered an important element in regulating environmental contaminants released through livestock excreta (Reijs et al. 2006). Such strategies should concentrate on nutrient management to prevent losses to the environment and enhance their cycling (Figure 1). Most of the recent studies (Beckman and Weiss 2005; Bartocci et al. 2006; Hayashi et al. 2009; Sultan et al. 2009) have therefore focused on balancing the supply of nutrients with the animal's requirements, although some studies were also carried out to determine nutrient cycling at the farm level (Haas et al. 2002; Børsting et al. 2003; Petersen et al. 2007).

Provision of an organic manure that meets the crops' nutritional requirements in time and in quantity is an important element for the design of a profitable organic farming system. Therefore the manipulation of the animals' diets in view of using their excreta as fertilizer for a variety of different cropping systems, and at the same time reducing the quantity and harmfulness of contaminants released to the environment is a focal point for ecological farming systems.

1.2.2. The effect of diet composition on nitrogen excretion

Livestock constitute a key element in the agricultural nitrogen fluxes, since dietary N is diverted into different products such as meat, milk and eggs, as well as into urine and faeces, and the latter two components are recycled into forages through the soil (Reijs et al. 2003). Given considerable N losses through gaseous emissions and leaching from manures (contributing by 65% to N_2O and 65% to NH_3 of global anthropogenic greenhouse gases production per year; FAO 2006), increasing the efficiency of nitrogen utilization is an important tool in reducing environmental pollution (Børsting et al. 2003). Tamminga (1996) stated that nitrogen losses occur at various sites in cattle's metabolism, whereby ruminal losses result from an imbalance between carbohydrates and proteins degraded in the rumen.

Moreover, imbalances between available net energy and amino acids cause further post-absorptive losses at the tissue level (Subnel et al. 1994). A high fraction of the ingested N may thus be excreted in urine and faeces, with reported values being as high as 70% (Tamminga 2006) and even 77.5% of the daily N consumption (Yan et al. 2007). Due to the highly positive correlation (r^2=0.92) between dietary protein concentration and N excretion, Yan et al. (2007) suggested that dietary N is a primary predictor for nitrogen excretion in beef cattle. A similarly high positive correlation (r^2=0.91) between dietary and excreted N and a positive relation between N excretion and dry matter (DM) intake was reported for lactating cows (Yan et al. 2006).

Nitrogen excreted in faeces can be distinguished into an organic (N_{org}) and an inorganic (N_{inorg}) component (van Vliet et al. 2007). Since plants can utilize nitrogen in its inorganic form only, N_{org} has to be mineralized into N_{inorg} to be ready for plant uptake, which requires a conversion of the organic matter. Soil microorganisms use part of the converted matter for assimilation of microbial tissue, and another part is oxidized to gain energy (Janssen 1996). If the amount of N is not sufficient for microbial assimilation requirements, inorganic nitrogen is taken from their environment in a process known as immobilization, or negative mineralization. A linear decrease of nitrogen mineralization was observed with an increasing dietary C:N ratio of organic material of similar decomposability (Janssen 1996). Sørensen et al. (2003) reported an elevated faecal nitrogen concentration with increased feed organic matter (OM) digestibility. Therefore, van Vliet et al. (2007) suggested that diet digestibility is probably the determining factor for the C:N_{org} ratio of slurry in dairy cattle.

From the above it appears that dietary nitrogen is an essential source of faecal nitrogen. Besides covering the amino acid (AA) requirements of the ruminant animal, the dietary protein should also cover the N and AA requirements of the microbial population in the rumen (Børsting et al. 2003). In an experiment, a positive correlation between crude protein (CP) intake and nitrogen excretion in manure (urine and faeces) was observed for 60 cattle heifers of various ages (Nennich et al. 2005). Moreover, an increase in urine-N concentration of cows with increasing concentration of dietary N in four iso-energetic diets of similar neutral detergent fibre (NDF) and DM contents was reported by Wang et al. (2007). Both dietary nitrogen and energy concentration were manipulated to test their effect on nitrogen concentration in manure slurry, which was positively affected by dietary nitrogen, but not by dietary energy (Van der Stelt et al. 2008). In another experiment with diets containing three levels of protein each at three levels of energy, faecal nitrogen digestibility was positively affected by nitrogen intake and dietary energy level; the linear effect on faecal N excretion with increased dietary CP concentration was attributed to increased DMI as well as excretion of undigested dietary CP (Broderick 2003). Moreover, increasing the CP concentration of the diet from 108 to 190 g kg^{-1} DM was found to increase total N and P concentration in the slurry by 56% and 48%, respectively (Van der Stelt et al. 2008).

Dietary energy plays an important role for nitrogen utilization in ruminants. Dijkstra et al. (1998) suggested that the availability of energy-yielding substrate is a limiting factor to microbial protein synthesis. Walsh et al. (2009) used iso-nitrogenous diets to test the effects of four levels of barley and wheat starch, and found a positive linear ($P<0.05$; barley and wheat) as well as a quadratic effect ($P<0.01$, barley) of the starch on N digestibility. They attributed the increase in N digestibility to the better availability of energy at the higher grain-to-straw ratio, while the lower concentration of acid detergent soluble nitrogen in straw compared to grain resulted in a higher efficiency with which the nitrogen was captured by rumen micro organisms, increasing N digestibility as the proportion of grain in the diet increased. In another study, faecal nitrogen output was not affected by different sources of energy, but there was a significant increase in urinary nitrogen output with highly degradable starch, which was attributed to an increased microbial protein synthesis compared to poorly degradable starch which provided less rumen fermentable energy (Castillo et al. 2001). Hart et al. (2009) found that the DM digestibility of grass had a direct effect on the CP digestibility, which could be attributed to the high concentration of metabolizable energy (ME) of the highly digestible diet compared to the poorly digestible one. In another experiment, the CP digestibility was affect by the level of rumen undegraded protein (RUD) but not by the level of energy in the diet; in the same study the yield of microbial nitrogen (MN) was positively affected by the concentrate level in the diet, demonstrating that the efficiency of MN synthesis is a useful indicator for the fraction of energy directed towards N deposition in microbes (Pina et al. 2009)

The excretion of nitrogen in manure is thus mostly a function of N intake, whereas diet composition affects nitrogen partitioning towards faeces or urine excretion (Weiss et al. 2009). James et al. (1999) found a significant increase in the percentage of nitrogen excreted via urine as the amount of diet protein increased, although this was accompanied by an increase in dietary NDF concentration. In dairy cows fed corn silage based diet, the percentage of consumed N excreted via faeces increased and the percentage excreted via urine decreased as compared to an alfalfa-based diet (Wattiaux and Karg 2004). A shift in nitrogen excretion from faeces towards urine was also observed as starch replaced NDF in isonitrogenous diets (Hirstov and Ropp 2003). As fibre replaces starch in the diet, the fermentation of carbohydrates shifts to the large intestine which may reduce urea recycling to the rumen; accordingly rumen microbial protein production reduces (Gressley and Armentano 2007). However, the shift could also be due to a reduced microbial capture of rumen degradable protein (RDP) in the rumen, since fibre is usually less fermentable than starch (Weiss et al. 2009).

Fibre-bound N (NDF-N) accounted for 13% – 22% of total N in faeces, 16% of which originated from undegraded feed N (Weisbjerg unpublished, cited by Børsting et al. 2003). In cows fed semi-synthetic diets, composed of straw, starch, molasses and urea and low in amino acids, the treatments were three levels of straw to concentrate ratios. It was found that 16%, 55% and 29% of faecal nitrogen originated from feed, microbial and endogenous N, respectively (Larsen et al. 2001).

1.2.3. The effect of dietary starch and fibre on excreta composition

Carbohydrates account for the largest fraction in the diets of herbivorous animals. It is therefore vital to understand the role of dietary carbohydrates in the digestion process, their influence on nutrient uptake and manure output. The main carbohydrate fractions in ruminants' diets are fibre and starch. As indicated in chapter 2.1.1., synchronization of the dietary supply of nitrogen and energy is essential for improving the ruminant's nitrogen utilization and reducing nitrogen losses (Hoover and Stockes 1991). Increased levels of concentrate in the diet supplied the rumen with easily fermentable substrates, raised ruminal volatile fatty acid (VFA) concentrations and lowered rumen pH (Beauchemin et al. 2001). A reduction in rumen pH can affect ruminal fibre and protein degradation and the efficiency of microbial protein synthesis (Rotger et al. 2006): in an experiment, the apparent digestibility of DM, OM, and starch decreased linearly ($P<0.01$) as the amount of starch increased in the diet (Sutton et al. 1997). In the same experiment, the ingested amount of starch as well as faecal starch concentrations were increased with increased concentrations of dietary starch. The authors interpreted the excreted faecal starch as a potential loss of ME and mainly glucogenic nutrients for the cow. In buffalo diets, the increase in the concentrate fraction from 12.5% to 50% significantly increased OM digestibility and reduced NDF digestibility although no effects were found in intermediate diets (Puppo et al. 2002). Arriaga et al. (2010) observed a negative relation between the dietary ratio of fibre to starch and the faecal and urinary N yield. They attributed this to the low dietary energy level of diets high in forages. In contrary, Weiss et al. (2009) could only determine a minor and insignificant difference in nutrient excretion when increasing dietary starch concentration from 22% to 30%.

In an experiment with dairy cows, Hindrichsen et al. (2006) found that with a high ratio of fibre-to-starch (1.5:1), urinary N excretion per cow was 59 g d^{-1} versus 160 g d^{-1} for a low fibre-to-starch ratio (1:1). In the same study, urine N excretion, expressed as a proportion of total N excretion, was higher with the low fibre-to-starch ratio although faecal nitrogen excretion was not affected by the treatments. This was explained by the dietary CP concentration, which was 156 g kg^{-1} DM for the high concentrate versus 113 g kg^{-1} DM for the high NDF and low net energy diet; both factors may work together in inhibiting nitrogen degradation in the rumen. The dietary gross energy deficiency in the low concentrate diet shifted the excreted N fraction towards the urine rather than the faeces.

1.2.4. The effect of faeces quality on microbial breakdown and nutrient release in the soil

Organic fertilizers are a valuable source of nutrients for crop growth and are usually applied according to crops' N requirements (Mohammadi et al. 2009). Efficiency of nutrient cycling could be enhanced by developing diets that satisfy the nutritional demands of livestock while producing excreta less subjected to losses when applied to soil for plant production. Manipulation of diets is a useful strategy for adjusting manure composition, since dietary N is positively correlated with the fertilizer N value of manure (Reijs et al. 2007). Several studies show the positive impact of livestock manure on plant nutrition, giving essential information and recommendations on rate and time of manure application to cropland.

The N contained in ruminant faeces consists of endogenous nitrogen, N from microorganisms or microbial products from the rumen, small intestine, hindgut, and N originating from the digestive tract itself and undigested fibre (Powell et al. 2009). In slurry, organic N is mainly derived from faecal N, and the concentration of N in ruminants' faeces is related to the feed digestibility (Reijs et al. 2007). The plant availability of manure-N may be reduced if the fraction of N excreted in urine is reduced. Moreover, soil manure turnover and manure N availability to the plant is influenced by feed properties rather than by the dietary protein concentration, since the feed composition is reflected in the ratio between total C and N in the slurry and affects the amount of residual slurry left in soil (Sørensen et al. 2003). After application to the soil, faecal endogenous N mineralizes more rapidly than faecal undigested N that occurs in the fibre fraction (Sørensen et al. 1994; Powell et al. 1999). After applying manure to soil there was a negative correlation between the dietary NDF concentration and microbial mineralization of manure N, which was on the other hand positively related to the dietary crude protein concentration (Sørensen et al. 2003).

In an experiment investigating the effects of different levels of dietary protein and energy in non-lactating dairy cows, there was a positive linear relation between the N application rate in slurry and N uptake by grasses. In the same experiment, slurries from high protein diets showed a significantly higher average Mineral Fertilizer Equivalent (MFE) compared to slurries from low protein diets, while there was no effect of dietary energy (Reijs et al. 2007). In an experiment conducted by Sørensen et al. (2003), a negative correlation ($r^2=0.67$; $P<0.001$) between the C:N ratio in the manure and MFE was observed

The net mineralization of faecal N from differently fed animals was significantly correlated with the N concentration in faeces, and the fluctuation in faecal N mineralization was due to variations in the composition of faecal C (crude fibre, NDF and ADF (acid detergent fibre) and the C:N ratio in the faeces (Kyvsgaard et al. 2000).

The type of forage fed to ruminant livestock can affect faecal N mineralization in the soil. Powell et al. (1999) found that the type and amount of the forage in the diet affects faecal fibre and N concentration, which impacts manure decomposition and N availability in soils. In an experiment, maize fertilized with slurry from cows fed alfalfa showed a higher N uptake than maize that received slurry of cows fed low-tannin birdsfoot trefoil. The total N recovery was highest from alfalfa plots, followed by high-tannin birdsfoot trefoil and red clover (Powell and Graber 2009).

While N is voided in urine and faeces, P is mainly voided in faeces by the adult ruminant (Tamminga 1996; Bravo et al. 2003). Therefore P availability to the plant is governed by soil and organic matter immobilization-mineralization processes (Powell et al. 1999). Application of livestock manure over years could increase the amount of P available to the plants (Mohammadi et al. 2009). Phosphorus mobility depends on the P adsorption capacity of a soil, which is influenced by properties such as permeability, pH, concentration of lime, iron or aluminium oxides, amorphous materials, and organic matter

Soil organic matter is a critical component of soil productivity. The organic matter of subtropical soils is comparatively low because of high temperature and intense microbial activity, and the application of OM occasionally will influence plant growth and physiology, providing growth-regulating substances and enhance soil physical condition (Ramesh et al. 2009). The average SOM content was found to increase as a result of cattle manure application to cropland, the increase being proportional to the application rate of manure (Mohammadi et al. 2009).

Even though the addition of excessive amounts of manure to the soil causes soil dispersion resulting from accumulated K^+, Na^+ and NH_4^+ ions in the soil and the production of water-repellent substances by decomposer fungi, on the positive side the increase in SOM through manure addition increases water holding capacity, porosity, infiltration capacity, hydraulic conductivity and water stable aggregation and decreases bulk density and surface crusting (Haynes and Naidu 1998).

1.2.5. Implications of manure application for greenhouse gas emissions and nutrient leaching

There is an increased global awareness on the necessity to protect the environment from contaminants such as carbon dioxide, methane, ammonia, nitrous oxide and other gases contributing to the greenhouse effect as well as the contamination of soil and groundwater with excessive amounts of P, N and K. The livestock industry is a major source of solid, liquid and gaseous emissions that can be environmentally harmful. Manure contains nitrogen and phosphorus, which are the most important plant nutrients, but are detrimental when applied to agricultural land in excessive amounts. This may pollute groundwater with nitrate (NO_3^-), surface water with phosphorous causing eutrophication, and soils with heavy metals that may be included in some feed formulation as growth promoters (FAO 2006).

The total contribution of animal production to greenhouse gases was estimated at 18% (FAO 2006). The greenhouse gas methane (CH_4) is produced mainly by microbial activity in extremely anaerobic ecosystems such as natural and cultivated wetlands, sediments, sewage and landfills (Xu et al. 2003). Globally about 20% of methane is produced from ruminants and animal wastes (Tamminga 2006). Methane has a relatively high Global Warming Potential (GWP) compared with CO_2 (23 vs. 1). Annual CH_4 release from enteric fermentation of domestic ruminants was estimated at 84.5 Mio t in 2004, to which 8.2 Mio t a^{-1} add from anaerobic manure fermentation (FAO 2006). A multitude of studies were and are carried out to find effective solutions to reduce methane production from ruminants. Feeding high concentrate diets or including monensin-sodium in the diet was found to shift the VFA production in the rumen towards propionate at the expense of acetate and consequently reduce methane production (Moss et al. 2000).

Nitrous oxide results from microbial nitrification and/or microbial or chemical denitrification in the soil. N_2O emissions are thus influenced by adding N to the soil through animal manure, mineral N fertilizer, crop residues or sewage water (Tamminga 2006). To reduce ammonia production, Tamminga (2006) suggested that primarily dietary N intake should be kept at its minimum; second, the degradation of protein and carbohydrates in the rumen should be synchronized to ensure that rumen degradable protein is captured in microbial protein; lastly the excretion of N should be shifted from faces to urine by increasing dietary fibrous feed to enhance hindgut fermentation, and in this way capture more of the recycled urea and transfer that to microbial protein which will appear in faeces. The management of manure is thus an important tool to reduce the detrimental effects of animals waste to the environment. For instance, manure produced by cattle is considered as an important source of ammonia emissions causing acidification and eutrophication, while the entertic fermentation of cattle is a significant source of methane responsible for global warming (Havlikova et al. 2008). After manure addition to the soil, the estimated amount of N available to the crops in the first year was about 50% for pig slurry, 30% for cattle slurry and 10 – 49% for broiler litter (Nicholson et al. 1999). Part of the manure nitrogen will be lost by denitrification through a series of soil microbial processes that convert ammoniacal nitrogen (NH_3-N) to nitrate (NO_3^-), di-nitrogen and nitrous oxide (N_2O) (Sandars et al. 2003). The nitrate remains in the soil, will be available to the next crop and accumulates in the soil over the years, but few farmers take into account the residual nitrogen from manure accumulating in the soil (Sandras et al. 2003).

Difficulties exist for the elaboration of efficient systems to reutilize N, P, and K from intensive animal production by primary (plant) production in developed countries, due to the limited availability of land (Tamminga 1996). According to FAO’s (2006) Livestock’s Long Shadow report, the livestock sector is undergoing a complex process of technical and geographical change and the production units are shifting from the countryside to urban and peri-urban areas, towards sources of animal feed, be it feed crop areas or transport and trade hubs where feed is distributed, and this especially applies to developing countries with poor infrastructure. Therefore, “environmental problems created by industrial production systems derive not from their large scale, nor their production intensity, but rather from their geographical location and concentration” (FAO 2006).

Tamminga (1996) concluded that animal production is driven by energy (biomass and fossil) and with this energy other essential nutrients (N, P, K) are mobilized through the soil-plant-animal system. To maintain the sustainability between the three components, the systems should be balanced with each other so that losses of CO_2, CH_4, N, P, and K are limited to a minimum. Within the animal component, regulation of energy and nutrient flows are primarily determined by nutrition.

1.2.6. Effects of manure application on plant growth

Manure is a valuable resource for organic crop production. It provides the soil with nutrients and organic matter and stimulates the microbial activity in the soil, thus enhancing soil fertility. Although manure is a source for OM, which may also renew the SOM depleting due to agricultural practices, it can on the other hand be a source of excessive nitrate release beyond the crops’ uptake potential. Furthermore, it can introduce undesirable microorganisms, pathogens and weed seeds (Eghball and Power 1999). Livestock manure may also contain considerable amounts of salts that may have detrimental effects on soil productivity, and salt accumulation in the soil will lead to salinization, decreased water availability, and consequently, lower crop yields (Hao and Chang 2002).

However, numerous studies have shown the positive impact of livestock manure on crop nutrition and production. Powell et al. (2009) found an increase in maize yield by 35% and in nitrogen uptake by 49% on plots manured with slurry from beef cattle that had been fed alfalfa as compared to the unmanured control plots. In the same experiment, the maize yield exceeded that of control plots by 17% and 29%, and the N uptake was higher by 29% and 36% on plots manured with slurries from cattle fed red clover and birdsfoot trefoil, respectively.

In another trial, increasing slurry application from 29 to 39 t ha^{-1} increased the average annual yield of a rotation of rape, barley, peas and field beans from 6.2 to 6.8 t DM ha^{-1}; the minimal yield increase indicated that the additional amount of nutrients was not utilized by the plants, and that phytotoxic substances might have been present in the slurry (Hansen 1996). Likewise, neither annual yield nor proportional N recovery were enhanced by increasing slurry application to timothy grass (*Phleum pratense* L.) from either 132 to 186 kg N ha^{-1} or from 134 to 267 kg N ha^{-1} (Anderson et al. 1993). On the other hand, enhancing the manure application level was found to improve the root length density in the upper soil, indicating that root proliferation was improved, possibly through improved physical properties and increased nutrient and water availability (Mosaaddeghi et al. 2009).

In another experiment, the concentration of CP in orchard grass and reed canarygrass was enhanced when increasing the application level of cattle manure; similarly, alfalfa CP concentration was positively affected but the effect on the grasses was more pronounced, which was explained by the legume's ability to fix N and ultimately alleviate the manure application effect (Min et al. 2002). Moreover, the authors found that cattle manure had a comparable or even greater effect on increasing plant CP concentrations than inorganic chemical fertilizer.

Crop productivity in organic farming is influenced by the source of nitrogen, particularly in non-leguminous crops, due to the crucial effect of the amount of N released during the period of rapid crop growth (Berry et al. 2002). In a three-year experiment in India, organic manures (well-composted cattle dung, poultry manure and vermicompost) were tested alone or in combination in four cropping systems; plots treated with mineral fertilizer of similar nutrient composition served as a control (Ramesh et al. 2009). In the rainy season, there was no quantitative difference in crop yields obtained with cow dung, the combination of the three organic manures and mineral fertilizer, respectively; across the three years, all organic treatments were superior to the control. At the end of the study, the application of organic manures had improved soil quality parameters, namely soil organic carbon, soil available nutrients (N, P and K), soil biological activity and microbial biomass (Ramesh et al. 2009).

Manure application was also shown to influence the quality of ensiled grass and grass-legume mixtures: a timothy-red clover mixture and orchard grass fertilized with farmyard manure at 25 t DM ha^{-1} contained more *Bacillus* spores than when the same crops received mineral fertilizer (Rammer et al. 1994). For the manured crops a decline in silage quality was observed, namely a high pH (>4.5), high ammonia N (>150 g kg^{-1} total N) and butyric acid concentration (6.3 g kg^{-1} water), low lactic acid concentration (<12 g kg^{-1} water) and high counts of *Clostridium* spores (>10^{5} g^{-1} water).

1.3. The global and regional importance of dairy buffalo husbandry

Global milk production has increased drastically in the last two decades, and buffaloes contribute about 13% to the world milk production (FAOSTAT 2010). Due to its morphological, anatomical and behavioural characteristics, the buffalo is well adapted to hot and humid climates and muddy terrain (Marai and Haeeb 2010). Two types of domesticated buffalo (*Bubalus bubalis*) are distinguished: the river buffalo and the swamp buffalo. Swamp buffaloes are used mainly for draught power in rice cultivation in the paddy fields of Southeast Asia. These buffaloes produce relatively small amounts of milk (1 - 2 kg d^{-1}) and are mainly used for meat and draught purposes (Thomas 2004). In the riverine water buffalo, various breeds are distinguished; their live weight ranges from 522 to 1500 kg in bulls and from 408 - 800 kg in mature cows (Moioli and Borghese 2007). Their milk production capacity is far higher than that of the swamp buffaloes; for instance Nili-Ravi buffalo cows yielded an average of 1,925 kg of milk during a 282 day lactation (NRC 1981). River buffaloes can adapt to a large range of environmental conditions; in India and Pakistan they are mostly confined to areas where the summer temperatures rise above maxima of 46℃ and the winter temperatures may fall below 4℃ (Marai and Haeeb 2010).

The domestic buffalo is widely distributed in Asia, but it has also been introduced to Europe, the Near East, China, South America, the former Soviet Union and the Caribbean (Figure 2). The current world population of buffaloes is 180.7 million (FAOSTAT 2010), of these, 174.2 million live in Asia. In many parts of the world, buffalo milk production is part of the traditional farming system, for example in the Caucasian countries, in Asia and Egypt, where local demand for fresh buffalo milk, butter and yoghurt is considerable (Thomas 2004). At present, buffalo numbers increase quicker than cattle numbers in Asia - reasons for this include customer preference for higher butterfat contents, longevity and consistency in milk yield in the buffalo under poor management conditions, and their high disposal value (Devendra and Thomas 2002).

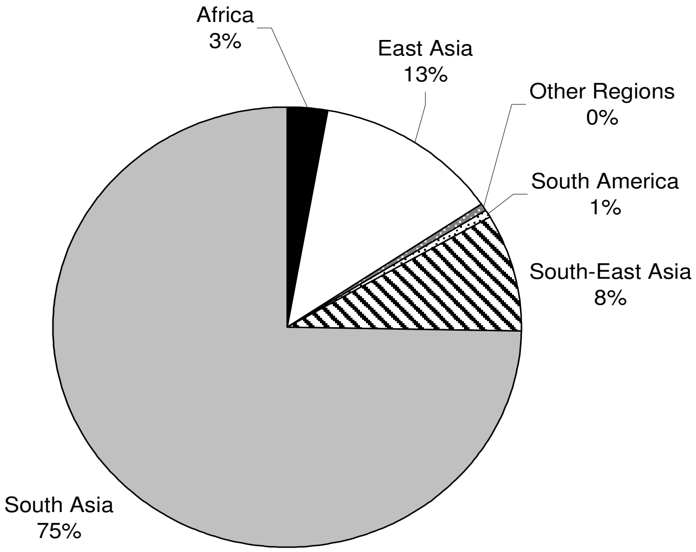

Figure 2: Distribution (%) of the global buffalo population across different regions (Source: FAOSTAT 2010)

As few as 0.02% of the world buffalo population is kept in the EU countries, mostly in Italy. There, buffalo production is mainly located in the central and southern regions that may be characterized as economically disadvantaged rural areas, where this activity plays an important role for improving the economy of these areas (Bartocci et al. 2006). The high demand for the famous buffalo mozzarella cheese fostered the country's buffalo milk production. Worldwide, an annual increase of 6.2% in buffalo milk production was observed between 1990 and 2002, demonstrating the increasing significance of the buffalo as a dairy animal (Nanda and Nakao 2003). The increasing demand for buffalo mozzarella cheese, not only in Italy but also in Europe (Germany, France, Great Britain) and the USA clearly demonstrates that this is an important commodity. However, the growth of buffalo production in Italy is not only due to the profitable price of buffalo milk, but also due to the replacement of cattle by buffalo as a result of EU restrictions on cow milk production (Puppo et al. 2002). The expanding market for buffalo dairy products introduced buffalo farming to other European countries such as Holland, Great Britain, Switzerland, and Spain (Bartocci et al. 2006).

Buffalo meat is popular in Asian countries; it is mainly produced from culled animals or surplus males that are slaughtered (Nanda and Nakao 2003). Buffalo meat contains less saturated fatty acids than beef, 40% less cholesterol, 11% more protein and 10% more minerals (Nanda and Nakao 2003), and the cost of fattening per kg bodyweight is lower in buffalo than in cattle (Chantalakhana 2001), which makes buffalo meat a valuable protein source for resource poor areas. Although the economic importance of buffaloes is evident, little work has been done to exploit the genetic potential of this species.

1.3.1. Digestive efficiency of buffalo as compared to cattle, and implications for the relation between feed and faeces quality

There is a general notion that (water) buffaloes posses an enhanced ability to utilize feeds with high concentrations of structural carbohydrates ('poor feed') better than cattle (Kawashima et al. 2006), although studies comparing the two species show a variation in their results concerning the digestive efficiency of cattle and buffaloes. In a comparison digestibility study between swamp buffaloes and Brahman cattle, digestibility of DM, OM and NDF were numerically higher in buffalo but statistically, differences were insignificant (Kawashima et al. 2006). Kennedy et al. (1992a) tested a diet containing rice straw and 5% of *Leucaena leucocephala* leaves plus mineral supplements, and in another experiment rice straw was offered together with urea or with urea plus sunflower meal. In the first trial, no difference was observed in OM digestibility between cattle and swamp buffaloes, while the digestibility of cell wall constituents (CWC) was significantly higher in cattle than in buffalo. In the second trial, both the apparent digestibility of OM and CWC was significantly higher in cattle than in buffalo. The authors attributed these results to the observed faster particulate passage rate in buffaloes as compared to cattle. However, in another comparison of cattle and buffalo, the *in vitro* digestibility of crude fibre from hay and grass silage was superior for buffaloes with both substrates (Batista et al. 1982). Likewise, the *in vivo* digestibility of cellulose and hemicelluloses was higher in riverine buffaloes than in sheep (Bartocci and Terramoccia 2006). On the other hand, rumen retention time and total tract retention time were similar in buffaloes and sheep, but as the variation of the structural carbohydrates in the diet increased, the rumen retention time of solid particles in buffalo proved to be more variable than in sheep i.e. the ruminal retention time in buffaloes was significantly affected by the level of dietary NDF (Bartocci and Terramoccia 2006). Bartocci et al. (1997) reported that buffalo retain particles longer in the rumen than cattle, while total tract retention time was shorter because of a low residence in the lower gastrointestinal tract (GIT).

As far as nitrogen use efficiency is concerned, buffaloes have been shown to maintain higher rumen ammonia levels through enhanced recycling of blood urea to the rumen, and have a more efficient net synthesis of ruminal microbial N than cows (Kennedy et al. 1992a). Together with an enhanced N utilization within the animal’s body, this is considered a crucial property in animal nutrition (Kennedy et al. 1992b). An *in sacco* trial carried out to test rumen degradability of protein and protein-free dry matter in buffalo, cattle and sheep (Terramoccia et al. 2000) showed superiority of buffalo in rumen CP degradability for all tested feed substrates, namely concentrate ($P<0.05$), alfalfa ($P<0.01$) and maize silage ($P<0.05$). The higher ruminal CP degradability in buffaloes was attributed to the longer residence time of feed particles in the rumen. The total amount of amino acids derived from undegraded feed protein and absorbed in the lower GIT was higher in cattle than in buffalo, while ammonia nitrogen concentration in the buffalo’s rumen was higher than in cattle and sheep.

The *in vitro* incubation of various feedstuffs (alfalfa hay, barely meal, beet pulp maize meal and silage, ryegrass hay and silage, as well as soya bean meal) yielded a higher gas production with rumen fluid from cattle rather than buffalo ($P<0.001$) for all substrates, and a lower VFA production, especially of acetic and butyric acid, with buffalo rumen fluid ($P<0.001$), although OM digestibility of the feeds was same for both species (Calabrò et al. 2004). The stoichiometric equation derived from data obtained in the experiment predicted that a greater proportion of the degraded OM is used for microbial biomass production at the expense of VFAs in buffalo compared to cattle, explaining also the earlier finding from a ruminal fluid study that for buffalo, diets with the same energy content but less protein are required (Sadhana et al. 1992).

The *in vitro* degradation of eight feeds inoculated with rumen fluid from either cattle or buffalo showed a significant influence of the type of rumen inocula in all substrates (Calabrò et al. 2008). The authors concluded that differences in rumen fermentation between the two species can be attributed to differences in microbial activity, differences in the amount of microbial cells within the otherwise similar population, or differences in the species of bacteria and protozoa constituting the microbial population.

Concerning the utilization of feed energy, buffaloes were found to have higher Gross Energy (GE) digestibility than cattle (Ichinohe et al. 2004). On the other hand, Kawashima et al. (2006) did not observe differences in energy intake, energy loss (faeces, urine, methane, heat production) and energy retention between the two species. Moreover, in a comparison of swamp buffaloes and Malaysian local cattle, it was found that the maintenance energy requirement of the buffalo was lower, but cattle were energetically more efficient with respect to fat deposition (Liang and Young 1995).

The superiority of buffaloes rather than cattle in utilizing dietary fibre could reduce the N excreted in urine and faeces. Although most reports did not show variations in OM digestibility between the two species, there were differences in the structural carbohydrates digestibility. These issues should be taken in account when using buffalo manure for crops fertilization.

1.4. Research objectives and structure of the thesis

Numerous studies have dealt with manipulation of cattle manure quality by means of nutrition, increasing crops' nutrient utilization efficiency and reducing the detrimental effects of manure on the environment.

In the last decade, the number of the water buffaloes has increased vastly around the globe, increasing the amount of manure produced from this species. In developed countries there is a general trend towards deploying organic manure in soil fertilization, especially in organic farming; contrastingly, in poor rural and remote areas of Asia where the majority of water buffaloes is kept, manure is used as a cheap nutrient source to fertilize cropland and as a secondary source of income through sale of manure and dung cakes (Nanda and Nakao 2003). This intensive use of manure together with the growing number of animals raises the need to enhance the quality of manure produced.

In view of the above, the main objective of this study was to improve the understanding of the factors affecting the excretion of nutrients in faeces of water buffalo, so as to determine which dietary manipulations might significantly affect manure quality and subsequent crop growth. To this end, this research project aimed at:

(i) Investigating the effects of the dietary C-to-N ratio and the ratio of NDF-to-soluble carbohydrates (SC) namely starch and sugars, on feed and nutrient digestibility and final faecal output in terms of quality and quantity;

(ii) Observing the influence of the above variables (i) on the particle rate of passage through the digestive tract.

Data presented in this study were collected on farm experiments in Sohar, Oman, during July 2007 to January 2009. In Chapter 2, the results of two digestibility trials with four test diets with an either high or low C/N ratio and an either high or low NDF/SC ratio are presented, while Chapter 3 examines the effects of these four rations on parameters of particle passage through the gastrointestinal tract. Based on these experiments, Chapter 4 discusses to which extent diet characteristics affect the excretion of nutrients via faeces, and whether such interdependencies can be used to improve nutrient use efficiency in the animal and crop component of organic farming systems.

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Chapter 2

Quality of water buffalo manure as affected by dietary C/N and NDF/soluble carbohydrates ratios

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Quality of water buffalo manure as affected by dietary C/N and NDF/soluble carbohydrates ratios

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2.1. Abstract

Optimizing composition of manure has the potential to increase crop yield and to reduce nutrient losses to the environment. In this study the effect of dietary ratios of carbon (C) to nitrogen (N) and of structural to non-structural carbohydrates was assessed in relation to manure quality. Two experiments were conducted with twelve water buffalo heifers each, using a full Latin square design. One control and four tests diets were examined during three subsequent 7 day experimental periods, preceded each by 21 days adaptation. Diets consisted of varying proportions of Rhodes grass hay, soybean meal, wheat bran, maize, dates, and a commercial concentrate to achieve (1) a high C/N and a high NDF (neutral detergent fibre)/SC (soluble carbohydrate) ratio (HH), (2) a low C/N and a low NDF/SC ratio (LL); (3) a high C/N and a low NDF/SC ratio (HL) and (4) a low C/N and a high NDF/SC (LH) ratio.

Organic matter (OM) intake and excretion of LL and LH treatments were significantly lower than of HH and LH treatments, respectively. Digestibility of N was highest in LH (88%) and lowest in HH (74%). While NDF digestibility was also highest in LH (85%), it was lowest in LL (78%). Faecal N concentration was positively correlated ($P<0.001$) with N intake, and was significantly higher in faeces of animals from the LL treatment in comparison to the HH treatment. Faecal fibre and starch concentrations were positively affected by fibre and starch content in the diet, with NDF being highest in HH (77%) and lowest in LL (63%) faecal OM. Faecal C/N ratio was positively related ($P<0.001$) to NDF intake; C/N ratios were 12 and 7 for HH and LL ($P<0.001$), while values for HL and LH were 11.5 and 10.6 ($P>0.05$).

Results demonstrate that the composition of buffalo manure (i.e. C/N ratio, NDF, ADF, ADL) is clearly affected by diet composition. Despite buffaloes' reportedly high fibre digestion capacity, digestive processes did not alleviate the manifestation of diet characteristics in faeces. Feeding diets of high NDF/SC ratios resulted in high manure NDF concentrations, which may slow down nutrient mineralization rates and grant a prolonged period of nutrient supply to crops.

Key words

Bubalus bubalis, dietary manipulation, faecal carbon, structural carbohydrates, Oman

2.2. Introduction

In smallholder production systems of tropical regions, ruminant species like sheep, goats and buffaloes are often of high economic importance. The worldwide population of buffaloes increased from 148 million head in 1990 to 181 million head in 2008. Especially in India and Pakistan, buffalo numbers increased tremendously by 22% and 72%, respectively. While the meat production from buffalo (*Bubalus bubalis* L.) enhanced only slightly, milk production was largely increased from 44 million tonnes to 89 million tonnes in the same time period. Again India and Pakistan were the drivers of this development, but also Italy and countries of the Near East, like Egypt and Syria, substantially invested in buffalo milk production (FAOSTAT, 2010). Besides the demand for the primary products milk and meat, the dung production of buffaloes also becomes increasingly interesting. While environmental pollution (e.g. nitrate leaching, greenhouse gas emissions) from intensive buffalo production systems has to be reduced, the use of buffalo manure as source of organic fertilizer in crop production should be optimised. Results obtained from studies with cattle cannot be transferred directly to buffalo production, because differences in the digestive processes of cattle and buffalo have been widely observed. It is accepted that the buffalo is superior to cattle in digesting poor-quality feeds like roughages (Robles et al., 1971; Devendra, 1992; Van Soest, 1994). Nevertheless, contradictory results are reported concerning the physiological reasons. Several authors obtained significantly better fibre digestibility in buffaloes compared to cattle (Grant et al., 1974; Hussain and Cheeke, 1996), while other studies showed that fibre digestibility was higher in cattle (Kennedy et al., 1992; Puppo et al., 2002). Less diverse results were obtained for the CP digestibility, as most authors reported similar CP digestibility in buffaloes and cattle (Sebastian et al., 1970; Devendra, 1992; Puppo et al., 2002). However, differences in N metabolism were observed between cattle and buffaloes, indicating that buffaloes are able to use ingested N more efficiently, due to higher N retention (Sebastian et al., 1970) as well as higher blood-urea concentration (Kennedy et al., 1987) and ammonia concentrations in the rumen (Kennedy et al., 1992a), resulting in lower urinary N excretion and a higher total N balance (Devendra, 1992).

The quality of organic fertilizers is mainly determined by their nitrogen (N) and carbon (C) content, usually expressed by the C-to-N ratio (C/N ratio). In addition, decomposition of organic fertilizers is influenced by the forms in which carbon is available in organic fertilizers, i.e. rapidly available soluble carbohydrates or slowly degrading cell wall components like lignin (Heal et al., 1997). Nevertheless, N is the most common limiting factor in decomposition and determines the growth and turnover rate of soil microbial biomass which mineralises the organic C (Kyvsgaard et al., 2000). In organic agriculture the use of mineral fertilizers is usually not allowed, therefore special importance is attached to the quality of livestock manure as organic fertilizer. The major aim is to supply sufficient amounts of nutrients in accordance to the growth process of the crops and avoid nutrient surpluses in the soil at times the crop cannot use them. Excess nutrients are prone to leaching and are lost to deeper soil layers inaccessible for crops or are converted into unavailable forms (Myers et al., 1997). In tropical environments an additional concern is to stabilise the soils by maintaining sufficient levels of soil organic matter (OM). High temperatures and high water availability, either in the humid tropics or in irrigated cropping systems, result in high rates of OM decomposition and thus rapid nutrient release (Fischer et al., 2006). Therefore, organic fertilizers used in tropical, organic cropping systems have to meet two requirements: (i) supply sufficient amounts of nutrients to the growing crop and (ii) have a long-term effect on the soil OM content.

The mineralization of nutrients in organic materials depends mainly on their C/N ratio (Kyvsgaard et al., 2000; Van Kessel et al., 2000). Janssen (1996) showed that for materials of similar decomposability, the net mineralization linearly decreased with increasing C/N ratio. Long-term effects of organic fertilisation on soil OM and organic N accumulation in the soil are mainly determined by the C form (Hassink, 1994). Correspondingly, considerable amounts of C should be supplied in form of slowly decomposable carbohydrates and lignin to facilitate long-term nutrient release and stabilisation of soil OM (Handayanto et al., 1997). A major source of organic fertilizer is livestock manure. The degradation of plant biomass to manure by livestock digestion processes changes its nutrient content and composition (Powell et al., 1999; Van Vliet et al., 2007). In intensive agricultural systems under temperate conditions, these changes are mainly studied in view of avoiding environmental pollution like nutrient leaching or greenhouse gas emissions (Tamminga, 1996; Jongbloed and Lenis, 1998). Several studies have also examined the effects of dietary manipulation on manure and slurry composition and the subsequent mineralization processes in the soil and therewith the fertilizer quality. Fertilizer quality was mainly defined by the N concentration and the C/N ratio. Most important impacts on manure or slurry quality could be related to N and fibre intake. A significant positive correlation (r = 0.50) between crude protein (CP) content of feeds and the fertilizer quality was found by Sørensen et al. (2003) and Powell et al. (2006), which also lead to a decreased C/N ratio in ruminant manure (Reijs et al., 2007; Van Vliet et al., 2007). Sørensen et al. (2003) also found a negative correlation between fibre content in feeds and the fertilizer quality of cattle slurry. Reijs et al. (2007), using the C/N ratio as manure quality indicator, were able to explain the causes of variance by a combination of CP content (84%) and neutral detergent fibre (NDF) content (12%) in the diet. Kyvsgaard et al. (2000) observed a negative correlation between fibre content of feed and the mineralization of N in sheep faeces in the soil.

However, studies investigating how the quality of buffalo manure is influenced by digestion processes and dietary manipulation are rare. Kennedy (1995) claimed that the buffalo's higher digestive efficiency may reduce the chances to influence the quality of manure through dietary manipulation. Challenging this perception we hypothesize that despite the buffalo's seemingly higher digestive efficiency, marked differences in diet quality will persist and be reflected in faeces composition and therewith manure quality. The objectives of the present study were therefore to clarify, (i) if the faecal composition is influenced by dietary manipulation in buffalo, and (ii) which faecal components or manure quality indicators are especially susceptible to dietary manipulation.

2.3. Materials and methods

Two feeding trials were conducted with Nili Ravi river buffalo heifers on a private organic farm in Sohar, Oman, in 2007 and 2008. In each trial, two experimental diets were fed, which differed in C/N ratio and in the ratio of NDF to soluble carbohydrates (SC = sugars and starch; NDF/SC ratio). These ratios were chosen to vary the components N and C, as well as the C sources, which are important criteria with regard to the use of manure as fertilizer in crop production.

2.3.1. Experimental site

Two feeding trials, subdivided into three experimental periods of 28 days each, were carried out on a private farm in Sohar, Oman, located 220 km North of Oman's capital Muscat. The first trial took place during September – November 2007 and the second trial during February – May 2008. The average daily temperature and relative humidity during the experimental periods are depicted in Table 1; no rainfall occurred during the trials.

Table 1.
Mean daily temperatures and relative humidity during three subsequent experimental periods of two feeding trials with river buffaloes in summer 2007 and spring 2008.

Trial	Date	Period	Mean ambient temperature (℃)	Relative humidity (%)
1	11.08. - 17.08.2007	1	33.4	69.5
	08.09. - 14.09.2007	2	31.7	71.9
	06.10. - 11.10.2007	3	27.8	38.8
2	08.03. - 14.03.2008	1	22.0	52.0
	05.04. - 11.04.2008	2	28.2	59.0
	10.05. - 16.05.2008	3	34.7	45.0

2.3.2. Animals

In each of the two trials, twelve Nili Ravi river buffalo heifers were selected randomly from a herd of more than 60 heifers. The animals were 1.7 – 2.0 years old and weighed 385 – 600 Kg at the start of the two trials; their live weight development during the trials was determined on a mechanical scale at days 1, 11, 21 and 28 of each trial period (Table 4). The animals were individually tethered under roofed paddocks with open sides and fed from large-size troughs.

2.3.3. Treatments and experimental design

In each of the two trials, two experimental diets were compared to one control diet. All diets contained Rhodes grass hay (*Chloris gayana*) and varying proportions of soybean meal (*Glycine max*), crushed maize (*Zea mays*), whole dates (fruits of *Phoenix dactylifera*), wheat bran (*Triticum* spp.), cotton seed meal (*Gossypum hirsutum*) and a commercial concentrate feed (for details see Table 2). The ingredients were combined in such a way that the ratio of carbon (C) to nitrogen (N; C/N) and the ratio of neutral detergent fibre (NDF) to soluble carbohydrates (sugars plus starch, SC; NDF/SC) was either high (H) or low (L), resulting in four treatments, namely HH and LL (tested in trial 1) as well as HL and LH (tested in trial 2). With the exception of hay and dates, the different feedstuffs were mixed to achieve the requested composition in the different treatments and were offered as concentrate mixture. As control diet (CD) the habitual diet of the buffalo on the private farm was retained. The chemical composition of the different diets is presented in Table 3.

Table 2.
Composition of the experimental diets (in g/kg DM) offered to water buffalo in two feeding trials conducted in Oman.

	Trial 1 (autumn 2007)			Trial 2 (spring 2008)		
Feedstuff Diet	HH	LL	CD	HL	LH	CD
Rhodes grass hay	899	401	677	612	681	677
Soybean meal	78	392		27	259	
Maize		207		361		
Dates			148			148
Wheat bran	23					
Cotton seed meal					60	
Concentrate mix			175			175

CD: control diet, HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC.

Table 3.
Chemical composition of experimental diets fed to water buffalo in two feeding trials conducted in Oman in autumn 2007 (trial 1) and spring 2008 (trial 2).

		Trial 1		Trial 2		Trials 1, 2
Component		HH	LL	HL	LH	CD
OM	(g/kg DM)	939.4	942.4	947.5	928.1	933.3
N	(g/kg OM)	15.4	36.6	17.6	29.5	14.0
C		419.9	403.4	414.5	408.8	403.4
P		2.7	4.3	2.5	3.8	3.6
NDF		705.4	366.8	501.2	550.7	541.9
ADF		368.8	192.4	259.3	307.3	289.7
ADL		32.4	16.2	21.2	32.1	28.6
Starch		12.2	122.6	197.2	60.0	66.9
Sugar		43.9	178.2	30.2	21.9	98.7
C/N	(g/g)	27.2	11.0	23.5	13.9	28.8
NDF/SC		12.6	1.2	2.2	6.7	3.3

CD: control diet, HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC.

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

Each trial was set up as a complete Latin Square design where each of the three diets offered per trial was fed to 4 animals at a time and to all 12 animals per trial within the 3 experimental periods. Based on their tabulated concentration of metabolizable energy (ME), all diets were offered at 1.45 times maintenance energy requirements of the animals. Due to the high proportion of crushed maize and soybean meal, respectively, in diets HL (36% maize) and LH (26% soybean meal), the metabolizable energy (ME) concentration of these diets was about 11.5 MJ and 11.0 MJ ME kg^{-1} DM. Water was offered *ad libitum* throughout the day. Of the 28 days per trial period, 21 days served the animals' adaptation to the diet, followed by 7 days of quantification of feed intake and faecal excretion.

The total amount of feed to be given was divided into two equal portions and offered daily at 7:00 and 14:30 h. At each meal, the mixed concentrates were offered in the first place; after one hour, eventual refusals were collected and then Rhodes grass hay was offered. Refusals were taken off and weighed immediately before the start of the second daily meal. Representative samples of feed offered and the total of refusals (separated according to feed components) were collected daily during the quantification period, weighed (electronic balance, range 0 - 30 kg, accuracy 0.1 kg) and dried to constant weight at ambient temperatures, weighed again and milled to 1 mm particle size before analysis.

During the 7 days of collection, faeces were quantitatively gathered into a plastic bowl whenever a defaecation occurred, whereby care was taken that faeces did not fall to the ground. If this occurred, any adhering dust was brushed away carefully before the excretion was weighed (electronic balance, range 0 - 30 kg, accuracy 0.1 kg). After homogenization of the excreted material, a representative sample of approximately 30 g fresh matter was taken and stored at -18°C until analysis. In this way, 3 to 4 faecal samples per animal and day were obtained, of which one (afternoon sample) was air dried and treated in the same way as described for the feeds.

2.3.4. Chemical analysis

Before analysis, samples were pooled as follows: For feed offered, one pooled sample was constituted per type of feed (concentrate, dates or hay) and 7-day sampling period; the same was done for the different types of residue samples for each individual animal. Likewise, the 7 dried faecal samples per animal were pooled into one composite sample. Following standard procedures (Naumann and Bassler, 1988), all dried samples were analyzed for concentrations of dry matter (DM) and organic matter (OM). The concentration of phosphorus (P) was measured according to a standard procedure (Naumann and Bassler, 1988) using a spectrophotometer (UVIKON 930, Kontron Instruments Ltd, Bletchley, UK). Concentrations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were measured by a modification of the method of Van Soest et al. (1991) using a semi-automated Ankom 220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) without using decalin or sodium sulphite. NDF, ADF and ADL values are expressed without residual ash. The determination of the concentration of sugar and starch followed standard procedures according to the LUFF-SCHOORL-method and the polarimetric EWERS-method, respectively (Naumann and Bassler, 1976). Carbon (C) and nitrogen (N) concentrations were analysed using a C/N–TCD analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) where 200 mg of sample material is combusted at 800°C and the concentration of gaseous C and N in the helium carrier gas is measured. Faecal samples used for the determination of C and N were morning samples of frozen faeces collected on days 2, 4 and 6. Samples were crushed, dried to constant weight at 60°C in a fan-assisted oven and ground to 1 mm particle size prior to analysis.

2.3.5. Statistical analysis

To account for seasonal differences between the two trials the control diet was included into both trials. A pre-test showed that the data obtained from the control diets were different between the two trials, so that these were analysed individually by analysis of variance (ANOVA). Apart from the control for experimental period, the data of the control diet were not of interest for in-depth analysis and discussion in the framework of this study.

The ANOVA was performed using the GLM procedure of SAS® 9.2 (SAS Institute Inc., Cary, NC, USA). Dependent variables (y) were analyzed for the influence of experimental diets (D: control and two test diets) and the effect of squares which were defined as blocks. Each square (block) consisted of a 3x3 Latin square design; since twelve buffaloes were used, four squares were defined; animals were grouped by weight and groups of 3 animals were assigned to each square. The following model was used:

$y_{ij(k)m} = \mu + \mathrm{SQ}_m + \mathrm{per(SQ)}_{im} + \mathrm{buf(SQ)}_{jm} + \mathrm{D}_{(k)} + \varepsilon_{ij(k)m}$ ($i,j,k = 1, \ldots, r$; $m=1, \ldots, b$),

where $y_{ij(k)m}$ = observation $ij(k)m$, μ = overall mean, SQ_m = effect of square m, $\mathrm{per(SQ)}_{im}$ = effect of period i within square m, $\mathrm{buf(SQ)}_{jm}$ = effect of buffalo j within square m; $\mathrm{D}(k)$ = effect of diet k; $\varepsilon_{ij(k)m}$ = random error, r = number of diets, periods and buffaloes within each square, b = the number of squares.

Means with significant *F*-values were tested by the Tukey-Test. The level of significance was chosen to be $P < 0.05$. For simple linear regression analysis the REG procedure of SAS® 9.2 was used.

2.4. Results

2.4.1. Feed intake and faecal output

Mean values of live weight (LW, kg) of the buffaloes were not different between the experimental treatments HH and LL in trial 1 and between HL and LH in trial 2 (Table 4). The mean LW over the experimental treatments and all periods was about 83 kg higher in trial 2 than in trial 1, resulting in a higher mean metabolic weight (MW) of 112.7 $kg^{0.75}$ in the second compared to 99.5 $kg^{0.75}$ in the first trial. The organic matter intake (OMI) and the organic matter faecal output (OMFO) were different between the experimental treatments in both trials. In trial 1, OMI was 68.1 and 60.4 $g/kg^{0.75}$ in treatments HH and LL, but only 52.5 and 50.8 $g/kg^{0.75}$ in treatments HL and LH in trial 2, displaying an approximately 20% lower OMI in trial 2. The OMFO in treatments HH and LL was 16.5 and 14.2% of the OMI. In trial 2, OMFO was 15.9 and 11.6% of the OMI in experimental treatments HL and LH, respectively.

Table 4. Mean live weight (LW), mean daily organic matter (OM) intake and mean daily OM faecal output obtained from four experimental diets (HH, LL, HL, LH) and one control diet (CD) fed to water buffalo in two feeding trials conducted in Oman.

Diet	LW (kg)		OM intake (g/kg$^{0.75}$/d)		Faecal output (g OM/kg$^{0.75}$/d)	
	Mean	SD	Mean	SD	Mean	SD
Trial 1						
HH	463.8	40.74	68.1	5.22	11.3	2.50
LL	459.8	44.75	60.4	2.63	8.6	1.36
CD	466.0	41.53	67.4	2.89	10.7	2.40
SE	*1.72*		*0.85*		*0.47*	
			P-values			
sqa	<0.001		0.022		0.210	
per(sqa)	0.005		0.370		0.012	
buf(sqa)	<0.001		0.110		0.503	
trt	0.064		<0.001		0.003	
Trial 2						
HL	542.5	47.58	52.5	0.89	8.3	1.67
LH	547.3	53.18	50.8	1.23	5.9	0.84
CD	554.0	50.58	52.8	1.05	7.2	1.05
SE	*2.97*		*0.28*		0.30	
			P-values			
sqa	<0.001		0.493		0.031	
per(sqa)	0.001		0.514		0.379	
buf(sqa)	<0.001		0.109		0.293	
trt	0.050		<0.001		<0.001	

CD: control diet, HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC; SD: standard deviation; sqa: square; per: period; buf: buffalo; trt: treatment.

2.4.2. Faeces chemical composition

Nitrogen (N) concentration in faecal organic matter of treatments HH (2.4%) and LL (4.0%) in trial 1 differed significantly (Table 5), while this was not the case for the N concentration of the intermediate treatments HL (3.1%) and LH (3.0%) in trial 2. On the other hand, the carbon (C) concentration was not different between treatments HH and LL (29.4 and 27.8%) in trial 1, but between the experimental treatments HL and LH (32.3 and 30.7%) in trial 2. No differences between treatments were observed for the phosphorus (P) concentration in both trials. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were highest in treatment HH (76.9 and 53.9%) and lowest in treatment LL (63.5 and 39.9%), while the treatments HL and LH of trial 2 showed intermediate concentrations. The acid detergent lignin (ADL) concentration was highest in treatment LH (23.5%), followed by HH (19.1%). Lower levels were determined analogically for treatments LL and HL. Significant differences were observed between HH and LL in trial 1, as well as between HL and LH in trial 2, for NDF, ADF and ADL concentrations. The concentration of starch in faeces also differed between the experimental treatments in both trials, being as low as zero in treatment LH and as high as 10.8% in treatment HL, with intermediate concentrations in treatments HH and LL in trial 1.The sugar concentration in faeces was similar in treatments HH and LL in trial 1 and it was about 0.4%, while it differed significantly between treatments HL (1.2%) and LH (0.2%). The faecal C/N ratio discerned between the experimental treatments in trial 1, but not in trial 2. The treatments HH and LL showed a C/N ratio of 12.2 and 7.0, while treatments HL and LH had a similar C/N ratio of 11.5 and 10.6, respectively.

Table 5. Chemical composition of faeces (values in g/kg OM) obtained from four experimental diets (HH, LL, HL, LH) and one control diet (CD) fed to water buffalo in two feeding trials conducted in Oman.

Diet	N	C	P	NDF	ADF	ADL	Starch	Sugar	C/N ratio
Trial 1									
HH	24.1	294.0	10.3	768.9	538.6	191.1	3.4	3.5	12.2
LL	39.8	278.3	11.4	635.7	398.7	156.0	29.8	4.6	7.0
CD	28.0	307.1	9.0	717.1	513.3	198.4	6.3	2.5	11.0
SE	*0.53*	*8.48*	*0.97*	*22.59*	*15.94*	7.43	*2.36*	*0.74*	*0.30*
P-values									
Sqa	0.890	0.627	0.856	0.315	0.570	0.903	0.534	0.271	0.523
per(sqa)	0.078	0.246	0.492	0.060	0.117	0.042	0.402	0.046	0.096
buf(sqa)	0.103	0.794	0.082	0.834	0.856	0.349	0.644	0.533	0.635
Trt	<0.001	0.089	0.237	0.003	<0.001	0.003	<0.001	0.162	<0.001
Trial 2									
HL	31.4	323.0	4.9	651.6	375.0	160.4	107.9	12.0	11.5
LH	29.7	307.2	5.2	721.6	526.5	235.2	0.0	2.0	10.6
CD	27.0	303.5	4.2	740.8	556.2	219.1	2.0	3.1	11.4
SE	*2.47*	*3.05*	*0.93*	*13.90*	*15.28*	8.74	*11.35*	*0.75*	*0.42*
P-values									
Sqa	0.265	0.0003	0.735	0.157	0.430	0.136	0.720	0.352	0.192
per(sqa)	0.255	<0.001	0.657	0.552	0.370	0.398	0.376	0.142	<0.001
buf(sqa)	0.379	0.006	0.739	0.099	0.252	0.584	0.297	0.723	0.202
Trt	0.467	0.001	0.714	0.001	<0.001	<0.001	<0.001	<0.001	0.273

CD: control diet, HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC;
sqa: square; per: period; buf: buffalo; trt: treatment; N: nitrogen; C: carbon; P: phosphorus; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

2.4.3. Intake, faecal excretion and digestibility of nutrients

The daily mean nutrient intake and daily mean nutrient faecal output were related to metabolic weight ($LW^{0.75}$) to balance for differences in LW between the two trials. The results of nutrient intake of trial 1 and trial 2 are presented in Table 6, the results of nutrient faecal output are shown in Table 7. The N intake (2.2 $g/kg^{0.75}/d$) as well as the faecal N output (0.34 $g/kg^{0.75}/d$) was highest in treatment LL. For the other treatments this relationship was not apparent. In both trials, the experimental treatments were different in N intake, but for the faecal N output, differences were only observed between treatments HH and LL. For the treatments HH and HL, which both were high in C/N ratio, a lower N digestibility was observed in comparison to treatments LL and LH (Table 8). With respect to C, the treatment with the highest intake (HH) showed the highest faecal C output, but also in this case this relationship did not manifest in the other treatments. Nevertheless, in both trials the experimental treatments were different from each other, both for C intake and faecal C output. Concerning phosphorus, no differences between treatments were observed in both trials, neither for intake nor for faecal output. The intake and faecal output of NDF was highest in treatment HH (48 and 8.8 g/kg$^{0.75}$/d), while the treatment LL with the lowest NDF intake showed the second highest NDF output (22.5 and 5.4 g/kg$^{0.75}$/d). The intermediate diets HL and LH had a lower faecal NDF output, even though NDF intake was higher than in LL. The same observation was made with respect to the ADF and ADL intake and faecal output. In both trials, NDF and ADF intake as well as faecal output differed significantly between the experimental treatments, whereas differences in ADL intake were significantly different only in trial 1, while no differences between treatments were determined for faecal ADL output. The NDF digestibility was lower in the treatments LL (78%) and HL (77%) in comparison to HH (82%) and LH (85%), probably due to the low NDF/SC ratio. The starch intake and faecal output were highest for the HL treatment (10.8 and 0.95 g/kg$^{0.75}$/d), followed by the LL treatment (7.3 and 0.26 g/kg$^{0.75}$/d). A correlation between quantitative starch intake and excretion was not established for HH (0.84 and 0.04 g/kg$^{0.75}$/d) and LH (2.97 and 0.0 g/kg$^{0.75}$/d). Only very small concentrations of sugar were found in faecal output; differences in sugar intake between the experimental treatments were significant in both trials, while the differences in faecal output of sugar were only statistically different for treatments HL and LH in trial 2. The C/N ratio of the ingesta differed between the treatments in both trials, but for the faecal output only in trial 1. Treatment HH had a C/N ratio in intake of 27.2 and of 12.2 in faecal output, equivalent to a decrease in the C/N ratio of 55% from feed to faeces. A similar decrease was obtained in treatment HL from 23.3 to 11.5 (51%). In treatments LH and LL the decrease was less distinct, namely from 14.1 to 10.6 (25%) and from 11.5 to 7.0 (38%), respectively. The NDF/SC ratio in relation to feed intake was different between experimental treatments in both trials, but differences could not be determined for the faecal output because for 6 and 11 animals receiving diets HH and LL (and 15 receiving CD), faecal NDF/SC ratios were >100 due to very low (<0.5%) concentrations of SC in faecal dry matter.

Table 6.
Mean nutrient intake (g/kg $LW^{0.75}$/d) of buffaloes offered four experimental diets (HH, LL, HL, LH) and one control diet (CD) in two feeding trials conducted in Oman.

Diet	N	C	P	NDF	ADF	ADL	Starch	Sugar	C/N ratio	NDF/SC ratio
Trial 1										
HH	1.05	28.60	0.19	48.00	25.09	2.09	0.84	2.17	27.15	15.95
LL	2.19	24.40	0.26	22.54	11.83	1.12	7.29	3.33	11.15	2.20
CD	0.95	27.19	0.24	36.55	19.53	1.86	4.55	6.50	28.77	3.30
SE	*0.03*	*0.35*	*<0.01*	*0.53*	*0.28*	*0.16*	*0.09*	*0.05*	*0.05*	*0.06*
					P-values					
sqa	0.076	0.024	0.023	0.069	0.065	0.964	0.087	0.008	0.498	0.867
per(sqa)	0.637	0.369	0.449	0.285	0.282	1.000	0.439	0.260	0.708	0.753
buf(sqa)	0.536	0.109	0.241	0.076	0.073	0.999	0.549	0.114	0.685	0.741
trt	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001
Trial 2										
HL	0.93	21.73	0.13	25.72	13.29	1.24	10.82	1.59	23.26	2.08
LH	1.47	20.77	0.19	28.20	15.71	1.52	2.97	1.12	14.11	6.92
CD	0.74	21.28	0.19	28.60	15.28	1.46	3.57	5.17	28.76	3.27
SE	*0.01*	*0.11*	*<0.01*	*0.12*	*0.06*	*0.08*	*0.10*	*0.02*	*0.08*	*0.06*
					P-values					
sqa	0.781	0.486	0.605	0.461	0.419	0.997	0.665	0.371	0.969	0.911
per(sqa)	0.353	0.518	0.422	0.648	0.648	0.848	0.500	0.550	0.394	0.369
buf(sqa)	0.170	0.110	0.114	0.288	0.240	1.000	0.394	0.425	0.370	0.309
trt	<0.001	<0.001	<0.001	<0.001	<0.001	0.065	<0.001	<0.001	<0.001	<0.001

CD: control diet, HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC;
sqa: square; per: period; buf: buffalo; trt: treatment; N: nitrogen; C: carbon; P: phosphorus; NDF: neutral detergent fibre; ADF: acid detergent fibre;
ADL: acid detergent lignin

Table 7.
Mean excretion of nutrients and cell wall constituents in faeces (g/kg $LW^{0.75}$/d) of buffaloes offered four experimental diets (HH, LL, HL, LH) and one control diet (CD) in two feeding trials conducted in Oman.

Diet	N	C	P	NDF	ADF	ADL	Starch	Sugar	C/N ratio
Trial 1									
HH	0.27	3.30	0.12	8.77	6.04	2.08	0.04	0.05	12.22
LL	0.34	2.37	0.10	5.44	3.42	1.49	0.26	0.04	7.01
CD	0.30	3.29	0.09	7.66	5.53	1.20	0.07	0.03	11.04
SE	*0.01*	*0.12*	*0.01*	*0.39*	*0.28*	*0.18*	*0.02*	*0.01*	*0.30*
					P-value				
Sqa	0.142	0.045	0.559	0.856	0.487	0.621	0.377	0.312	0.523
per(sqa)	0.001	0.001	0.031	0.003	0.010	0.500	0.210	0.030	0.100
buf(sqa)	0.216	0.086	0.273	0.271	0.558	0.982	0.760	0.316	0.635
Trt	0.002	<0.001	0.303	<0.001	<0.001	0.073	<0.001	0.367	<0.001
Trial 2									
HL	0.26	2.71	0.04	5.39	3.05	1.32	0.95	0.10	11.46
LH	0.18	1.82	0.03	4.27	3.10	1.41	0.00	0.01	10.58
CD	0.20	2.17	0.03	5.32	4.01	1.55	0.02	0.02	11.44
SE	*0.02*	*0.12*	*0.01*	*0.21*	*0.16*	*0.09*	*0.11*	*0.01*	*0.42*
					P-value				
Sqa	0.442	0.725	0.616	0.706	0.671	0.854	0.566	0.467	0.193
per(sqa)	0.320	0.561	0.627	0.374	0.634	0.664	0.459	0.452	0.001
buf(sqa)	0.504	0.074	0.952	0.033	0.122	0.184	0.369	0.556	0.203
Trt	0.062	<0.001	0.330	0.003	0.001	0.181	<0.001	<0.001	0.273

CD: control diet, HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC;
sqa: square; per: period; buf: buffalo; trt: treatment; N: nitrogen; C: carbon; P: phosphorus; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

Table 8. Digestibility (%) of chemical components of four experimental diets (HH, LL, HL, LH) and one control diet (CD) by water buffalo in two feeding trials conducted in Oman.

Diet	OM	N	C	P	NDF	ADF	Starch	Sugar
Trial 1								
HH	83.4	74.1	88.4	43.0	82.4	75.0	94.9	97.9
LL	85.8	84.4	90.3	60.8	77.7	69.6	96.5	98.8
CD	84.2	68.3	87.9	61.4	80.4	73.9	98.5	99.6
SE	*0.50*	*1.00*	*0.45*	*2.65*	*1.05*	*1.56*	*0.92*	*0.40*
				P-values				
Sqa	0.072	0.079	0.037	0.125	0.440	0.667	0.925	0.422
per(sqa)	<0.001	0.001	0.003	<0.001	0.021	0.025	0.088	0.086
buf(sqa)	0.059	0.120	0.057	0.003	0.541	0.643	0.275	0.307
Trt	0.012	<0.001	0.006	<0.001	0.021	0.065	0.048	0.028
Trial 2								
HL	84.1	72.2	87.5	69.6	79.1	77.0	91.3	93.7
LH	88.3	88.1	91.2	84.9	84.9	80.2	100.0	98.9
CD	86.4	73.7	89.8	84.3	81.4	73.8	99.5	99.6
SE	*0.61*	*2.66*	*0.51*	*3.51*	*0.75*	*1.03*	*1.00*	*0.50*
				P-values				
Sqa	0.980	0.471	0.687	0.603	0.752	0.668	0.662	0.409
per(sqa)	0.683	0.380	0.510	0.532	0.365	0.632	0.618	0.440
buf(sqa)	0.067	0.619	0.061	0.868	0.036	0.101	0.339	0.409
Trt	<0.001	0.001	<0.001	0.012	<0.001	0.002	<0.001	<0.001

CD: control diet, HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC;
sqa: square; per: period; buf: buffalo; trt: treatment; OM: organic matter; N: nitrogen; C: carbon; P: phosphorus; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

2.4.4. Relationship between nutrient intake and faecal output

Simple regression analysis was applied to determine relationships between chemical components in intake and in faecal output (Table 9). Only regression equations with r^2-values of 0.30 and above are presented. The concentrations of N and starch in faeces were positively and moderately related to the N and starch concentrations in intake with r^2-values of 0.60 and 0.58, respectively. The NDF and ADF concentrations in faeces were related to NDF and ADL concentration in intake, and the ADF concentration in faeces was additionally influenced by the starch concentration in intake ($r^2 = 0.55$). The ADL concentration in faeces was related only to the ADL concentration in intake. The C/N ratio of faecal output was weakly correlated to the N and NDF concentration in intake, and to a lesser extend to the C/N ratio of intake.

Table 9. Significant linear regression equations with r² values >0.3 between the concentration of chemical components in intake and in faecal output (g/kg OM) of water buffalo fed four different experimental diets (n=12/diet).

Linear regression equations	r²	SE	P	n
N_FOM = 18.458+0.5132 x I_N	0.60	0.0508	<0.001	71
STA_FOM = -27.545+0.5909 x I_STA	0.58	0.0606	<0.001	72
NDF_FOM = 418.823+0.4992 x I_NDF	0.42	0.0728	<0.001	72
NDF_FOM = 454.246+8.7314 x I_ADL	0.46	1.1219	<0.001	72
ADF_FOM = 219.969+0.4954 x I_NDF	0.33	0.0838	<0.001	72
ADF_FOM = 228.376+9.6728 x I_ADL	0.46	1.2536	<0.001	72
ADF_FOM = 575.736-1.0242 x I_STA	0.55	0.1099	<0.001	72
ADL_FOM = 86.172+4.0424 x I_ADL	0.33	0.6859	<0.001	72
CN_FOM = 14.271-0.1728 x I_N	0.34	0.0287	<0.001	72
CN_FOM = 2.993+0.0143 x I_NDF	0.31	0.0026	<0.001	72
CN_FOM = 6.399+0.1904 x I_CN	0.29	0.0361	<0.001	72

X_FOM: concentration of X in faecal organic matter (OM); I_X: concentration of X in organic matter intake; N: nitrogen; STA: starch; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; CN: carbon-to-nitrogen ratio;

2.5. Discussion

At an allowance of 1.45 times maintenance ME requirements, the amount of feed offered to the treatments HL and LH (LW averaging 545 kg) were significantly lower than that of the roughage-rich diet HH (10.5 MJ ME kg^{-1} DM) offered to animals of 464 kg LW and the energy-richest diet LL (11.9 MJ ME kg^{-1} DM) offered to animals of 460 kg LW. The four experimental diets were formulated to differ from each other in C/N ratio (high, low) and NDF/SC ratio (high, low). Due to the experimental design only the diets HH (high C/N and NDF/SC ratio) and LL (low C/N and NDF/SC ratio) could be compared statistically (trial 1), as well as the diets HL (high C/N and low NDF/SC ratio) and LH (low C/N and high NDF/SC ratio) in trial 2. The control diet was not considered explicitly in the presentation of results and in the discussion as they were only used to control for the effect of trial period.

Mean values of the C/N ratios in faeces were lower than those of the respective diets in all treatments, but to different extents. While the C/N ratio was strongly reduced by 55% and 50% in diets HH and HL with high dietary C/N ratios, the reduction was less strong in the diets LL (38%) and LH (25%) with low dietary C/N ratios. Reduced C/N ratios in faeces have also been observed by Delve et al. (2001) in cattle faeces and in cattle slurries (Reijs et al., 2007). The N concentration of the diet had the strongest influence on the faecal C/N ratio

(Table 9, r^2 = 0.34), because it also determined the N concentration of faeces (Table 9, r^2 = 0.60). In all treatments, the faecal N concentration increased compared to the corresponding diets. The increase in faecal N concentration was higher in diets with low N concentrations or high C/N ratios, respectively. Diets with high C/N ratios also showed a lower apparent OM, N and C digestibility than the diets with a low C/N ratio. Apparently, an increased proportion of undigested dietary N accumulates in faeces, probably to a great extend in form of NDF-bound N, resulting in relatively higher faecal N concentrations of diets with a high C/N ratio. This is in accordance with Delve et al. (2001) who reported that ruminal digestion decreased the high C content of low quality plant material to a larger extend than the relatively lower C concentration of high quality plant material, while fibre-bound N increased in faeces, resulting in a narrower faecal C/N ratio especially with fibrous diets.

The comparable low apparent OM, N and C digestibility of the high C/N diets obviously resulted from the high fibre contents and the low N concentrations. However, with respect to the recommendations regarding the N requirements of river buffaloes as reviewed by Ranjhan (1992), the buffaloes in the present study were supplied with sufficient amounts of N. As mentioned above the main determinant of faecal N concentration was the N concentration of the diet, this has also been reported in other studies (Sørensen et al., 2003; Powell et al., 2006). Nevertheless, the possibility to increase the faecal N content in order to increase the amount of N available after manure application to the soil is limited, since increasing the N concentration of diets results only in moderate increases in faecal N, while excess N is excreted via urine (Kebreab et al., 2001; Castillo et al., 2001; Broderick, 2003).

The NDF/SC ratio being the second determinant in diet formulation, was not calculated for the faeces, because very high digestibilities (>90%) were observed for sugar and starch in the experimental diets. Only for the HL diet, sugar and starch digestibility was slightly lower, but still above 90%. This might be due to the high maize content (36%) in the HL diet. Starch resistance to rumen degradation has often been observed in maize diets, and certain amounts also escape the duodenal digestion and are excreted with faeces (Hindle et al., 2005; Svihus et al., 2005). The starch concentration in faeces was positively correlated with the starch concentration in intake (Table 9, r^2 = 0.58). This shows that the concentration of soluble carbohydrates in faeces can be influenced by choosing feed sources rich in starch resistant to fermentation.

Of interest from the point of view of manure quality is how the diverse fibre fractions, described here by NDF, ADF, ADL, are influenced by the diet composition. The fibre fractions are digested to different extents in the ruminant digestive system and will be decomposable to different extents when faeces are applied as fertilizer to the soil. In the present study, all fibre fractions showed an accumulation in faeces. Since ADL is almost indigestible for ruminants (Van Soest, 1994), a very strong increase in its concentration from the diet to the faeces was observed. The concentration of ADL in faeces was correlated significantly only with its intake (Table 9, $r^2 = 0.33$). The picture is not that clear for NDF and ADF, as different cell wall constituents of different ruminal degradation patterns are summarised under these terms. The NDF digestibility was significantly lower in diet LL (78%) compared to HH (82%) and was lower in diet HL (79%) compared to LH (85%). This demonstrates that NDF in diets with a low NDF/SC ratio or a high concentration of SC in relation to NDF concentration respectively, is less digestible. This has also been observed in several *in vivo* and *in vitro* studies (Kennedy and Bunting, 1992; Olsen et al., 1999; Sveinbjörnsson et al., 2006) and was attributed to a decreasing pH in the rumen with increasing SC content of the diet, which negatively affects the activity of cellulolytic bacteria. On the other hand, rumen microorganisms prefer to use starch first, so that the lag time for fibre degradation increases and less fibre is degraded during the period the digesta remains in the reticulo-rumen (Kennedy and Bunting, 1992), whereby especially the digestibility of the hemicellulose is sensitive to starch-associated changes in the ruminal environment. This is confirmed by the results of the present study: the digestibility of hemicellulose, calculated as the difference between NDF and ADF, was significantly lower (P=0.012) in LL (81%) compared to HH (88%) and in tendency also in HL (84%) compared to LH (89%) (P=0.075; data not shown). The faecal ADF concentration was also influenced by the NDF/SC ratio of the diets, with higher ADF digestibility in diets with a high NDF/SC ratio. This indicates that high amounts of soluble carbohydrates also influenced the digestibility of cellulose. This is confirmed by the moderately negative correlation between faecal ADF concentration and starch intake (Table 9, $r^2 = 0.55$), indicating the influence of starch intake on ADF digestibility, which was also observed by Mertens and Loften (1980).

Organic fertilizers with C/N ratios below 20 to 25 have been observed to show fast mineralization of N after application to the soil (Senesi, 1989; Myers et al., 1994). All manures in the current study had C/N ratios below this threshold, and also in other studies C/N ratios of faeces were well below this point (Sørensen et al., 2003; Powell et al., 2006). Delve et al. (2001) determined an extreme C/N ratio of 86 in barley straw and a C/N ratio of 27 in the faeces of cattle fed only on this straw. Overall it has to be accepted that livestock manures are organic fertilizers with fast N mineralization rates, which can be hardly influenced by dietary manipulation. It has also to be taken into account that diets with a very high C/N ratio are physiologically not balanced, as they mainly contain fibre and only very low amounts of CP, so that energy concentration and OM digestibility may be limiting for producing ruminants.

The N concentration of faeces strongly depends on the N concentration of the diet, so that the faecal N concentration can be influenced by dietary manipulation. In temperate, intensive systems this relationship is used to reduce N emissions from livestock production systems (Børsting et al., 2003). Excess supply of N is avoided and the N efficiency is attempted to be maximized. In tropical, organic crop production the problem of excess N is probably less severe. However, it is obvious that the timing of fertilizer application is crucial so that mineralized N is supplied to the growing crop rather than lost by leaching. Therefore the dynamics of N mineralization of livestock manures need to be understood; they were also examined for the manures produced in the present study (Siegfried et al., 2010).

The form of C, which decides about its decomposition in the soil, can be influenced by dietary manipulation. Lignin is the least decomposable cell wall fraction and almost indigestible for ruminants, so that an increase in lignin concentration results in increased lignin concentrations in faeces. To achieve long-term effects with regard to the stability of soil OM, moderate lignin concentrations in manures are desirable. However, lignin is strongly negatively correlated to the OM digestibility of ruminant diets (Van Soest, 1994) and therefore possibilities to increase manure lignin concentrations are limited. It was also demonstrated that high fibre intake (NDF and ADF) resulted in high faecal output of fibre. The observed negative influence of soluble carbohydrates on NDF and ADF digestibility adds to the accumulation of fibre in faeces, but faeces derived from diets with high NDF/SC ratio still contain more fibre than diets high in SC. The effect of the SC on fibre digestion is therefore negligible with regard to manure quality. When aiming at manures rich in medium- to long-term decomposable fibre, low digestible fibre needs to be fed (Siegfried et al., 2010); this may in many cases contradict the aims of ruminant nutrition and production.

2.6. Conclusions

The results of the present study suggest that manure quality of river buffalo heifers can be considerably influenced by diet composition. Despite the reportedly high fibre digestion capacity of buffalo, digestive processes did not alleviate the manifestation of diet characteristics in faeces. This is of importance when aiming at a specific manure quality for fertilization purposes in crop cultivation. Although there was a strong correlation between ingestion and faecal excretion of nitrogen, the correlation between the diet and faecal C/N ratio was weak. To impact on manure mineralization, the dietary NDF and N seem to be the key control points, but modulating effects are achieved by the inclusion of starch into the diet. To improve the understanding of the fertilization potential of buffalo manure, further studies dealing with this resource might be indicated.

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Chapter 3

Interdependency of particulate digesta passage with different dietary ratios of NDF, nitrogen and soluble carbohydrates and their impacts on feed digestibility and quality of faecal excreta in water buffalo

Interdependency of particulate digesta passage with different dietary ratios of NDF, nitrogen and soluble carbohydrates and their impacts on feed digestibility and quality of faecal excreta in water buffalo

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3.1. Abstract

The present study determined the interdependencies of digesta passage through the gastrointestinal tract of water buffalo on feed digestibility and quality of faecal excreta as affected by the dietary ratios of carbon (C) to nitrogen (N) and of neutral detergent fibre (NDF) to soluble carbohydrates (SC). One control and four experimental diets were offered to four Nili Ravi heifers each in an adaptation period of 21 days followed by an experimental phase of 7 days. Diets contained varying proportions of Rhodes grass hay, soybean meal, wheat bran and maize, to achieve a (1) high C/N and high NDF/SC ratio (HH), (2) low C/N and low NDF/SC ratio (LL); (3) high C/N and low NDF/SC ratio (HL) and (4) low C/N and high NDF/SC (LH) ratio. At the beginning of the experimental phase each animal received an oral pulse dose of fibre particles marked with Ytterbium (Yb). Particle passage was modelled based on quantitative Yb excretion using a one-compartment age-dependent Gamma-2 model.

Dietary N concentration was positively affecting faecal N concentration (r = 0.82; $P \leq 0.001$) but was negatively correlated with faecal NDF concentration (r = -0.50; $P \leq 0.05$) and faecal NDF/N and C/N ratios (r ≥ 0.83; $P < 0.001$). Particle passage through the mixing compartment (λ) was lower ($P \leq 0.05$) for HL (0.033 h^{-1}) than for LL treatment (0.043 h^{-1}), while values of 0.034 h^{-1} and 0.038 h^{-1} were obtained for LH and HH treatments. At 55.4 h, total tract mean retention time was lower ($P \leq 0.05$) in LL than in all other treatments. This was probably due to the high N concentration in diet LL that was negatively correlated with time of first marker appearance in faeces (r = -0.84, $P \leq 0.001$), while dietary C concentration was negatively correlated with λ (r = -0.57, $P \leq 0.05$).

Although λ and the derived passage parameters had a significant influence on the C concentration in faeces, they did not affect faecal NDF and ADF concentrations and showed no correlation to any digestibility coefficient of the proximate diet constituents. Despite this, diet quality clearly affected the quality of faeces. Within the limits determined by the animals' requirements for energy and nutrients, diet formulation should take into account the abiotic and biotic manure turnover processes in the soil and the nutrient requirements of the crops to which the manure is applied, so as to increase nutrient use efficiency along the continuum of the feed, the animal, the soil and the crop in farming systems.

Keywords: C/N ratio, fibre, G2 one-compartment model, Oman, rumen mean retention time, soluble carbohydrates, ytterbium

3.2. Introduction

The global stock of water buffalo (*Bubalus bubalis* L.) increased from 148 million head in 1990 to 181 million head in 2008. While global production of buffalo meat rose only gradually, from 2.3 million tons per annum in 1990 to 3.4 million tons in 2008, annual production of buffalo milk increased from 44 million tons to 89 million tons during the same period (FAO 2010). Even though this is again mainly due to increased buffalo milk production on the Indian subcontinent, the development in Italy (+412%) as well as in Egypt (+111%) and Syria (189%) point to the growing demand for mozzarella cheese on the European (Puppo et al., 2002) and Near and Middle East markets.

Beside milk products, the recycling of the animals' dung to crop and forage production sites is often, and especially on organic farms, an integral part of the farming system (Steinshamn et al., 2004). In view of an efficient use of scarce resources such as phosphorus (Reddy et al., 1999), and a substantial reduction of negative environmental effects of dung application, such as greenhouse gas emissions or nitrate leaching to aquifers (Byrnes, 1990), recycling of organic matter and nutrients comprised in the dung is not sufficient. Rather, well-timed matching of nutrient release from dung with crops' requirements is needed (Fatondji et al., 2009). With regard to this, Al Asfoor et al. (2010) conducted a feeding trial that determined the effects of different dietary ratios of carbon (C) to nitrogen (N) and neutral detergent fibre (NDF) to soluble carbohydrates (SC) on buffalo manure quality; the subsequent nutrient release from this manure to field crops was analyzed by Siegfried et al. (2010).

In dairy cattle, the major factor determining slurry C/N ratio is diet digestibility (van Vliet et al., 2007), whereby faecal nitrogen concentrations increase as feed organic matter digestibility improves (Sørensen et al., 2003). At constant dietary concentrations of energy and NDF, a positive correlation also exists between crude protein intake and urine nitrogen concentration (Nennich et al., 2005; Wang et al., 2007; Van der Stelt et al., 2008). On the other hand, Castillo et al. (2000) reported a significant increase in urinary nitrogen output with increasing dietary levels of highly degradable starch, while faecal nitrogen output was not affected by different energy sources; this was attributed to an increased microbial protein synthesis with increasing availability of rumen fermentable energy. However, increased dietary concentrate levels that supply the rumen with easily fermentable substrates enhance the ruminal concentration of volatile fatty acids and lower rumen pH (Beauchemin et al., 2001). The latter effect may reduce ruminal fibre and protein degradation as well as the efficiency of microbial protein synthesis (Rotger et al., 2006): the apparent digestibility of dry matter (DM), organic matter (OM) and starch decreased linearly as the amount of starch increased in the diet of cattle (Sutton et al., 1997). In buffaloes, however, increasing the concentrate fraction in the ration from 12.5% to 50% significantly increased OM digestibility but reduced NDF digestibility (Puppo et al., 2002). Especially with feeds rich in fibre, the retention time of digesta in the rumen is considered to limit the extent of the overall digestion process (Richter and Schlecht, 2006). Particle retention and passage rate in the gastrointestinal tract are, among others, influenced by animal species, feeding level, forage to concentrate ratio, particle size and environmental temperature (Bartocci et al., 1997). Bartocci and Terramoccia (2006) found that although rumen retention and total tract retention time of particles were similar in buffaloes and sheep, the *in vivo* digestibility of cellulose and hemicelluloses was higher in the buffaloes than in the sheep.

To examine the extent to which marked differences in the C/N as well as NDF/SC ratio of diets affect the gastrointestinal retention time of fibrous particles and translate into differences in dung quality, the water buffalo was chosen as an experimental animal, due to its relative importance as a dairy animal and its avowed high fibre digestion efficiency, which might probably even out quality differences introduced with the diet.

3.3. Materials and methods

3.3.1. Site, animals, and treatments

The study consisted of two separately conducted experiments (Exp. I, Exp. II) that were carried out in a roofed animal pen on a private farm near Sohar, located 220 km North of Oman's capital Muscat. According to continuous registries (30 min intervals) of a HOBO Pro data logger (Model H08-032-08, Onset Corp., Bourn, MA, USA), daily temperatures during Exp. I (October 2007) and Exp. II (March 2009) averaged 27.8℃ and 23.6℃, while average air humidity was 38.8% and 60.4%, respectively.

Twelve Nili Ravi river buffalo heifers aged 1.75 to 2 years were randomly allocated to two treatment groups and one control group each per experiment; their live weights ranged from 285 to 535 kg (Table 3).

Two times two test diets and a control diet that was identical in the two experiments were composed of Rhodes grass hay (*Chloris gayana* Kunth.), soybean meal (*Glycine max* L.), wheat bran (*Triticum aestivum* L.), crushed maize (*Zea mays* L.), whole date palm fruit (*Phoenix dactylifera* L.), cotton seed cake (*Gossypum hirsutum* L.*)* and a commercial concentrate (Table 1). The feedstuffs were combined in such a way that the ratio of carbon (C) to nitrogen (N) and the ratio of neutral detergent fibre (NDF) to soluble carbohydrates (SC; sugars plus starch) was either high (H) or low (L). This resulted in the four test diets (Table 2) HH and LL (Exp. I), HL and LH (Exp. II). These were not supposed to represent practical buffalo heifer diets but intended to represent a high variation in C/N and NDF/SC ratios. The control diet (CD) corresponded to the normal ration of buffalo heifers on the farm and only served to control the effects of experimental period on the dependent variables.

Based on the tabulated concentration of metabolizable energy of their constituents, all diets were offered at 1.45 times maintenance energy requirements of the individual animal; water and a mineral plus vitamin lick were offered *ad libitum* throughout the day. Animals were fed two times per day, at 7:00 a.m. and 2:30 p.m. At each meal, the concentrate mixture was offered initially and after one hour eventual refusals were collected and weighed. Subsequently the hay was offered with eventual refusals collected at 2 p.m. and at 7 a.m. To minimize feed loses, the hay was offered in three subsequent portions per meal.

Of the 28 days per experiment, 21 days served the animals' adaptation to the diet followed by 7 days of quantification of feed intake and faecal excretion. The animals' live weight (LW) was determined on a mechanical scale on day 1, 10 and 20 of the adaptation period, and on the last day of the experimental week.

During the experimental period, representative samples of feed offered and refused were collected daily, weighed (electronic balance, range 0 - 30 kg, accuracy 0.01 kg), dried to constant weight at ambient temperatures, weighed again and milled to 1 mm particle size before analysis. Whenever a defaecation occurred, this was quantitatively gathered at the anus into a plastic bowl whereby care was taken that faeces did not fall to the ground. If this occurred, any adhering dust was brushed away carefully before the individual excretion was weighed (electronic balance, range 0 - 30 kg, accuracy 0.01 kg). After thorough homogenization of the excreted material, a representative sample of 200 g fresh matter was placed in a paper bag and air dried to constant weight under a shading roof. Air dry samples were milled to 1 mm particle size and stored in air-tight containers prior to chemical analyses (sections 3.3.2., 3.3.3.).

Table 1. Ingredients of one control and four test diets offered to water buffaloes in two rate-of-passage experiments (Exp.) at Sohar, Oman. Values in g DM per kg DM of overall diet.

	Exp. 1 (autumn 2007)		Exp. 2 (spring 2009)		Exp. I & II
Feedstuff	HH	LL	HL	LH	CD
Rhodes grass hay	899	401	612	681	677
Soybean meal	78	392	27	259	
Wheat bran	23				
Maize (crushed)		207	361		
Dates (whole)					148
Cotton seed cake				60	
Concentrate premix					175

DM: dry matter;

Diets: CD: control; HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC;
HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC;

Table 2. Chemical composition of one control and four test diets fed to water buffaloes in two rate-of passage experiments at Sohar, Oman, in autumn 2007 and spring 2009.

Year	2007		2009		2007, 2009
Component	HH	LL	HL	LH	CD
OM ($g\ kg^{-1}$ DM)	939.4	942.4	947.5	928.1	933.3
N ($g\ kg^{-1}$ OM)	16.4	38.8	18.6	31.8	15.0
C ($g\ kg^{-1}$ OM)	447.0	428.1	437.5	440.5	432.2
NDF ($g\ kg^{-1}$ OM)	705.4	366.8	501.2	550.7	541.9
ADF ($g\ kg^{-1}$ OM)	368.8	192.4	259.3	307.3	289.7
Starch ($g\ kg^{-1}$ OM)	12.2	122.6	197.2	60.0	66.9
Sugar ($g\ kg^{-1}$ OM)	43.9	178.2	30.2	21.9	98.7
C/N ratio ($g\ g^{-1}$)	27.2	11.0	23.5	13.9	28.8
NDF/SC ratio ($g\ g^{-1}$)	12.6	1.2	2.2	6.7	3.3

ADF: acid detergent fibre; C: carbon; DM: dry matter; N: nitrogen; NDF: neutral detergent fibre; OM: organic matter; SC: soluble carbohydrates (sum of sugar and starch fraction);

Diets: CD: control; HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC;
HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC.

3.3.2. Marker fabrication, application and analysis

The passage of feed through the gastrointestinal tract (GIT) was determined from a pulse dose of fibre particles marked with Ytterbium (Yb). Coarsely milled particles of Rhodes grass hay that remained on a 5 mm sieve were boiled in EDTA-free neutral detergent solution for one hour and then rinsed repeatedly with tap water. After drying (70℃), particles were soaked for 24 h in 12.4 mmol/l aqueous solution of $Yb(CH_3COO)_3 . 4H_2O$ (Teeter et al., 1984; Villalobos et al., 1997) and rinsed with tap water. To discard unabsorbed Yb, the marked particles were then soaked for 6 h in 100 mmol/l solution of acetic acid, rinsed and dried

(70°C). To determine the concentration of marker in the fiber, representative subsamples (25 g) were kept of each of the two batches (Exp. I, II).

In the morning of the first experimental day, each animal was dosed with 683 mg of marked fibre (14.5 mg Yb g^{-1} OM) per kg LW. Depending on the animal's ease to consume the marked fibre it was mixed with 50 – 500 g of wheat bran meal. In case of marker loss and/or incomplete ingestion, this was quantified as far as possible. The individual starting time (t_0) for marker excretion was defined as time when the animal had completely ingested the marked fibre; in case that marker ingestion took longer than 30 minutes (12 out of 24 animals) the halftime of marker consumption was defined as t_0. Due to difficulties in manually stimulating the defecation of the buffaloes, representative faecal samples of 200 g fresh matter for Yb analysis were taken from each defecation during the first 2 days after marker application, while on days 3 to 7 one sample was kept from the first excretion in the morning (5 - 11 a.m.) and the evening (4 – 10 p.m.), respectively. The obtained samples were supposed to reflect the marker concentration at the mid-point between the previous and the present excretion. In addition to the samples kept for Yb analysis, one faecal sample was collected at noon on days 2, 4, and 6 to determine the proximate composition of faeces. To this end, 30 g of ground air dry material of each of these three samples were pooled and analysed as described in section 3.3.3.

Of all samples of marked fibre and faeces, 200 mg air-dry material was digested in closed Teflon® vessels with 3 ml of 65% (v/v) nitric acid at 190°C during 10 h according to Heinrichs et al. (1986). After digestion the residues were filtered into 50 ml flasks. The filter paper was rinsed three times with distilled water into the flasks which were afterwards filled up to 50 ml. The Yb-concentration of the solution was determined at 396.419 nm by an inductive coupled plasma atomic emission spectrometer (CIROS ccd, Spectro, Kleve, Germany); the results are an average of 5 measurements.

3.3.3. Proximate analysis of feeds and faeces

Before chemical analysis, samples were pooled as follows: for feed offered, one pooled sample was constituted per type of feed and 7 day sampling period; the same was done for feed residue samples obtained from individual animals. Following standard procedures (Naumann et al., 2004), dried and ground (1 mm) samples of feeds, refusals and the pooled samples of dried faeces (section 3.3.2.) were analyzed for concentrations of dry matter (DM) and organic matter (OM). Concentrations of ash-free neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using a modification of the method of Van Soest et al. (1991) and a semi-automated Ankom 220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) without using decalin or sodium sulphite, and overnight incineration of the residual at 550°C. The determination of the concentration of sugar and starch in feeds offered and refused followed the standard LUFF-SCHOORL-method and the polarimetric EWERS-method, respectively (Naumann and Bassler, 1976). Carbon and nitrogen concentrations were analysed with a C/N – TCD analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) where 200 mg of sample material are combusted at 800℃ and the concentration of gaseous C and N in the helium carrier gas is measured.

3.3.4. Evaluation of marker data and statistical analysis

Particle passage through the gastrointestinal tract was modelled using a one-compartment age-dependent Gamma-2 model for the mixing compartment (Susmel et al., 1996); this type of model has been shown to be well applicable to the buffalo by Amici et al. (1997). Marker and digesta movements through non-mixing segments of the GIT were assumed to be characterized by a laminar flow or time delay (TT) before the marker appeared in the faeces, using the models published by Richter and Schlecht (2006). In 21 of the 24 individual curves of faecal Yb concentration plotted against time, one large peak for the marker occurred at 8 to 20 hours after dosing. In three cases (1 x CD, 2 x LL) an immediate appearance of a low concentration of marker was observed very early (<6 hours) after marker dosing, which was either due to marker disassociation and outflow with the soluble phase or to a low stability of the bound Yb to various types of ligands in small particles as elaborated by Richter and Schlecht (2006). In these three cases the Type-D model (Richter and Schlecht, 2006) that accounts for marker disassociation was applied; for all other excretion curves the Type-N model was used.

SAS® 9.2 software (SAS Institute Inc., Cary, NC, USA) was used to run the model calculations (PROC NLIN method=dud). The calculation of TT, the 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}, $0.8392\times2/\lambda$) and particle mean retention time, both in the mixing compartment (CMRT, $2/\lambda$) and the total tract (TMRT, $2/\lambda +$ TT) was based on the cumulative amount of marker excreted (marker concentration in faeces times faecal mass) at time t_i.

To control possible time-depended (seasonal) differences between the two experiments, a pre-test compared the effects of experiment (I, II) on the dependent variables (qualitative and quantitative excretion of faeces, digestibility and rate of passage parameters) obtained for the Exp. I and Exp. II control group. This comparison showed that the effects of experimental period were significant ($P \leq 0.05$) in only 3 out of 21 dependent variables tested to this end. The variable ‘experimental period’ was therefore not accounted for in further statistical analyses. Not all of the dependent variables were normally distributed; rather than normalizing the various variables by a set of different approaches, the data of the four test diets were submitted to the Kruskal–Wallis one-way analysis of variance using the NPAR1WAY procedure in SAS; significance was declared at $P \leq 0.05$. When effects of the independent variable ‘diet’ were significant, differences between individual treatments were analysed using the Mann-Whitney U test. Spearman correlation statistics and probabilities were computed using the CORR procedure.

3.4. Results

Due to the generally very low concentration of SC in faecal DM (sugars <1.1% for all treatments, starch average 0%, 0% and 3% for HH, LH and LL, 9% for HL; Al Asfoor et al., 2010) this fraction, although accounted for in diet formulation, was not considered with respect to its qualitative and quantitative excretion via faeces.

3.4.1. Feed intake and faecal excretion

Given the experimentally pre-determined differences in the C, N and NDF concentrations of the four experimental diets, the intake (per kilogram of metabolic weight) of OM from Rhodes grass hay and the four supplement mixtures differed significantly between all diets, while total OM intake differed between the two extreme diets (HH, LL) and each of these and the intermediate diets (HL, LH) only, but was similar for the latter two (Table 3). Total intake of N ranged from 1.0 g $kg^{-0.75}$ d^{-1} (HL) to 2.4 g $kg^{-0.75}$ d^{-1} (LL) and was significantly different between all diets. Likewise, all groups differed significantly in their total intake of NDF, whereby the highest value was observed in HH (47 g $kg^{-0.75}$ d^{-1}) and the lowest in LL (22 g $kg^{-0.75}$ d^{-1}). Total intake of C and ADF did not differ significantly between animals consuming the HL and LH diets, but values were different between the two exceptional diets as well as between each exceptional diet and the intermediate ones. The deliberate differences in diet composition manifested in the C/N, NDF/N and ADF/N ratios of the actually consumed ration, with a high (>25 g g^{-1}) but nevertheless statistically different C/N ratio in the ingesta of groups HH and HL, and a low (<12.5 g g^{-1}) C/N ratio in the ingesta of groups LL and LH (Table 3). While in group HH (518.6 g kg^{-1} OM) and LL (491.9 g kg^{-1} OM) a slight (P>0.05) and in group LH (523.1 g kg^{-1} OM) a more pronounced ($P\leq$0.05) enrichment of C relative to N in the excreted faeces was observed, a significant reduction ($P\leq$0.05) of the C/N ratio was determined for group HL (16.8 g g^{-1}) (Table 4). The faecal NDF/N ratio - as compared to the ingested diet (Figure 1) - narrowed in groups HH (P>0.05) and HL ($P\leq$0.05) and widened in groups LL and LH ($P\leq$0.05), while the ADF/N ratio widened in all groups ($P\leq$0.05) except for HL (P>0.05).

The concentration of N and NDF in faeces differed significantly between the treatments HH and LL, whereas the concentrations of C and ADF were only significantly different between diet HL and LL. The ingestion of the two intermediate diets resulted in faeces of very similar quality (Table 4). The differences in the ingested amount of OM, N, C and cell wall constituents resulted in significant differences in the quantitative excretion of OM, C, NDF and ADF via faeces between diets HH and HL on the one hand and diet LH and LL on the other hand (Table 4). In contrast, the quantitative excretion of C and ADF only differed between the two exceptional diets as well as between each of the exceptional diets and the two intermediate ones. The concentrations of N, C, NDF and ADF in faeces as well as the faecal C/N, NDF/N and ADF/N ratios were all significantly related to the respective diet quality parameters (Table 6): with increasing N concentration of the ingesta, an increase in faecal N concentration and a decline in faecal concentrations of C, NDF and ADF as well as a decrease in the C/N, NDF/N and ADF/N ratios was observed. Conversely, increasing C, NDF and ADF concentrations and widening C/N, NDF/N and ADF/N ratios in the ingesta was paralleled by a significant reduction of the faecal N concentration. While faecal concentrations of C (Table 6) and ADF (not shown) were not correlated to any of the variables characterizing diet quality, faecal ratios of C/N, NDF/N and ADF/N were strongly correlated to the C, NDF and ADF concentrations and the C/N, NDF/N and ADF/N ratios of the ingesta (Figure 1).

Table 3. Quantitative intake of roughage and concentrate as well as of proximate diet constituents and qualitative characteristics of four test diets fed to water buffaloes at Sohar, Oman. Treatment means and standard error of the mean (SEM).

Parameter Treatment	HH	HL	LH	LL	SEM	*P*≤
Live weight (kg)	341^{a}	338^{ab}	370^{a}	481^{b}	18.25	0.05
Intake (g $kg^{-0.75}$ d^{-1})						
Rhodes grass OM	59.5^{a}	36.7^{b}	31.9^{c}	23.9^{d}	3.43	0.01
Supplement mixture OM	7.1^{a}	16.2^{b}	20.0^{c}	35.0^{d}	2.62	0.01
Total OM	66.6^{a}	52.9^{b}	51.9^{b}	58.9^{c}	1.54	0.01
Total N	1.3^{a}	1.0^{b}	2.1^{c}	2.4^{d}	0.15	0.01
Total C	34.2^{a}	26.0^{b}	25.7^{b}	27.5^{c}	0.89	0.01
Total SC	3.7^{a}	12.0^{b}	4.2^{c}	17.7^{d}	1.50	0.01
Total NDF	46.8^{a}	29.3^{b}	26.4^{c}	21.8^{d}	2.45	0.01
Total ADF	24.5^{a}	15.3^{b}	15.0^{b}	11.4^{c}	1.25	0.01
Ingesta C/N ratio (g g^{-1})	27.1^{a}	25.5^{b}	12.4^{c}	11.4^{d}	1.87	0.01
Ingesta NDF/SC ratio (g g^{-1})	12.5^{a}	2.4^{b}	6.2^{c}	1.2^{d}	1.14	0.01
Ingesta NDF/N ratio (g g^{-1})	37.2^{a}	28.7^{b}	12.8^{c}	9.0^{d}	2.98	0.01
Ingesta ADF/N ratio (g g^{-1})	19.4^{a}	14.9^{b}	7.2^{c}	4.7^{d}	1.52	0.01

ADF: acid detergent fibre; C: carbon; DM: dry matter; N: nitrogen; NDF: neutral detergent fibre; OM: organic matter; SC: soluble carbohydrates, namely sugars plus starch.

Diets: HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC;
HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC.

Within rows, means with different superscripts differ at the indicated probability level.

Table 4. Quantitative excretion of organic matter, nitrogen, carbon and fibre fractions and proximate composition of faeces excreted by water buffaloes offered four test diets at Sohar, Oman. Treatment means and standard error of the mean (SEM).

Parameter Treatment	HH	HL	LH	LL	SEM	$P<$
Faecal excretion (g kg$^{-0.75}$ d^{-1})						
OM	9.4^{a}	10.1^{a}	7.7^{b}	8.1^{b}	0.32	0.05
N	0.2^{a}	0.3^{b}	0.2^{c}	0.3^{bc}	0.01	0.01
C	4.9^{a}	5.3^{a}	4.0^{b}	4.0^{b}	0.18	0.05
NDF	6.3^{a}	5.9^{a}	4.4^{b}	4.8^{b}	0.23	0.05
ADF	4.4^{ab}	4.7^{a}	3.5^{b}	3.3^{ab}	0.20	0.05
Faeces quality						
N (g kg^{-1} OM)	20.1^{a}	31.6^{b}	32.0^{b}	35.8^{c}	1.53	0.01
C (g kg^{-1} OM)	518.6^{ab}	530.0^{a}	523.1^{ab}	491.9^{b}	4.48	0.05
NDF (g kg^{-1} OM)	671.1^{a}	584.5^{b}	573.0^{b}	589.9^{b}	12.37	0.05
ADF (g kg^{-1} OM)	464.7^{ab}	466.1^{a}	459.8^{ab}	413.9^{b}	11.45	0.05
C/N ratio (g g^{-1})	25.9^{a}	16.8^{b}	16.3^{b}	13.8^{c}	1.20	0.01
NDF/N ratio (g g^{-1})	33.5^{a}	18.5^{b}	17.9^{bc}	16.5^{c}	1.84	0.01
ADF/N ratio (g g^{-1})	23.2^{a}	14.8^{b}	14.3^{b}	11.6^{c}	1.23	0.01

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

Diets: HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC;
HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC.

Within rows, means with different superscripts differ at the indicated probability level

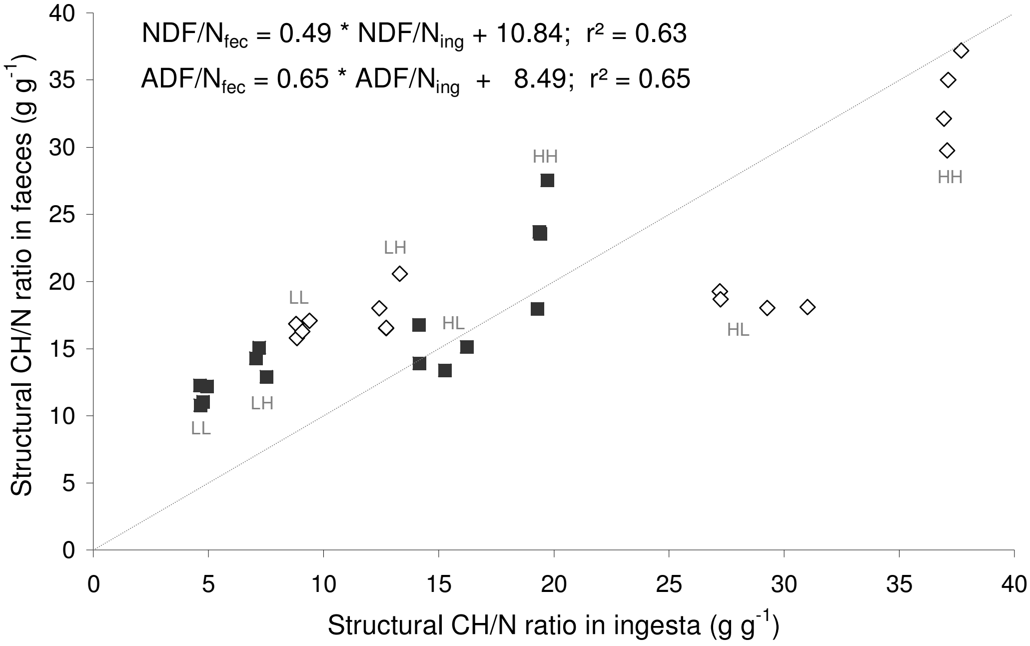

Figure 1.
Linear correlation equations and graphical depiction of the relation between ingesta ratios of NDF/N (white diamonds) and ADF/N (black squares), respectively, and faecal NDF/N and ADF/N ratios. The dotted line indicates equality in ingesta and faecal ratios of structural carbohydrates (CH; i.e., NDF and ADF) to N. The tested treatment groups (see Table 2) are indicated by the respective labels.

3.4.2. Feed digestibility and gastrointestinal passage of particles

The digestibility of N, NDF and ADF differed significantly between diets HH and LL (Table 5), whereby N digestibility was lower and fibre digestibility was higher in the HH group. Varying closely around 86%, the digestibilities of OM and C were similar for diets HH and LL. All digestibility coefficients differed significantly between diets HH and HL, but were similar for diets LL and LH. With the exception of the NDF digestibility that was only numerically but not statistically higher for diet HL, diets HL and LH differed significantly in the various digestibility coefficients. While the digestibility of OM, N and C, respectively, was not correlated to any of the proximate constituents of faeces, the digestibilities of NDF and ADF were positively correlated to faecal NDF concentration and the C/N and NDF/N ratios, while a negative correlation was observed with respect to the faecal N concentration (Table 6).

At 9 h, laminar flow (TT) of the fibre particles through the non-mixing GIT segments was lowest ($P \leq 0.05$) for diet LL, while for all other diets the first marked particles appeared at 14 h after dosing or later (Table 5). At 0.043 h^{-1}, the outflow of particles from the mixing compartment (λ) was highest for treatment LL ($P \leq 0.05$), but even with the other diets that contained more structural carbohydrates, average values of λ were >0.03 h^{-1}. The average residence time of particles in the mixing compartment (CMRT) was also lowest for diet LL (47 h) and highest for diet HL (62 h). However, since the coefficient of variance of CMRT was very high for diets HL (25.0%) and LH (19.4%), the numerical differences in the average CMRT of treatments HH, HL and LH were not statistically significant. Treatment differences in the derived parameter T_{50} (half time of particles in the mixing compartment) paralleled those of CMRT, and those of the total tract mean retention time (TMRT) were similar to the ones described for TT.

While TT was significantly affected by the quantitative intake of N (r=-0.71, $P \leq 0.001$), ADF (r=0.68, $P \leq 0.01$), NDF (r=0.74, $P \leq 0.01$) and SC (r=-0.70, $P \leq 0.01$) as well as by the dietary concentrations of N, C, SC, NDF and ADF, and the C/N, NDF/SC, NDF/N and ADF/N ratios of the ingesta (Table 6), TMRT was only influenced by the quantitative intake of N (r=-0.58, $P \leq 0.05$), sugar (r=-0.70, $P \leq 0.01$) and C (r=0.51, $P \leq 0.05$). The latter two variables also affected λ and T_{50} (intake of SC: r=0.61 for λ and r=-0.61 for T_{50}, $P \leq 0.05$; intake of C: r=0.55, $P \leq 0.05$). Reciprocally, the faecal C concentration was significantly correlated with all rates of passage parameters except for TT.

Table 5. Digestibility of organic matter, nitrogen, carbon and fibre fractions and parameters of gastrointestinal passage of feed particles in water buffaloes offered four test diets at Sohar, Oman. Treatment means and standard error of the mean (SEM).

	Digestibility (%) of feed components					Parameters of particle passage				
Treatment	OM	N	C	NDF	ADF	TT (h)	λ (h^{-1})	CMRT (h)	T_{50} (h)	TMRT (h)
HH	85.9^{a}	85.1^{a}	85.8^{a}	90.7^{a}	82.2^{ac}	18.1^{a}	0.038^{ab}	52.7^{ab}	44.2^{ab}	70.8^{a}
HL	81.0^{b}	68.9^{b}	79.5^{b}	83.9^{b}	69.1^{b}	16.2^{a}	0.033^{b}	62.4^{b}	52.4^{b}	78.7^{a}
LH	85.2^{a}	88.1^{c}	84.4^{a}	86.6^{ab}	76.4^{c}	14.0^{a}	0.034^{ab}	61.2^{ab}	51.4^{ab}	75.2^{a}
LL	86.3^{a}	88.0^{c}	85.6^{a}	84.7^{b}	70.8^{bc}	8.9^{b}	0.043^{a}	46.5^{a}	39.1^{a}	55.4^{b}
SEM	0.65	2.08	0.74	0.81	1.56	1.03	0.0016	2.85	2.39	3.10
$P\leq$	0.05	0.01	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

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

TT: time of first marker appearance in faeces; λ: rate of particle passage through the mixing compartment; CMRT: particle mean retention time in the mixing compartment; T_{50} half time of marker in the mixing compartment; TMRT particle mean retention time in the total gastrointestinal tract.

Diets: HH: high C/N, high NDF/SC; LL: low C/N, low NDF/SC; HL: high C/N, low NDF/SC; LH: low C/N, high NDF/SC.

Within columns, means with different superscripts differ at the indicated probability level.

Table 6. Spearman correlation coefficients and significance levels of the individual relationships between variables characterizing diet quality and digestibility with time of first marker appearance (TT), and between diet quality, digestibility and rate of passage parameters with quality of faecal excreta of water buffaloes offered four test diets at Sohar, Oman.

Variable Dependent / Independent	Parameter of passage TT (h)	Quality of faeces N (g kg^{-1} OM)	C (g kg^{-1} OM)	NDF (g kg^{-1} OM)	C/N ratio (g g^{-1})	NDF/N ratio (g g^{-1})	ADF/N ratio (g g^{-1})
Diet quality							
N (g kg^{-1} OM)	-0.75§	0.82§	n.s.	-0.50*	-0.86§	-0.83§	-0.82§
C (g kg^{-1} OM)	0.68†	-0.86§	n.s.	n.s.	0.84§	0.77§	0.83§
NDF (g kg^{-1} OM)	0.77§	-0.87§	n.s.	0.57*	0.90§	0.88§	0.86§
ADF (g kg^{-1} OM)	0.67†	-0.82§	n.s.	n.s.	0.82§	0.78§	0.83§
SC (g kg^{-1} OM)	-0.68†	0.85§	n.s.	n.s.	-0.82§	-0.73†	-0.85§
C/N ratio (g g^{-1})	0.77§	-0.87§	n.s.	0.57*	0.90§	0.88§	0.87§
NDF/SC ratio (g g^{-1})	0.64†	-0.82§	n.s.	n.s.	0.81§	0.74†	0.81§
NDF/N ratio (g g^{-1})	0.77§	-0.87§	n.s.	0.57*	0.90§	0.88§	0.86§
ADF/N ratio (g g^{-1})	0.77§	-0.87§	n.s.	0.57*	0.90§	0.88§	0.86§
NDF digestibility (%)	n.s.	-0.50*	n.s.	0.67§	0.50*	0.61*	n.s.
ADF digestibility (%)	n.s.	-0.53*	n.s.	0.62†	0.50*	0.59*	n.s.
Parameter of passage							
TT (h)	n.a.	-0.84§	n.s.	n.s	0.86§	0.71†	0.85§
λ (h^{-1})	n.s.	n.s.	-0.57*	n.s.	n.s.	n.s.	n.s.
CMRT (h)	n.s.	n.s.	0.57*	n.s.	n.s	n.s	n.s
TMRT (h)	n.a	n.s.	0.69†	n.s.	n.s.	n.s.	n.s.
T_{50} (h)	n.s.	n.s.	0.57*	n.s.	n.s.	n.s.	n.s.

n.a.: derived values, correlation analysis not applicable.

Significance levels: n.s. $P>0.05$; * $P\leq0.05$; † $P\leq0.01$; § $P\leq0.001$.

ADF: acid detergent fibre; C: carbon; N: nitrogen; NDF: neutral detergent fibre; OM: organic matter, SC: soluble carbohydrates, namely sugars plus starch.

TT: time of first marker appearance in faeces; λ: rate of particle passage through the mixing compartment; CMRT: particle mean retention time in the mixing compartment; T_{50}: half time of marker in the mixing compartment; TMRT: particle mean retention time in the total gastrointestinal tract.

3.5. Discussion

The present experiment focused on determining the effects of diet composition and nutrient intake on particle passage through the gastrointestinal tract, and on the composition of faecal excreta. Diet formulation aimed at establishing marked differences in the C/N and NDF/SC ratios of the four diets: while diets HH and HL on the one hand and diets LH and LL on the other hand were expected in each case to be similar in their C/N ratios, similarity in the NDF/SC ratio was intended between diets HH and LH as well as between HL and LL.

As appears from the quality of the ingesta (section 3.4.1., Table 3), these criteria were met relatively well in all four treatments. Therefore, the question underlying the present study could in principle be answered with the chosen experimental setup. However, C/N and NDF/SC ratios are rarely used as criteria for diet formulation in experiments with cattle and buffalo, and this applies even more to their multi-factorial combinations. Rather, the roughage to concentrate ratio (Bartocci et al., 1997; Terramoccia et al., 2000; Puppo et al., 2002) or the ratios of NDF to N-containing fractions such as rumen degradable (RDP) or rumen undegradable protein (UDP) are common diet evaluation criteria (Sultan et al., 2009), which constricts direct comparison of our to other findings.

3.5.1. Effects of diet quality on digestibility coefficients and quality of faeces

Due to the high proportion of crushed maize and soybean meal, respectively, in diets HL (36% maize) and LH (26% soybean meal), the metabolizable energy (ME) concentration of these diets was about 11.5 MJ and 11.0 MJ ME kg^{-1} DM. Feed was offered at 1.45 times maintenance ME requirements of individual buffalo heifers, therefore the amount was significantly lower with the two intermediate diets fed to animals of an average LW of 338 kg (HL) and 370 kg (LH) than with the roughage-rich diet HH (10.5 MJ ME kg^{-1} DM) offered to animals of 341 kg LW and the energy-richest diet LL (11.9 MJ ME kg^{-1} DM) offered to animals of 480 kg LW. Despite the resulting significant treatment differences in OM intake, the quantitative excretion of faecal organic matter was similar for groups HH and HL, as well as for groups LH and LL. These results suggest that the dietary ratio of C/N rather than of NDF/SC affected faecal excretion: the amount of C excreted via faeces was higher for the high C/N (HH, HL) than for the low C/N (LH, LL) diets, whereby differences in faecal C concentration were much less pronounced. Together with the NDF concentration in faeces, which was similar in all but in the HH group, the effect of the carbon contained in the NDF fraction of the diet on faecal NDF concentration was correspondingly high, whereas the dietary SC concentration did not affect faecal C concentration.

Increased dietary concentrations of C, NDF and ADF, as well as increased ratios of C/N, NDF/N and ADF/N enhanced the faecal concentrations of the respective fractions, while an increased diet concentration of N decreased the concentrations of NDF, C/N, NDF/N and

ADF/N in faeces. The NDF/N and ADF/N ratios, which were no criteria considered *a priori* in diet formulation, decreased linearly from HH to HL, LH and LL. Since NDF and N concentrations in the offered rations were both low for diet HL and both high for diet LH, one would expect the NDF/N ratio to be similar for these two diets. This was, however, not the case, due to the lower NDF and higher N intake of the four animals in group LH as compared to group HL. Digestibilities of NDF and ADF were highest in group HH followed by group LH, and were poorest in group HL, indicating that the digestibility of fibrous diet components improves when the nitrogen concentration of the buffalo diet increases (Jetana et al., 2009). Sultan et al. (2009) showed that at a constant diet N concentration of about 2.7 g kg^{-1} DM, a decreasing RDP to RUP ratio increased buffaloes' DM intake but decreased DM and NDF digestibility. Thus, it is the availability of rumen degradable sources of protein that foster NDF digestibility. However, rumen microbial protein synthesis seems to be higher (Puppo et al., 2002; Calabrò et al., 2004), and ammonia-nitrogen seems to be used more efficiently in buffalo than in cattle (Calabrò et al., 2004). Despite a similar quantitative excretion of N per kilogram of metabolic weight, the N concentration in faeces differed significantly between groups HH and LL, which points to a higher contribution of microbial mass to faeces from diet LL and to a slight relative enrichment effect given the better digestibility of diet LL as compared to diet HH. In contrast, faeces from the two intermediate diets were similar in their N concentrations, despite distinctly different N concentrations of the two diets and significant differences in quantitative N intake of the two groups. This indicates that the combination of a low C/N and a high NDF/SC ratio (diet LH) improved fibre digestibility, as high concentrations of structural carbohydrates were matched with high concentrations of nitrogen. On the other hand, the moderate concentration of fibre in diet HL was not matched by a sufficiently high level of nitrogen, leading to a comparatively poor digestibility of NDF and ADF. In a 120 h *in vitro* incubation, Calabrò et al. (2008) used rumen fluid of buffalo fed a 0.6 : 0.4 hay to concentrate diet containing 22 g N and 368 g NDF kg^{-1} DM to determine the digestibility of ryegrass hay; their OM digestibility of 79% compares well to the OM digestibility of diet HL. While these authors determined a strongly negative correlation between the dietary fraction of structural carbohydrates and *in vitro* OM degradability, a correlation between diet NDF or ADF concentration and *in vivo* OM digestibility could not be establish in the present study.

In addition to the effects of a suboptimal diet NDF/N ratio, the presence of starch from the maize component in diet HL might have further affected its fibre degradation (Hindle et al., 2005). Maize starch is known for its low degradability (Huntington, 1997), which is partly reflected in the low C digestibility of diet HL as compared to the other diets. According to Calabrò et al. (2004), the buffalo seems to be less adapted than cattle to fermenting substrates low in fibre and rich in starch, to which our diets HL and LL resembled. On the other hand, with concentrate-supplemented straw-based diets the buffalo's cellulose digestion was little inhibited by the presence of soluble carbohydrates, which was ascribed to the higher number of cellulolytic bacteria and an earlier onset of cellulolysis compared to cattle (Calabrò et al., 2004). With four isonitrogenous (22.4 g N kg^{-1} DM) buffalo diets of increasing forage to concentrate ratios (roughage fraction: 0.5, 0.625, 0.75, 0.875), an increase in the average numbers of total ruminal bacteria per gram of dry rumen content was observed as the concentrate fraction increased (Puppo et al., 2002). With increasing inclusion of concentrate, the *in vivo* OM and CP digestibility increased linearly from 66% to 68%, while the NDF digestibility decreased from 53% to 48%. This was primarily attributed to an increased cellulose digestibility at an almost unaltered hemicellulose digestibility with increasing contribution of roughage to the ration (Puppo et al., 2002). In contrast to that, Calabrò et al. (2004) showed that even in the buffalo higher dietary concentrations of little lignified cell wall paralleled by a higher concentration of starch yielded a more complete OM degradation and a faster fractional rate of substrate degradation than pure (and poor) roughage diets. Although the buffalo is often assumed to be a more efficient digester of fibre than cattle (Kennedy et al., 1992; Franzolin, 1994) and sheep (Bartocci and Terramoccia, 2006), the differences in diet composition could not be cancelled out by the digestion process in buffalo. As shown in companion studies, differences in manure composition originating from LL and HH diets also manifested in differences of cabbage growth and yield (Siegfried et al., 2010).

3.5.2. Parameters of particle passage

In buffaloes offered four diets of increasing forage to concentrate ratio (roughage fraction: 0.5, 0.625, 0.75, 0.875), Amici et al. (1997) predicted ruminal and total tract particle passage by Gamma-2, Gamma-3, and Gamma-4 (G2, G3, G4) age-dependent one-compartment models, a Gamma-2 age-dependent / age-independent two-compartment model (G2G1) and the multi-compartment model (MC) developed by Dhanoa et al. (1985). Although the MC model fitted best to the marker excretion curves, the parameters predicted were not significantly different from the ones of the G2 model. G2 and G3 models gave better fits than the G1G2 model. Hence, we felt confident applying the G2 model presented by Richter and Schlecht (2006) to the cumulative Yb-excretion curves obtained from the buffalo heifers. As CMRT, T_{50} and TMRT are derived from λ and TT, the discussion focuses on the latter two variables.

At values of 0.033 h^{-1} to 0.043 h^{-1}, particle passage through the mixing compartment (λ) was quite high compared to other buffalo studies. However, in buffaloes fed a 0.9:0.1 mixture of chopped Rhodes grass and alfalfa, a rumen mean retention time of particles of 31 h (equivalent to a particle passage of ~0.065 h^{-1}) was determined by the infusion of *Ruthenium phenanthroline* (McSweeney et al., 1989), a value even lower than the shortest CMRT determined in our study (46.5 h for group LL). Low retention times in buffalo were attributed to a low rumen digesta load and a large rumen pool of fine particles (Kennedy et al., 1987), generated by intense rumination activity of this species (Kennedy, 1995). Other buffalo studies, however, reported much slower rumen-reticulum particle passage: in swamp buffaloes on a 0.4:0.6 and 0.2:0.8 concentrate to pineapple-waste-silage ration, which from its fibre concentrations (334 – 373 g NDF, 201 – 225 g ADF kg^{-1} DM) was similar to diet LL, the fractional outflow of Chromium (Cr) labeled 2 mm particles from the mixing compartment as determined by the bi-exponential time-independent model of Grovum and Williams (1973) was 0.028 – 0.029 h^{-1}, while outflow from the non-mixing compartment was 0.059 h^{-1} and TMRT was 58 - 60 h (Jetana et al., 2009). While (age-independent) particle outflow from the mixing compartment did not compare well to λ determined for diet LL, the reported TMRT matched relatively well. However, the reported digestibilities of 75% to 78% for OM and N, and of 64% to 72% for NDF (Jetana et al., 2009) were all lower than the respective values determined for diet LL.

With the above-described four diets of increasing forage to concentrate ratios (Puppo et al., 2002; section 3.3.1.), Terramoccia et al. (2000) determined rumen-reticulum passage (MC model) of Cr-labeled alfalfa hay particles (2.5 mm) in buffalo of 0.0280 h^{-1} to 0.0224 h^{-1}, with the higher values pertaining to the diets with a higher proportion of roughage. From concomitant determination of *in situ* disappearance (72 - 120 h) of the alfalfa hay particles, degradation rate constants of 0.035 h^{-1} and 0.079 h^{-1} were obtained for the diet with the lowest and highest proportion of roughage. The marker-determined particle passage rate constant from the buffalo rumen was highly, though not in all cases significantly related to the *in situ* degradation rate constant of the protein and the protein-free dry matter of alfalfa hay, maize silage and concentrate feed (Terramoccia et al., 2000), whereby an increasing dietary proportion of concentrate increased rumen degradability of protein and protein-free dry matter. From their comparison of buffalo data with those simultaneously obtained from cattle and sheep, the authors concluded that the buffalo is more similar to sheep than to cattle in terms of ruminal particle breakdown and flow mechanisms.

While rumen-reticulum particle passage is always assumed to represent the flow characteristics of the whole diet, the degradation rate constant is specific to the incubated feedstuff (Terramoccia et al., 2000). A similar disparity of the two parameters was observed by Ichinohe et al. (2004) who fed three buffaloes with a 0.5 : 0.3 : 0.20 corn silage, brewer's grain and concentrate mixture characterized by NDF/N and ADF/N ratios similar to diet HL (NDF/N: 25 g g^{-1}, ADF/N: 13 g g^{-1}). *In vivo* digestibility coefficients of OM, NDF, ADF and CP averaged 0.73, 0.71, 0.64 and 0.68 and, with the exception of ADF and CP digestibility, were much lower than the values obtained for group HL. Rumen-reticulum passage rate (G1 model) of rare earth element labeled particles of undisclosed size averaged 0.024 h^{-1}, 0.029 h^{-1} and 0.031 h^{-1} for corn silage, brewer's grain and the concentrate mixture, resulting in respective rumen retention times of 50.6 h, 40.8 h and 38.7 h. At 0.037 h^{-1}, 0.041 h^{-1} and 0.050 h^{-1}, the *in situ* degradation rate constants of corn silage, brewer's grain and the concentrate mixture were considerably higher than their rumen passage rate, but were lower than the *in situ* DM degradation rates of wheat straw (0.054 h^{-1}), rice straw (0.045 h^{-1}) and oat hay (0.065 h^{-1}) determined in buffalo by Bhatia et al. (1995).

Ground to >5 mm, the size of particles we used to determine the outflow rate of particles from the rumen was at the upper end of values reported in literature. This is, however, in agreement with the observations of Bartocci et al. (2007) who also observed relatively high rumen-reticulum passage of solids and a prolonged post-ruminal flow of large-size particles in buffalo as compared to sheep and cattle. In our study, the post-ruminal particle flow (TT) was moreover strongly affected by all diet quality parameters, but not by any digestibility coefficient, including those of NDF and ADF. The independence of TT from fibre digestion supports the notion that TT truly represents the post-ruminal laminar flow of digesta (Richter and Schlecht, 2006). Despite only numerical and not statistically significant differences in TT determined for diets HH, HL and LH, there is a clear trend for a decrease in TT with an increasing N concentration of the diet, which is also reflected in the high correlation between diet N concentration and TT. On the other hand, TT prolonged as diet C/N, NDF/N and ADF/N ratios widened. This seems to be in line with the notion of Ichinohe et al. (2004) that differences in physical coarseness of the diet and rumen mat consistency are factors explaining differences in the ruminal passage rate constants between different feedstuffs in buffalo. While a high dietary concentration of sugar accelerated particle passage through the mixing compartment, dietary starch and total SC concentration were not related to this and the derived parameters in the present study; this is inconsistent with the findings of Sutton et al. (1997), Calabrò et al. (2004) and Hindle et al. (2005). However, our data shows a large variation in λ, with the coefficient of variance being 5.0% for diet LL, and 12.2%, 20.5% and 22.0% for diets HH, HL and LH, respectively. Similarly high variation of values determined for rate of passage parameters can also be found in other studies with buffalo (Kennedy et al., 1992; Bartocci et al., 1997; Terramoccia et al., 2000), which adds to the notion of sometimes contradictory results obtained from studies where not only the tested diets but also the marker substance and the modeling approach applied to determine passage parameters vary (Schlecht et al., 2007).

3.6. Conclusions

Rumen-reticulum passage rate of particles λ and the derived variables CMRT and T_{50} are expected to be related to the digestibility of feeds and feed components, with a longer CMRT (that is lower λ and lower T_{50}) leading to a more efficient digestion, especially of fibrous feedstuffs (Kennedy et al., 1992; Bartocci et al., 1997). Although λ, CMRT, T_{50} and TMRT had a significant influence on the C concentration in faeces (negative for λ, positive for the others), they did not affect faecal NDF and ADF concentrations and showed no correlation to any of the digestibility coefficients obtained for the proximate constituents of the diets. The long TT determined in the present study could at least partly be due to the longer particle size that was used in the present trial. A further source of variation of our and of reported parameters of particle passage is due to the type of the model used. For a more conclusive picture and a valid comparison of differences in such parameters between a wide variety of feedstuffs and diets it might therefore be indicated to harmonize the methodological approaches throughout experimental setup (type of marker, particle size, sampling scheme and duration) including the mathematical evaluation of rate of passage experiments. Despite such recognized limitations to conclusive data interpretation, diet composition clearly affected the composition of faeces. Qualitative differences between rations were not cancelled out by the claimed digestion efficiency of the buffalo. Within the margins determined by the animals' metabolic and (re)productive requirements for energy and nutrients, diet formulation should take into account the abiotic and biotic manure turnover processes in the soil and the nutrient requirements of the crops to which the manure is applied so as to increase nutrient use efficiency along the continuum of the feed, the animal, the soil and the crop in (organic) farming systems.

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Chapter 4

General Discussion and Conclusions

4.1. General discussion

Most of the water buffaloes worldwide are kept in resource-constrained households in Asia, where a range of primary and secondary products of livestock origin contribute to household income (Denvendra and Thomas, 2002). Given the fact that manure is a major nutrient source for such households' crop land, improving manure quality with respect to specific crop requirements without affecting the animals' productivity would improve crop land productivity and farmers' income. Additionally, excess nutrient losses to the environment could be avoided by such a strategy (Reijs et al. 2006).

4.1.1. Effects of diet on quality of faeces

Although some studies suggested that, due to the buffalo's superiority in utilizing low quality feed (Kennedy, 1995), it might be difficult to manipulate the quality of its faeces, the present results (Chapters 2, 3) clearly show that differences in diet quality translate into qualitative differences of faeces.

The four tested experimental diets were formulated to differ from each other in the C/N as well as NDF/SC ratio. While the faecal C/N ratio was strongly reduced with a high dietary C/N ratio, the reduction was less strong with a low dietary C/N ratio. Similarly reduced C/N ratios have also been observed by Delve et al. (2001) in cattle faeces and in cattle slurries (Reijs et al., 2007). The N concentration of the diet had the strongest influence on the faecal C/N ratio because it directly affected the N concentration of faeces. In all treatments, the faecal N concentration increased compared to the corresponding diets, whereby the increase in faecal N concentration was higher for diets with low N concentrations or high C/N ratios, respectively. Diets with high C/N ratios also showed a lower apparent OM, N and C digestibility than diets with a low C/N ratio. Apparently, an increased proportion of undigested dietary N accumulates in faeces probably to a great extent in form of NDF-bound N - resulting in relatively higher faecal N concentrations of diets with a high C/N ratio. This is in accordance with results of Delve et al. (2001), Sørensen et al. (2003) and Powell et al. (2006). Nevertheless, the possibility to increase the faecal N content in order to increase the amount of N available after manure application to the soil is limited, since excess dietary N is excreted via urine (Kebreab et al., 2001; Castillo et al., 2001; Broderick, 2003). In tropical (organic) crop production, the problem of excess N is probably less severe. However, it is obvious that the timing of manure application to the field crop is crucial to supply mineralized N to the growing crop rather than losing it by leaching or gaseous emission (Siegfried et al., 2010).

Another point of interest with respect to manure quality is the effect of diet composition on the diverse fibre fractions (NDF, ADF, ADL). These are digested to different extents in the ruminant digestive system and are also decomposable to different extents when manure is applied to the soil. In the present study, all fibre fractions, particularly ADL, showed an accumulation in faeces. Faecal concentration of ADL was correlated significantly with ADL intake, while the results were not that clear for NDF and ADF. To achieve long-term effects of manuring with regard to the stability of soil OM, moderate lignin concentrations in manures are desirable. However, since lignin is strongly negatively correlated to the OM digestibility of ruminant diets (Van Soest, 1994), possibilities to increase manure lignin concentrations are limited.

The NDF digestibility was significantly lower in diets LL and HL compared to HH and LH. This demonstrates that in diets with a high concentration of SC in relation to NDF, the NDF fraction is less digestible. This has also been observed in several *in vivo* and *in vitro* studies (Kennedy and Bunting, 1992; Olsen et al., 1999; Sveinbjörnsson et al., 2006) and was attributed to a decreasing pH in the rumen with increasing SC content of the diet, which negatively affects the activity of cellulolytic bacteria; hemicellulose (that is, NDF minus ADF) digestibility is particularly sensitive to starch-associated changes in the rumen environment. Additionally, rumen micro organisms prefer to use starch first, so that the lag time for fibre degradation increases and less fibre is degraded during the period the digesta remains in the reticulo-rumen (Kennedy and Bunting, 1992).

4.1.2. Effects of diet on particulate passage

As far as the effects of diet composition and nutrient intake on particle passage through the gastrointestinal tract are concerned (Chapter 3), particle passage through the rumen (λ) was quite high compared to other buffalo studies. Similarly short rumen mean retention times were determined by McSweeney et al. (1989) and Kennedy et al. (1987). Other buffalo studies, however, reported much slower rumen-reticulum particle passage (Bhatia et al., 1995; Terramoccia et al., 2000; Jetana et al., 2009). However, the present data showed a large variation in λ, with average coefficients of variance between 5 and 22%. Similarly high variation in values of rate of passage parameters were reported by Kennedy et al. (1992), Bartocci et al. (1997) and Terramoccia et al. (2000). Further reasons for disparities of results may be due to differences in particle size, marker substances and type of mathematical model used in the different studies.

The post-ruminal particle flow (TT) was strongly affected by all diet quality parameters, but not by any digestibility coefficient, including those of NDF and ADF. The independence of TT from fibre digestion supports the notion that TT truly represents the post-ruminal laminar flow of digesta (Richter and Schlecht, 2006). There was a clear trend for a decrease in TT with an increasing N concentration of the diet, which was also reflected in a high correlation between diet N concentration and TT. On the other hand, TT prolonged as diet C/N, NDF/N and ADF/N ratios widened. This is in line with the notion of Ichinohe et al. (2004) that differences in physical coarseness of the diet and rumen mat consistency are factors explaining differences in the ruminal passage rate constants between different feedstuffs in buffalo. While a high dietary concentration of sugar accelerated particle passage through the mixing compartment, dietary starch and total SC concentration were not related to λ which contradicts the findings of Sutton et al. (1997), Calabrò et al. (2004) and Hindle et al. (2005). This adds to the notion that the sometimes quite contradictory results of different studies where not only due to the tested diets but also to the methodological and mathematical approaches used to determine passage parameters (Schlecht et al., 2007).

4.1.3. Effects manure quality on crop growth

The companion study that tested the effects of manures LL and HH on nutrient availability to crops and crop growth (Siegfried et al., 2010) found that under the subtropical conditions of Sohar, Oman, stems and canopies of irrigated cauliflower receiving LL manure were larger than those of plants receiving HH manure. Likewise, yield of radish was higher with faster mineralizing LL manure. The authors concluded that, although mineralization was higher from LL manure, this potential disadvantage (high environmental losses of nutrients) was adequately compensated by an optimal timing of manure application. On the other hand, the growth of carrots was fostered by HH manure with its slower release of nutrients. To achieve a good crop yield, the choice of the manure should therefore take into account the nutrient requirements of the individual crop and its physiological characteristics (Siegfried et al., 2010). Another crucial aspect that should be considered to increase the efficiency of organic fertilizer use is application time (Fatondji et al., 2009). Under Oman's sub-tropical conditions, the N mineralization from manure applied to regularly irrigated cropland was higher than in temperate climate (Siegfried et al., 2010). This points to the necessity to elaborate specific application patterns for organic manures according to climatic conditions and irrigation practices, so as to ensure timely and adequate nutrient supply and avoid nutrient leaching. For the irrigated vegetable production under subtropical conditions, the carbon rather than the nitrogen concentration of the manure seemed to be the factor determining crop growth and nutrient turnover (Siegfried et al., 2010).

4.2. Conclusions and implications

The present results suggest that manure quality of river buffalo heifers can be considerably influenced by diet composition. Despite the reportedly high fibre digestion capacity of the buffalo, digestive processes did not alleviate the manifestation of diet characteristics in faeces. This is of importance when aiming at a specific manure quality for fertilization purposes in crop cultivation. Although there was a strong correlation between ingestion and faecal excretion of nitrogen, the correlation between the diet and faecal C/N ratio was weak.

Rumen-reticulum passage rate of particles is expected to be related to the digestibility of feeds, with a lower λ leading to a more efficient digestion, especially of fibrous feedstuffs

(Kennedy et al., 1992; Bartocci et al., 1997). Although λ had a significantly influence on the C concentration in faeces, it did not affect faecal NDF and ADF concentrations and showed no correlation to any of the digestibility coefficients obtained for the proximate constituents of the diets.

To impact on manure mineralization, the dietary NDF concentration seems to be a key point. When aiming at manures rich in medium- to long-term decomposable fibre, low digestible fibre needs to be fed (Siegfried et al., 2010). This may contradict the aims of ruminant nutrition, since diets with a very high C/N ratio are physiologically not balanced, as they mainly contain fibre and supply only very low amounts of crude protein to the animal. Where dietary energy concentration and OM digestibility become limiting for producing ruminants, feeding for manure quality must firstly consider the animals' metabolic and (re)productive requirements and only secondly try to adjust manure quality to the environmental conditions (climate, soil) and the crop's requirements.

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Affidavit

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

(Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt und keine anderen als die in der Dissertation angegebenen Hilfsmittel benutzt habe. Alle Stellen, die aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, habe ich als solche kenntlich gemacht. Kein Teil dieser Arbeit ist in einem anderen Promotionsverfahren verwendet worden.)

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