Aus dem Institut für Tierernährung und Stoffwechselphysiologie
der Christian-Albrechts-Universität zu Kiel

Effect of grazing intensity on feed intake and productivity of sheep in the Inner Mongolian steppe

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Meinem Mädchen

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List of Abbreviations

ADF	Acid detergent fiber
ADG	Average daily gain (live weight)
ADL	Acid detergent lignin
CA	Crude ash
CP	Crude protein
Cr_2O_3	Chromic oxide
DM	Dry matter
DMI	Dry matter intake
DOM	Digestibility of organic matter
DOMI	Digestible organic matter intake
GI	Grazing intensity
HM	Herbage mass
IVDOM	In vitro digestibility of organic matter
ME	Metabolizable energy
NDF	Neutral detergent fiber
OM	Organic matter
OMI	Organic matter intake
OMO	Organic matter output
SEM	Standard error of the mean
TiO_2	Titanium dioxide

1 General Introduction

1.1 Objectives and structure of the dissertation

Li and Yong (1993) described the winter cold temperate Eurasian steppe as the largest grazed grassland in the world. The Mongolian loess plateau ecoregion is a vast area belonging to the Eurasian steppe. This region consists of a plateau being 1000-1700 metres above sea level. The climate is influenced by Mongolian high pressure centres in the winter, which cause a cold and dry weather. The summer is influenced by the south east monsoon from the Pacific resulting in rainy and warm weather. The land use is dominated by grazing sheep, goats and cattle for meat production. As reported by Tong et al. (2004) the Inner Mongolian steppe has severe ecological problems mainly caused by overgrazing, which leads to a degradation of the grassland and increased wind erosion. Ni (2002) showed the high importance of winter cold grasslands to the global carbon cycle. Carbon turnover times are relatively long due to the dominance of the cold and dry winter. Thus, carbon is accumulating in the soil of these grasslands under natural conditions. Cui et al. (2004) reported that grasslands of various types cover approximately 25.4% of the total land area, but store about 39% of the terrestrial carbon inventory. According to Jia et al. (2006) the total terrestrial carbon is twice the atmospheric CO_2 pool. This underlines the high importance of the carbon stored in grasslands for the global greenhouse effect. Li et al. (1998) showed in a ten years study that in an ungrazed Leymus chinensis steppe in the Xilin River Basin (Inner Mongolia, Autonomous Region of China), in average 19.88 g/m² carbon per year were stored. However, due to overgrazing about 12.4 % of the carbon in soil had been lost over the last four decades in this region. According to Li et al. (2006) the main reason for this change of the grassland from a carbon sink to a carbon source for the atmosphere is overgrazing, which destroys the vegetation cover and thus leads to a reduced photosynthesis of the grassland. This shows that the severe regional ecological problems mainly caused by overgrazing have also a significant influence on the global greenhouse effect. Wang et al. (2004) stated in their review that the Mongolian plateau is a main source for dust storms in China. The dust is most likely from degraded grasslands. According to Zhao et al. (2005) severe overgrazing of the grassland is responsible for the degradation.

The present study was conducted as a part of the Sino-German research collaboration "**MA**tter Fluxes of **G**rasslands in **I**nner **M**ongolia as influenced by stocking rate" (MAGIM) founded by the German Research Foundation (DFG, research unit no. 536), which consists of

nine subprojects incorporating agricultural and environmental sciences. The main goals for this research group are:

1. Characterisation of the water cycle both on the plot and the regional scale;
2. Determination of C- and N-cycling processes below- and aboveground and C and N-trace gas exchange with the atmosphere;
3. Description of grassland vegetation and growth rate;
4. Investigation of the stability and mineralisation kinetics of soil organic matter;
5. Analysis of redistribution of nutrients due to wind/water erosion as well as grazing management;
6. Investigation of the effect of low doses of fertiliser application on primary productivity of *Leymus chinensis* steppe;
7. Networking of biogeochemical, hydrological and erosion models for regionalisation of site results;
8. Scenario analysis on the site and the regional scale for various grassland management systems;
9. Establishment of a common GIS database of project results with access for all project participants;
10. Determination of biomass production, quality of grasslands, feed intake and animal productivity.

The present study belongs to a subproject of the MAGIM research group carried out by the Institute of Animal Nutrition and Physiology (Christian-Albrechts-University of Kiel), which contributes mainly to objective 8 and 10. We conducted in close corporation with the subproject administered by the Institute of Crop Science (Christian-Albrechts-University of Kiel) in 2005 a grazing experiment with six different grazing intensities of sheep in the Xilin River Basin of Inner Mongolia to measure their effect on herbage mass on offer, quality of herbage offered and ingested as well as feed intake and live weight gain. In this dissertation the results of this grazing experiment are shown in Chapter 3. Chapter 2 deals with a methodical aspect to measure the feed intake of grazing animals: Titanium dioxide as an inert marker for estimation of fecal output in grazing sheep. Chapter 1 gives an overview of land use and ecological problems of Inner Mongolia and the influence of grazing intensity on herbage on offer, quality of ingested and offered herbage as well as animal performance. For

the methodical part of this work presented in Chapter 2 overviews of methods for measuring feed intake of grazing animals and the use of inert markers are given in Chapter 1 as well.

1.2 Ecological problems in the Inner Mongolian steppe

1.2.1 Ecological and agricultural characteristics of Inner Mongolia

According to Meyer (2006) grasslands cover 40% of China's total land area. They are the largest ecosystem threatened by desertification in the world. Beside the Tibet plateau ecoregion the Autonomous Region Inner Mongolia is the largest steppe region of China. Yu et al. (2004) gave a detailed summary of the ecology and agriculture of Inner Mongolia. This autonomous region in the north of China has an area of nearly 1.2 million km², of which 73% is grassland. As already described the climate of this winter cold region is dry and cold in the winter and wet and warm in the summer. Most of the rainfall occurs from May to September. Inner Mongolia has a sharp annual rainfall gradient from 100 mm in the west to 600 mm in the east (Figure 1.1).

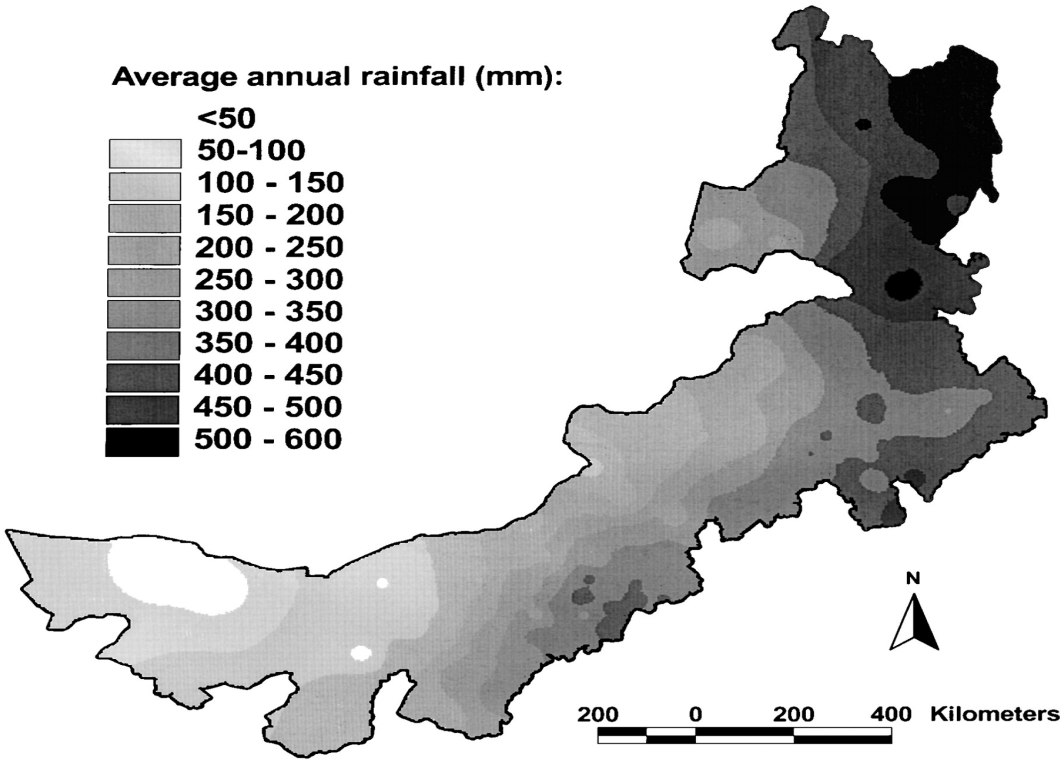

Figure 1.1. Spatially interpolated map of average annual rainfall (mm) in the Inner Mongolia Autonomous Region, 1982 – 1991 (Yu et al., 2004)

According to the rainfall gradient the vegetation of Inner Mongolia can be classified from east to west as mountain forest, meadow steppe, typical steppe, desert steppe, desert and sandy scrubland. Figure 1.2 shows the vegetation map of Inner Mongolia.

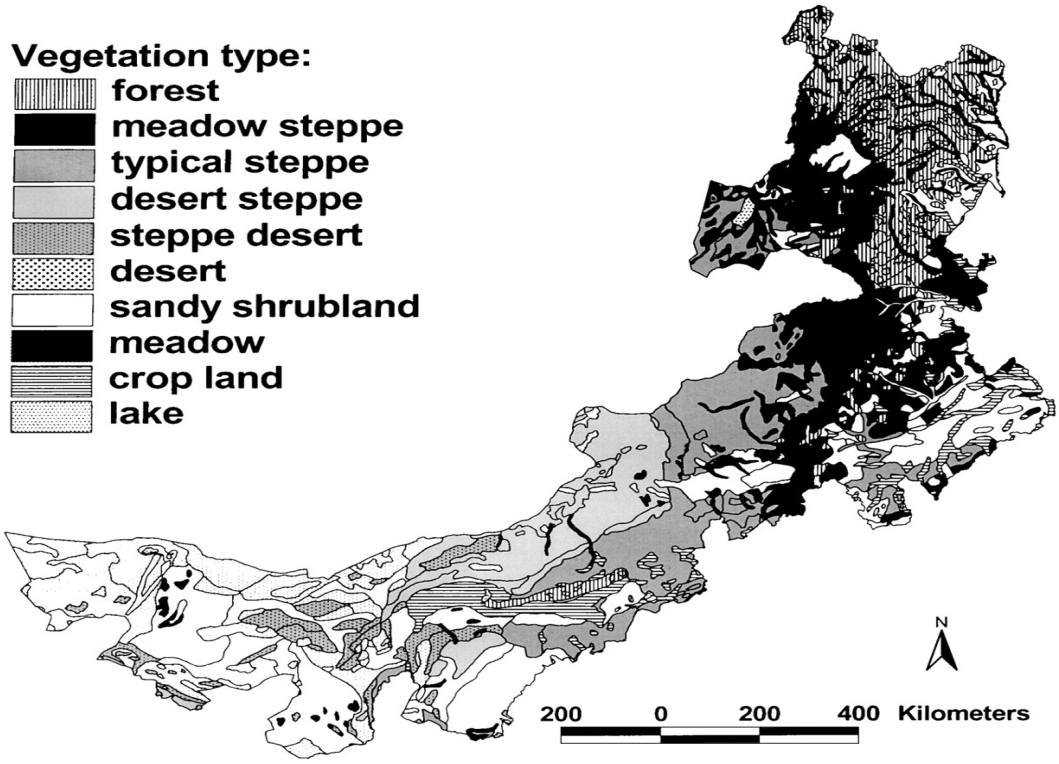

Figure 1.2. Vegetation map of the Inner Mongolia Autonomous Region (Yu et al., 2004)

The above ground net primary production is strongly related to the annual precipitation and varies from 0.1 to 4 t DM per hectare and year. This indicates that water is the limiting factor for grassland productivity in the steppe of Inner Mongolia (Figure 1.3).

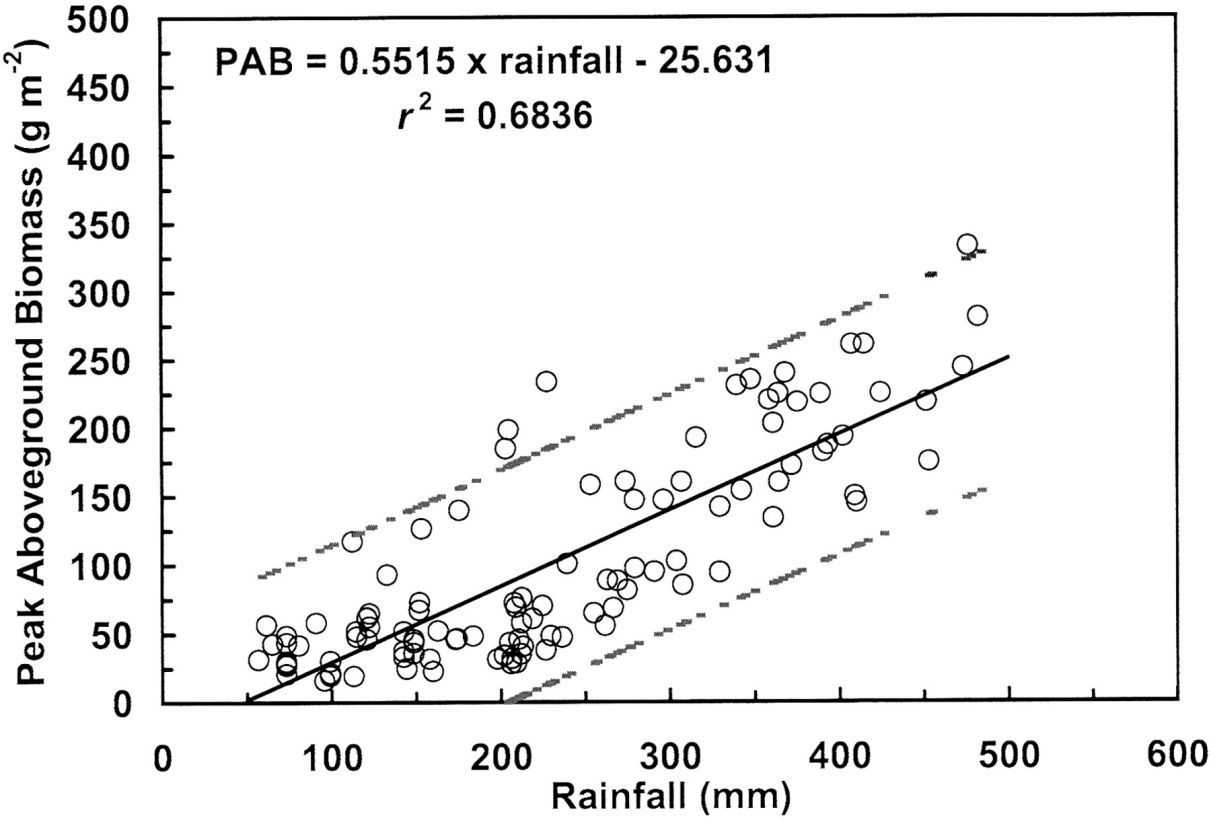

Figure 1.3. Relationship between annual rainfall (mm) and peak aboveground biomass (PAB) in the Inner Mongolia Autonomous Region (solid line = regression line, broken line = 95% confidence limits) (Yu et al., 2004)

Due to the steppe as dominant vegetation type in Inner Mongolia, grazing for meat production of sheep, goats, cattle and camels is the most important land use form in this part of China. The livestock density decreases from west to east according to the productivity of the vegetation. However, in the cold north with small human population and poor infrastructure the livestock density is low (Figure 1.4).

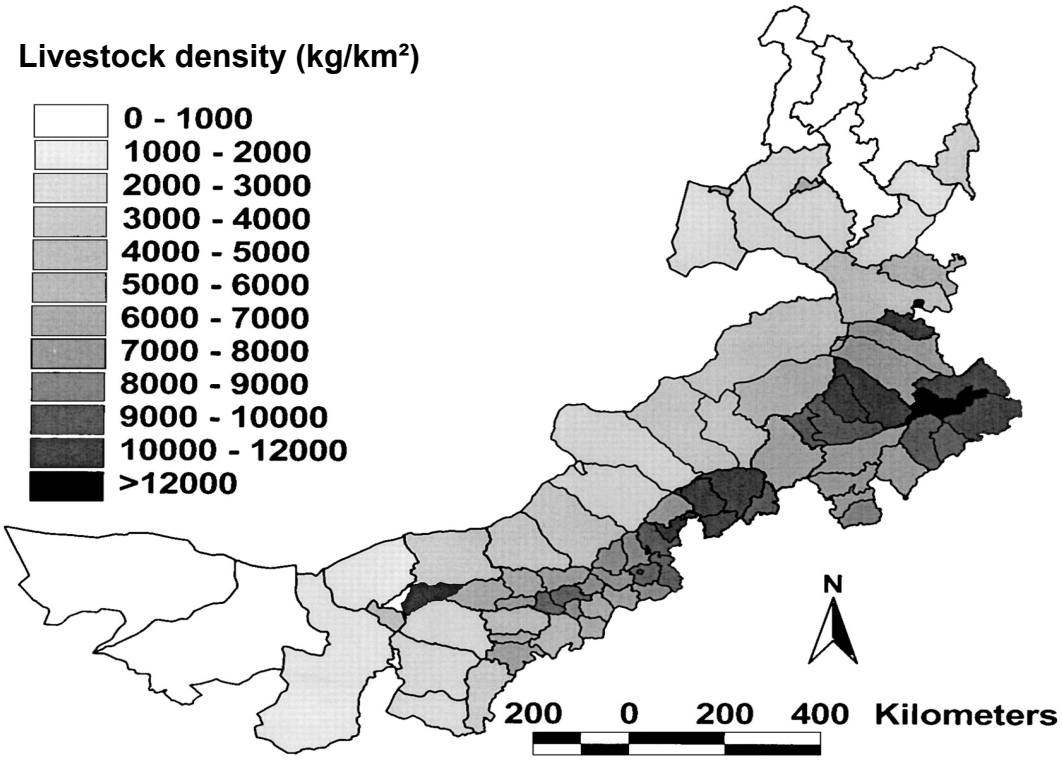

Figure 1.4: County average livestock density (kg/km², based on total area) in the Inner Mongolia Autonomous Region, 1982 – 1991 (Yu et al., 2004)

1.2.2 Specification of the ecology and agriculture in the Xilin River Basin

The MAGIM project was conducted in collaboration with the Institute of Botany, the Chinese Academy of Sciences, Beijing. This institute administers the "Inner Mongolia Grassland Ecosystem Research Station" (IMGERS). The station is located in the Xilin River Basin in the north eastern part of Inner Mongolia, which is about 600 km north of Beijing. The Xilin River basin covers an area of about 10.000 km² and is located 900 to 1500 m above sea level (Figure 1.5).

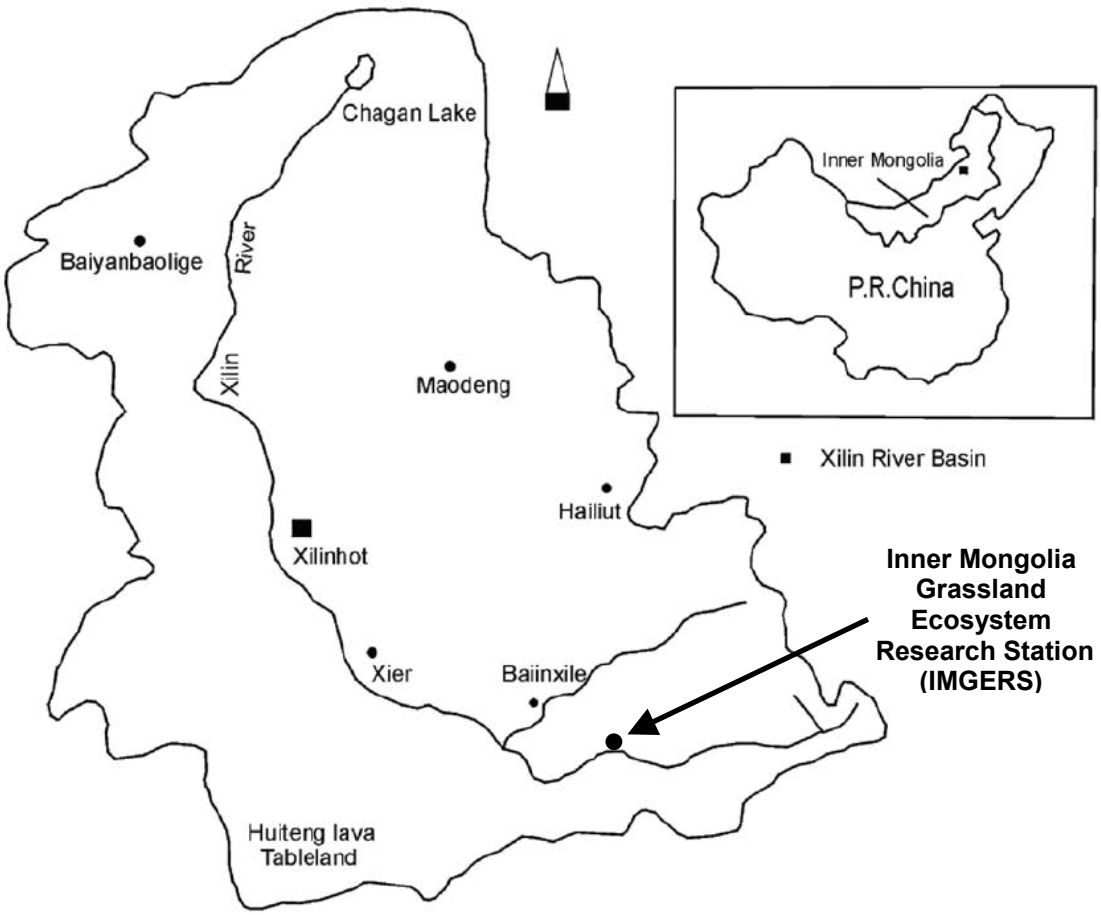

Figure 1.5. Map of the Xilin River Basin, Inner Mongolia, China (Tong et al., 2004)

The mean precipitation and temperature from 1982 to 2003, measured at a weather station near IMGERS were 343 mm and 0.7 °C, respectively (Figure 1.6). Most of the rain occurs in the period of May to September (summer wet steppe). Xiao et al. (1995) described a high variation of the annual precipitation between the years. They found a coefficient of variation of 22%.

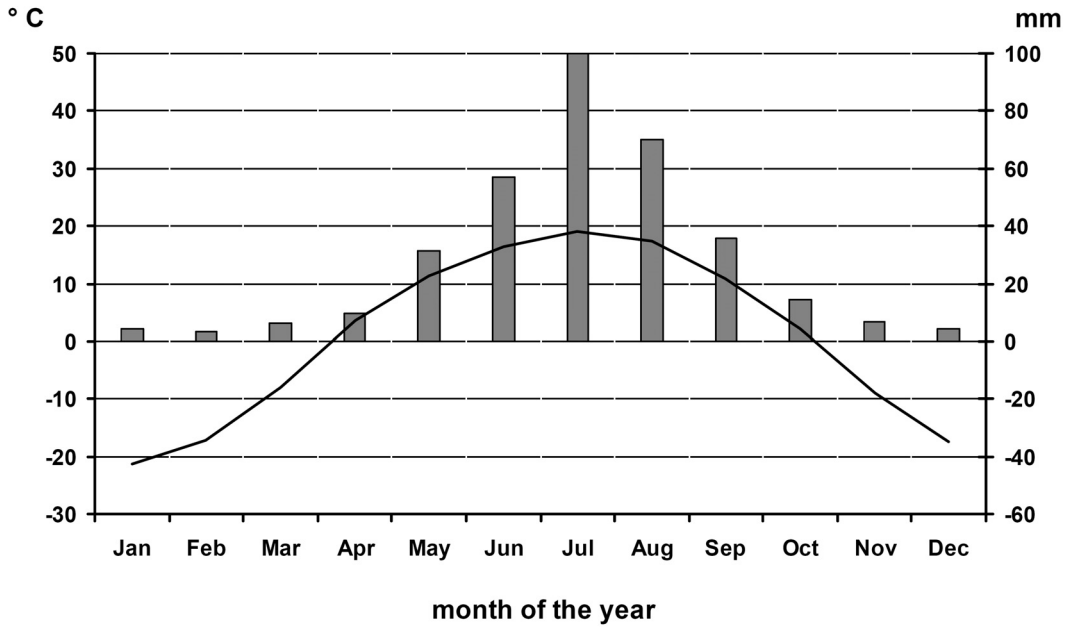

Figure 1.6. Average air temperature (°C, line) and precipitation (mm, bars) near the "Inner Mongolian Grassland Ecosystem Research Station" (IMGERS) administered by the Institute of Botany, The Chinese Academy of Sciences, Beijing (Means of 1982 to 2003)

Tong et al. (2004) reported that the vegetation of the semi arid Xilin River Basin is dominated by the perennial rhizome grass *Leymus chinensis* and the perennial bunchgrass *Stipa grandis*. The dominant plant communities of the Xilin River Basin are the Leymus chinensis steppe and the Stipa grandis steppe. The first type dominates in areas with higher water availability. The two community types represent the most widely distributed grassland communities in the Eurasia steppe. According to Bai et al. (2004) the growing season in the Xilin River basin starts at early April and ends at late September for perennial plant species, whereas annual plant species usually germinate in early July following the rains. Tong et al. (2004) showed in their study from 1980 to 1989 that the average peak above ground live biomass for a Leymus chinensis steppe and Stipa grandis steppe undisturbed by grazing was 183 and 144 g DM/m², respectively. However, caused by the high variation in annual precipitation the yield of above ground biomass varies greatly between years (Figure 1.7). Bai et al. (2004) concluded that January-July precipitation is the primary climatic factor causing fluctuations in biomass production.

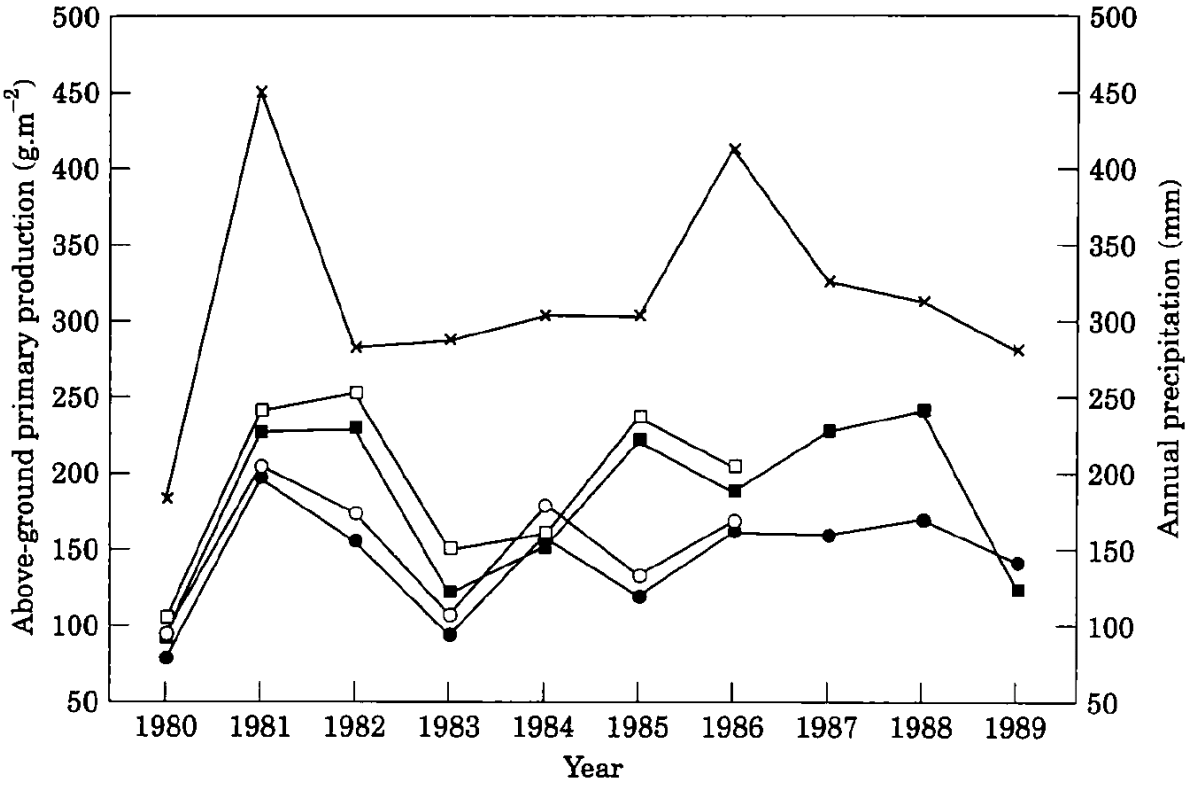

Figure 1.7. Interannual variation in peak above ground live biomass (PALB) and peak standing crop (PSC) of an ungrazed Leymus chinensis steppe and Stipa grandis steppe from 1980 to 1989 as influenced by precipitation. PALB (-■-) and PSC (-□-) = Leymus chinensis steppe; PALB (-●-) and PSC (-○-) = Stipa grandis steppe; annual precipitation (-x-), (Xiao et al., 1995)

Corresponding to large parts of the Inner Mongolian steppe the Xilin River Basins has severe ecological problems caused by overgrazing. According to Kawamura et al. (2005) livestock number of the Bainyinxile Livestock Farm, which covers approximately 33% of the central Xilin River Basin, increased from 1950 to 2001 (Figure 1.8). Horses were the most dominant animals in the farm just after its foundation in 1950. Livestock numbers increased steeply from 1959 to 1967 and then experienced two sharp declines in 1968 and 1977 through two severe storms. After 1983 ownership of the land altered from governmental to private and since then, stock numbers have been increasing. In December 2001 the total livestock number of the Bayinxile livestock farm was 252,700 sheep units. Thus, the average stocking rate was 0.76 sheep units per ha, including mowing land. Tong et al. (2004) examined the steppe degradation in the Xilin River basin. Their results showed that the total area of degraded steppe increased from 7191 km² in 1985 to 7689 km² in 1999, which means 67% and 72% of

the total basin, respectively. Further the four geological formations exhibited increasing degrees of degradation in the following order: low mountains < lava tablelands < hills < high plains.

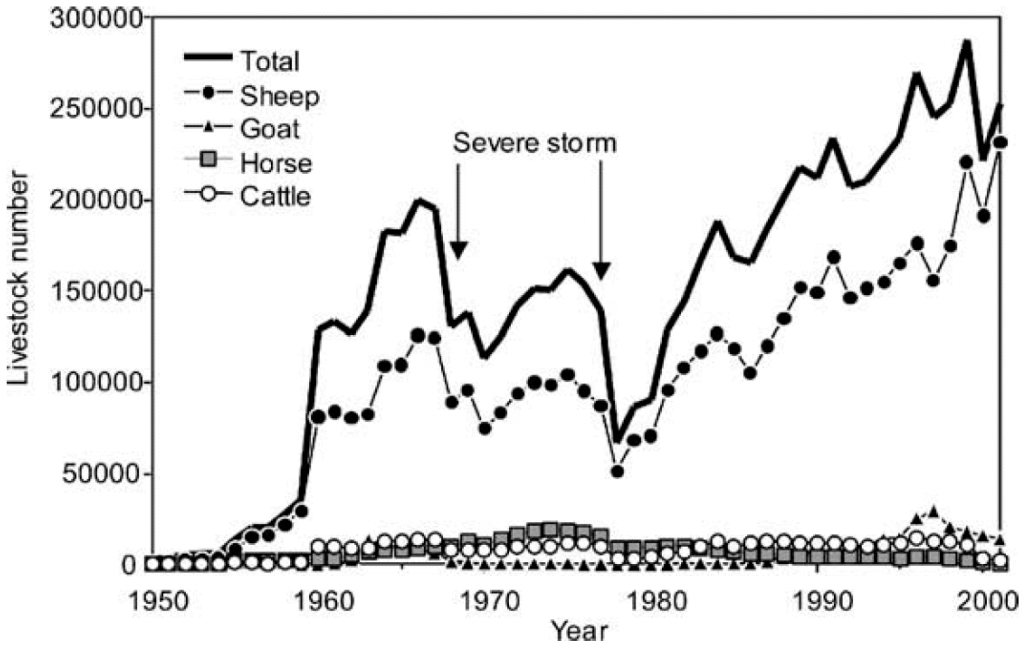

Figure 1.8. Changes in Livestock numbers during the years of 1950 to 2001 at the Baiyinxile livestock farm. Total numbers are equivalent to sheep units (SU) where one horse is 6 SU, one cattle 5 SU and one goat 0.8 SU (Kawamura et al., 2005)

1.2.3 Causes, mechanism and consequences of grassland degradation

In former times the Inner Mongolian steppe was inhabited by nomadic people, which used the grassland mainly by grazing livestock. The land use system was sustainable due to small livestock densities and moving the herds through large areas. Hay making did not play a significant role. The grazing stress of the grazed pastures were low. However, in the late 1940s the nomadism changed to settlement in Inner Mongolia with the consequence that only the areas close to the settlements were used for grazing and the areas far away for hay making. Thus, the grazing stress of the grazed areas increased, whereas no nutrient recycling occurred on the hay areas. Moreover, as already shown in the last chapter the stocking rate increased in Inner Mongolia, especially after the change of the ownership from governmental to private in the 1980s. Private ownership in China means not the same than in western

countries. The government is still the owner of the land, but the farmers are free to decide how to use the land. However, the negotiations between farmers and the local governments are only valid for a few years. This means in practice that the farmer strive for a short term maximal economic output. The long term view, which would require a sustainable use of the grassland is not the main interest of the farmers, because they are not certain to keep the land for more than a few years. Han et al. (2000) conducted a one year grazing experiment with five grazing intensities of sheep and found a decreasing live weight gain per sheep with increasing grazing intensity but an increasing live weight gain per hectare. This indicates that from a short term view high grazing intensities are able to maximize the economic gain of the grassland. However, the subsequent increase of grazing intensity up to severe overgrazing has long term negative economical and environmental effects.

The relationship between grazing intensity and wind erosion was described by Zhao et al. (2005). They showed in a sandy rangeland that heavy grazing leads to a decrease in vegetation cover and height. Furthermore the hoof impact of the animals increased by more animals per hectare and more grazing activity per sheep. This intensifies the degradation of the plant cover. Without the protecting plant cover the surface of the steppe is vulnerable to wind erosion in the cold and dry winter. The results are supported by Li et al. (2000), and Zhang et al. (2004). Su et al. (2005) further found a higher roughness of the unprotected soil by sheep trampling, which enhanced wind erosion. Li et al. (2005) showed that the decrease in vegetation cover and vegetation height decreases the roughness of the vegetation, which is able to lower wind speed and therefore wind erosion as illustrated in Figure 1.9. Zhang et al. (2004) and Zhao et al. (2005) stated that soils with a low organic carbon content are more sensible to wind erosion due to the lack of biological aggregates. The enhanced wind erosion causes desertification of the Inner Mongolian steppe and therefore an increase of dust and sand storm frequency. As reported by Wang et al. (2004) the sand and dust storms are responsible for high economic damages in large parts of China. Furthermore, health injuries occur for people, which have to stand sand and dust storms. Especially the densely populated region of Beijing is concerned.

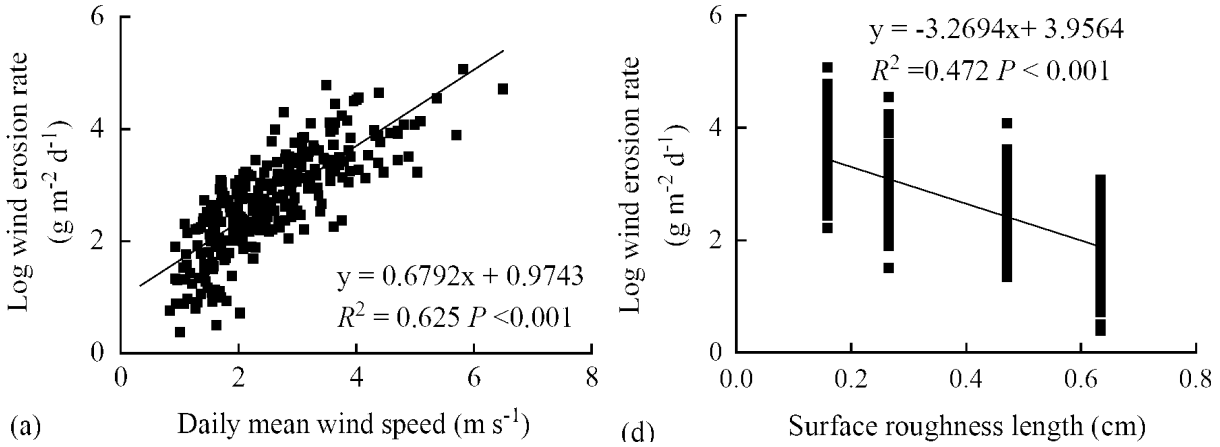

Figure 1.9 Relationships between daily wind erosion rate and either daily mean wind speed at 0.25m height; or surface roughness length (Li et al., 2005)

Overgrazing leads to a decrease in the productivity and forage value of the grassland. Zhao et al. (2007) showed that increasing grazing intensity changes the biodiversity of the plant species. High palatable plant species decrease and species of a low palatability and high resistance against grazing increase. Further some species alter their morphology to increase the resistance against grazing, which was also reported by Zhang et al. (2006). Zhang et al. (2004), Cui et al. (2005), and Zhao et al. (2005) found a decrease in standing biomass, when grazing intensity increased. Wang (2004) showed beside a decreasing herbage mass a decreased shoot and tiller density. Further the study reported that a positive correlation is existing between shoot and tiller density on the one hand and soil organic matter content and soil moisture on the other hand. The soil bulk density and soil-pH decreased with increasing shoot and tiller density. These relations show that the productivity of the grassland is not only decreasing due to degraded sward characteristics but also to changed soil characteristics. Trlica and Cook (1971) reported for desert plants in the semi arid climate of Utah, USA a decreasing carbon reserve, when defoliation is increasing. This was also observed by Wang (2004) in the inner Mongolian steppe together with decreased reproduction organs. Zhan et al. (2007) stated that grazing reduces the seed banks. Therefore for the long term sight grassland productivity decreases under severe grazing due to low reproduction and regrowth of the plant species. Livestock farming is a main source of income for the people of Inner Mongolia. The decrease in productivity of the grassland leads to severe consequences on the prosperity in this region.

As already mentioned the land use of the grassland has an influence on the carbon cycle of the soil. Su et al. (2005) reported that stronger wind erosion in overgrazed grasslands leads to losses in organic carbon and nitrogen in the soil. Cui et al. (2005) compared grazed and ungrazed areas and found no decreased organic carbon content in the soil of the grazed areas. They stated that a compensatory plant growth in the grazed areas could be responsible for this lack of losses in soil carbon. However, they assumed that severe grazing reduces the organic carbon content in the soil and the losses are difficult to determine. Li et al. (1998) showed in a ten years study in the Xilin River Basin that about 12.4% of the carbon originally stored in the soil was withdrawn due to overgrazing. Li et al. (2006) reported that the main reason of soil carbon losses is the degraded vegetation cover, which leads to weak photosynthesis of the grassland.

1.3 Animal response to varying grazing intensities

The results of our studies were received within only one grazing season in 2005. Therefore, in this chapter short term effects of grazing intensity are discussed. However, it should be considered that long term effects play an important role for the animal response as well.

1.3.1 Quality of herbage ingested

According to O'Reagain and McMeniman (2002) sheep grazing on rangelands are highly selective and prefer palatable plant species while avoiding or rejecting others. Spedding (1965) states that forage selection of grazing sheep can lead to large differences between quality of herbage offered and herbage ingested. Garcia et al. (2003) found that sheep prefer to maintain the quality of the diet ingested rather than to maintain a high herbage intake, when herbage availability is low. These findings agree with those of Ombabi et al. (2001) who harvested perennial ryegrass and Italian ryegrass at different stages of maturity, which was fed to sheep for ad libitum intake. They observed a decrease of organic matter digestibility from 80% to 70% with proceeding maturity. However, the effect on herbage intake was less pronounced. Ramirez (1999) stated that sheep select herbage on pasture to obtain adequate supply of protein and minerals, but these diets are often not sufficient in energy, when herbage allowance is low. Moreover, Animut et al. (2006) assumed that high grazing intensities decrease the amount of herbage offered and therefore limits the potential

for forage selection. Thus, the digestibility of herbage ingested can be expected to be only slightly affected if grazing intensity increases until a critical point of herbage availability is reached. Beside strong selection of herbage by sheep the characteristics of the sward influence the digestibility of the herbage ingested. Spedding (1965) stated that the quality of the offered herbage is less important for selection, if the amount of offered herbage is sufficient. However, if the herbage mass is limited, the quality becomes more important. Increasing grazing intensity can lead to higher quality of the sward as shown by Kristensen (1988) and Schlegel et al. (2000b). This shows that also an increase of herbage quality at increasing grazing intensity can contribute to maintain diet digestibility of grazing ruminants in high grazing intensities. However, considering that the studies were conducted in temperate grassland, it is questionable, if less sustainable semi-arid grasslands react in a similar way. As reported by Garcia et al. (2003) a decrease in herbage quality can occur in low grazing intensities due to maturing of the herbage. Sheep can compensate a decrease of herbage quality in biomass accumulating swards by grazing in small patches where a high herbage quality is maintained.

1.3.2 Herbage intake

Many studies (e. g. Harkess et al., 1972, Gillen et al., 1998, Schlegel et al. 2000a, and Braga et al., 2006) showed that increasing grazing intensities cause a decrease in herbage mass on offer. Thus, grazing sheep have greater effort to maintain herbage intake in high grazing intensities. Schlecht et al. (1999) hypothesized that grazing animals are conditioned by evolution to optimise rather than to maximize herbage intake. Animals reduce feed intake, when energy requirements for access and herbage ingestion are high. This is supported by Garcia et al. (2003), who stated that sheep can maintain herbage intake by increasing grazing time per day but they do not maximize herbage intake, when herbage availability is low and, therefore, the energy requirement for grazing is high. Fierro and Bryant (1990) even found a negative correlation between grazing time per day and herbage intake in a study with sheep grazing on a natural grassland in the Andes of Peru. A decreasing herbage intake of ruminants with increasing grazing intensity was also found by Harkess et al. (1972), Milne et al. (1979), Kristensen (1988), and Common et al. (1997). Gibb et al. (1997) compared three grazing intensities in lactating cows and found the maximal herbage intake at medium intensity. They concluded that the herbage intake at the lowest grazing intensity was limited by higher

demand of ruminating, which decreased the time available for grazing. This could be due to accumulation and senescence of herbage, which causes increased fibre contents in herbage. However, no significant differences between grazing intensities on dry matter, NDF, and ADF content of herbage was found in this study. Curll and Wilkins (1982) identified a further reason for decreased herbage intakes in high grazing intensities which is damage of the sward by hoof impacts and pollution with excreta of the animals.

1.3.3 Live weight gain

As mentioned above herbage intake is a more important factor for live weight gain of grazing ruminants than diet digestibility. Lippke (1980) found a stronger correlation between live weight gain and dry matter intake than between live weight gain and quality of herbage ingested. According to Spedding (1965) a similar growth rate can be achieved on different pastures as long as the herbage mass offered is sufficient. Schlegel (2000a) showed in a grazing trial with steers that live weight gain increases with increasing herbage allowance until the herbage mass offered starts to senescence and quality to decrease (Figure 1.10). Similar results were found by Osoro et al. (2002).

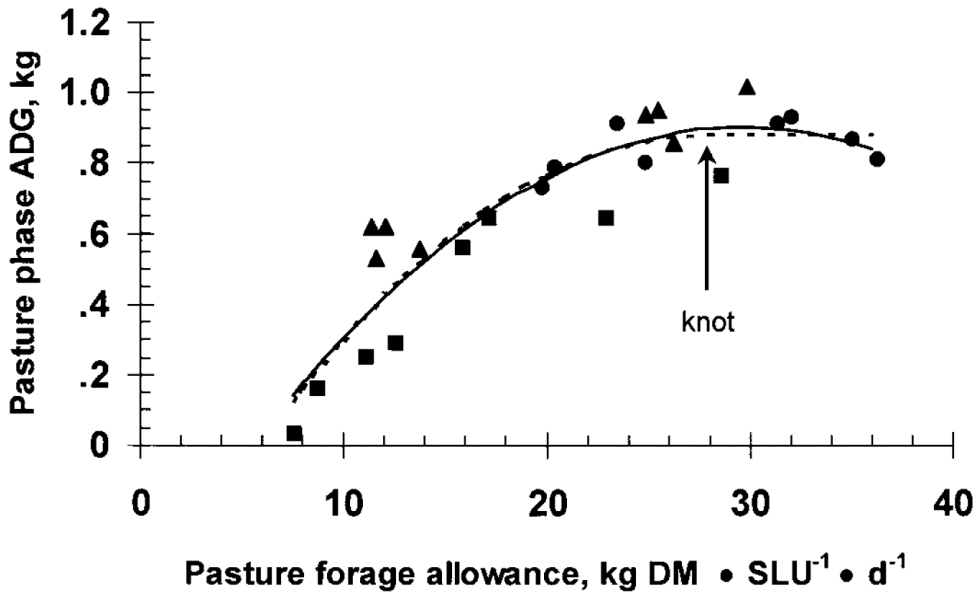

Figure 1.10. Relationship between pasture forage allowance and pasture-phase ADG of steers using data from 1989 (■), 1990 (▲), and 1991 (●), SLU = standard livestock unit; ADG = average daily gain), (Schlegel et al., 2000a)

Beside the strong influence of herbage intake on live weight gain of grazing ruminants the energy requirement for herbage intake should be considered. Lachia and Aguilera (2005) reported that sheep need to walk longer distances in pastures with low herbage allowance, which are decreased by high grazing intensities. This extra-energy expenditure can be an important contribution to the energy requirement of grazing ruminants. According to NRC (1981) the energy requirement for grazing activities of goats is 25% of maintenance for light activity, 50% in semi-arid rangeland and 75% in steep mountainous rangeland with a low vegetation cover. Contrary to this assumptions Animut et al. (2006) found no decrease in growth efficiency (daily live weight gain/ dry matter intake) by increasing grazing intensity. However, their grazing experiment in sheep was conducted in temperate grassland with only three grazing intensities. It is questionable, if the highest grazing intensity was high enough to cause a distinct increase of grazing activity.

In many studies (e.g. Harkess et al., 1972; Common et al., 1997; Schlegel et al., 2000a ; Virkajärvi et al., 2002 ; and Animut et al., 2006) a decrease in the individual performance with grazing intensity was observed. However, this decrease can be compensated by the increased number of animals per ha. Han et al. (2000) conducted an experiment with five grazing intensities in sheep on a *Stipa breviflora* desert steppe in the middle-west of Inner Mongolia from July to November. Although they found decreasing individual live weight gain with increasing grazing intensity, live weight gain per ha increased (Figure 1.11).

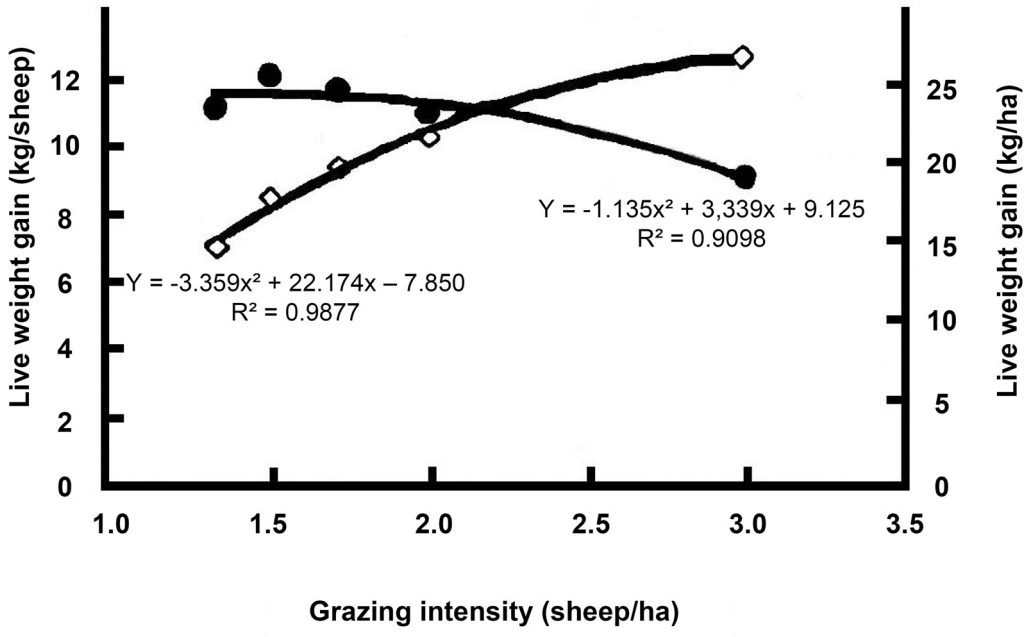

Figure 1.11. Live weight gain of sheep as influenced by grazing intensity (Han et al., 2000)

1.4 Methods for estimating herbage intake of grazing ruminants

Performance of ruminants on pasture mainly depends on digestible nutrient intake (Lippke, 2002). Although diet digestibility is an important component of this relationship, the performance is more related to herbage intake, because grazing animals are able to maintain diet digestibility in a certain range as described in Chapter 1.3. According to Mayes and Dove (2000) the direct measurement of herbage intake of grazing ruminants is not practical. Thus, indirect methods to estimate the herbage were developed. This Chapter gives an overview about common indirect methods for estimation of feed intake in grazing ruminants, which means generally intake of dry matter or organic matter per day.

1.4.1 Herbage disappearance method

According to Smit et al. (2005) the herbage disappearance method is the classical method to estimate herbage intake of grazing animals. It was used in several grazing experiments (e.g. Harkess et al., 1972; Kristensen, 1988; and Dougherty et al., 1989). A representative area of the total grazed area is harvested before and after grazing. The disappeared herbage mass corrected for regrowth allows to calculate average herbage intake per animal. An estimation of the individual herbage intake is not possible except when only one animal is grazing on the sampled plot. In order to receive reliable corrections for regrowth of the herbage during the grazing period this time should be short. Dillon (1993) stated that a correction for regrowth is not needed for a very short grazing period (12 to 24 hours). Furthermore, the herbage disappearance method provides reliable estimations of herbage intake, if a large proportion of the offered herbage is consumed within the grazing period.

1.4.2 Energy requirement method

Herbage intake of grazing dairy cows was estimated by energy requirement of the animals in the studies of Smit et al. (2005) and Macoon et al. (2003). Milk yield, live weight change, requirement for maintenance as well as for grazing, and the energy concentration of the herbage ingested were determined to derive herbage intake. Macoon et al (2003) estimated the net energy requirement for grazing by observation of the grazing behaviour of the animals, whereas Smit et al. (2005) assumed 20% of the requirement for maintenance. In both

studies energy content of the herbage ingested was estimated by the chemical composition of hand-plucked samples of forage, which were similar to those by the animals.
The estimation of herbage intake by the energy requirement of the animals is more often used for lactating cows, because the energy requirement can be estimated precisely. For non-lactating animals at low growth rates energy requirement is difficult to determine due to variation in composition of live weight gain.

1.4.3 Estimation by intake rate and grazing time

The herbage intake of grazing ruminants can be estimated by the intake rate (g/h) and the grazing time (h). The intake rate depends on the bite rate (bites/h) and the bite size (g/bite). Thus herbage intake can be calculated by the following equation:

$$\text{Intake (g DM/day)} = \text{bite rate (bites/h)} \times \text{bite size (g DM/bite)} \times \text{grazing time (h/day)} \quad [1]$$

However, according to Mayes and Dove (2000) the measurement of intake rate is inaccurate due to high variation of bite size, the time of observation below 24h per day, and the effect of observation on the behaviour of the animals. Despite of these limits some authors estimated herbage intake by measuring intake rate and grazing time. Gibb et al. (1997) for example measured intake rate of cattle during one hour grazing by determining live weight at the beginning and the end of this period. Furthermore grazing time of the whole day was recorded. Herbage intake was subsequently calculated by multiplication of feed intake rate and grazing time of the whole day. Beside the high effort of this methods it is necessary to discuss the reliability of this method, because intake rate is probably influenced by many factors as temperature and herbage offered. Changes of these parameters request current calibration of feed intake rate. In contrast an observer followed the grazing animal and counted and categorized the bites due to the grazed plant species in the study of Agreil and Meuret (2004). Afterwards the observer simulated the bites of every category and the feed intake rate was calculated. The authors reported that the observed animals were not disturbed in their grazing behaviour. It is to assume that the estimation of herbage intake by intake rate and grazing time has severe limits and is not applicable in grazing experiments with larger numbers of animals.

1.4.4 Estimation by internal markers

Internal inert markers are often used in indoor digestibility trials to avoid total collection of feces. According to Schneider and Flatt (1975) the digestibility of a diet can be determined by the following equation (ratio method):

$$\text{Digestion coefficient of a nutrient} = \frac{\text{concentration of marker in feed} \times \text{concentration of nutrient in feces}}{\text{concentration of marker in feces} \times \text{concentration of nutrient in feed}} \quad [2]$$

However, due to feed selection of grazing animals it is difficult to receive reliable values for marker concentration in the feed ingested. Furthermore, it was observed that type of forage influenced fecal recovery of the marker (Tamminga et al., 1989). Wallace and Van Dyne (1970) tried to solve the problem by determining the fecal recovery of the internal marker lignin in stall-feeding trials for the same feed, but even after adjustment the digestibility estimates remained invalid. Momont et al. (1994) examined the use of the internal marker alkaline peroxide lignin in sheep. Although a high fecal recovery of 97.8% ± 1.9 was found, the accuracy of the digestibility estimates were variable and adversely influenced predictions of dry matter intake. Thus, internal marker play no significant role in estimation diet digestibility of grazing ruminants with the exception of internal n-alkanes. They will be discussed more in detail in Chapter 1.5.1.

1.4.5 Estimation by determination of fecal output and digestibility of the diet

Cordova et al. (1978) as well as Mayes and Dove (2000) showed in their reviews that the intake of a nutrient by grazing ruminants can be estimated by using the equation for the calculated digestibility of nutrients:

The equation for e.g. OM:

$$\text{digestibility of OM} = \frac{\text{intake of OM} - \text{fecal output of OM}}{\text{intake of OM}} \quad [3]$$

can be transformed to:

$$\text{intake of OM} = \frac{\text{fecal output of OM}}{(1 - \text{digestibility of OM})} \quad [4]$$

Thus, for the estimation of herbage intake two values are needed: fecal output and digestibility of dry matter or organic matter ingested. This chapter gives an overview about different methods for estimating fecal output and digestibility of herbage ingested in grazing ruminants.

1.4.5.1 Determination of fecal output

According to Mayes and Dove (2000) two methods are often used in research to determine the fecal output of grazing mammalian herbivores: The direct measurement by a total feces collection by feces bags and the indirect estimation by inert markers.

Direct measurement by feces bags

There are only few grazing studies, which measure the fecal output of grazing ruminants by a total collection of feces with feces bags attached to the animals with harnesses (e.g. Common et al., 1991 and Ayantunde et al., 1999). According to Mayes and Dove (2000) the main concerns of this methods are uncontrollable losses of feces and the influence on animals, which could alter the grazing behaviour. Studies reviewed by Cordova et al. (1978) indicate that animals fitted with feces bags may experience weight loss. Additionally the high effort in work by catching the animals two times per day and by handling huge amounts of samples has to be mentioned. Cordova et al. (1978) calculated that about 70 man-hours of field work is needed to obtain one individual fecal output value. Common et al. (1991) were further concerned that the withdrawing of large amounts of feces from the grazed area effects nutrient recycling. This would bias long term measurements. However, Hatfield et al. (1993) did not observe differences in plasma cortisol, forage intake, fecal output, and live weight gain, when wethers were fitted with feces bags.

Indirect estimation by inert markers

To avoid a total collection of feces in stall-feeding and grazing trials inert markers can be used to estimate the fecal output. The advantage is that only the concentration of inert markers in feces has to be determined. This requires a representative feces sample of the total feces. In common grab samples from the rectum are obtained and analysed. Alternatively also

feces samples from the sward can be collected as described by Kotb and Luckey (1972). Compared to total feces collection field and laboratory work is reduced and the stress of the animal is smaller. According to Schneider and Flatt (1975) and Owens and Hanson (1992) an ideal inert marker must:

(1) not be absorbed in the digestive tract of the animal;
(2) not affect or be affected by the digestive tract or its microbial population;
(3) flow parallel with the digesta;
(4) have a specific and sensitive method of analysis.

The fecal output can be estimated by the following equation:

$$\text{fecal output (g DM/day)} = \frac{\text{intake of marker (mg/day)}}{\text{fecal marker concentration (mg/g DM)}} \qquad [5]$$

Markers can be divided into external and internal markers, which are discussed subsequently.

Internal markers

Internal markers occur naturally in feedstuffs. According to Titgemeyer (1997) the most common used in animal nutrition studies are acid-soluble ash, long-chain n-alkanes and indigestible ADF , whereas lignin is not a suitable internal marker due to an incomplete fecal recovery. Since fecal recovery of internal markers are often influenced by the diet, the fecal recovery has to be determined for the respective diets. Table 1.1 gives a detailed overview about internal markers. However, internal markers are difficult to use for fecal output estimation in grazing ruminants, because according to equation [5] the intake is not known. Santos and Petit (1996) used acid insoluble ash to determine fecal output of wethers fed grass silage indoors. They found a great variation of the results and no significant relation to the direct measurement of fecal output by total collection. Also Tamminga et al. (1989) reported poor estimations of the digestibility by internal markers.

Table 1.1. Overview of internal inert markers used in herbivores (Mayes and Dove, 2000)

Marker	**Type**	**Analysis**	**Recovery**	**Digesta association**	**Uses**[2)]
Lignin	Fibre fraction	Extraction	Variable	Solid-phase	D, RP
Acid-detergent lignin	Fibre fraction	Extraction	Variable	Solid-phase	D
Indigestible ADF	Fibre fraction	Extraction	Variable	Solid-phase	D, RP
Indigestible NDF	Fibre fraction	Extraction	Variable	Solid-phase	D
PIC[1)]	Fibre fraction	Extraction	Variable	Solid-phase	D
Acid-insoluble ash	Silicaceous	Extraction	High	Solid-phase	D
Silicia	Silicaceous	Various	High	Solid-phase	D
Chromogen	Plant pigments	Colorimetric	Variable	Uncertain	D
Long chain fatty acids	Plant-wax compounent	Gas chroma-tography	High	Mainly solid-phase	D
Long-chain N-alkanes	Plant-wax compounent	Gas chroma-tography	Medium/ high	Mainly solid-phase	D, RP, C, DF

1) Potentially indigestible cellulose

2) Estimation of D = digestibility, RP = rate of passage, C = diet composition, and DF = digesta flow

N-alkanes are the only internal marker, which are used often in grazing trials to estimate feed intake, however in combination with the use of external n-alkanes, which is discussed in more detail in Chapter 1.5.1.

External inert markers

External markers normally do not occur naturally in feedstuffs. It is necessary to administer them to the animals to enable the determination of fecal output based on its fecal concentration. The use of external inert markers is the most common method to estimate fecal output of grazing ruminants. Owens and Hanson (1992) described the different ways to administer external marker to ruminants:

(1) The marker is homogenous blended with a supplement.
(2) The marker is given continuously to the animals by infusion pumps.
(3) The marker is released continuously by a controlled release device in the rumen .
(4) The marker is administered to the animals as a daily dose (e.g. via a gelatine capsule containing the marker and given orally or through rumen fistulae)

The most frequently marker used in grazing experiments with ruminants are chromic oxide (Cr_2O_3), n-alkanes and titanium dioxide (TiO_2). Table 1.2 gives an overview of external markers. According to Kotb and Lukey (1972) Cr_2O_3 has been the most widely used fecal marker. It can be administered to grazing ruminants by route (3) and (4) like the n-alkanes. For TiO_2 no controlled-release devices are existing yet. Since this marker was used in our grazing experiment to predict fecal output of grazing sheep and it plays an important methodical role in this thesis, Chapter 1.5 will discuss the evaluation of the external marker Cr_2O_3, n-alkanes and TiO_2 more in detail.

Table 1.2. Overview of external markers used in herbivores (Mayes and Dove, 2000)

Marker	**Type**	**Analysis[3)]**	**Recovery**	**Digesta association**	**Uses[4)]**
Cr_2O_3	Insolubile oxide	AA or XRF	Very high	None, Dense	FO,RP
TiO_2	Insolubile oxide	AA or XRF	Very high	None, Dense	FO, RP
$BaSO_4$	Insoluble salt	XRF	Very high	None, Dense	FO
Ce, Dy, Er, Eu, Yt, Yb	Soluble rare earths	AA or XRF	Medium/high	Mainly solid-phase	FO, DF, RP
Ru-phenanthroline	Soluble complex	AA or XRF	High	Mainly solid-phase	FO, DF, RP
Cr-mordanted fibre	Bonded to fibre	AA or XRF	Very high	Solid-phase	FO, RP
Plastic particles	Insoluble polymer	Physical	Very high	None	FO, RP
Artificial n-alkanes	Insolubile wax	GC	Medium/high	Mainly solid-phase	FO, RP
CrEDTA[1)]	Soluble complex	AA or XRF	Medium/high	Liquid-phase	FO, DF, RP
CoEDTA[2)]	Soluble complex	AA or XRF	Medium/high	Liquid-phase	FO, DF, RP
Polyethylene glycol	Soluble polymer	Turbidity	High	Liquid-phase	FO, DF, RP

1) Complex of chromium and ethylenediamine tetra-acetic acid

2) Complex of cobalt and ethylenediamine tetra-acetic acid

3) AA = Atomic absorption spectroscopy, XRF = X-ray fluorescence spectroscopy

4) Estimation of FO = fecal output, RP = rate of passage, and DF = digesta flow

1.4.5.2 Estimation of the digestibility of herbage ingested

Sward cutting methods

Quality of herbage ingested is influenced by the quality of offered herbage. Thus, determination of the digestibility of herbage offered could give information about the quality of ingested herbage. However, as shown in Chapter 1.3.1 the difference between quality of herbage offered and herbage ingested can be very large due to herbage selection of the animals. Moreover, it is difficult to get representative herbage samples in heterogeneous pastures to estimate its digestibility in vitro. Therefore, Brand et al. (1997) harvested ten replicate transects (1.0 m × 0.25 m) on an oat stubble sward. Lee et al. (1995) measured the digestibility of the offered herbage in vitro, but included biomass availability to estimate diet digestibility of grazing sheep, because herbage selection is related to herbage allowance as well. Hodgson and Wilkinson (1968) as well as Ramirez-Perez et al. (2000) tried to get representative herbage samples for the diet of grazing cattle and sheep by obtaining herbage samples from the sward immediately in front of the grazing animal by hand plucking. The authors gave no information wether the sampling affected the animals in their behaviour. Therefore, it can not be excluded that the method influenced herbage selection and intake of the animals.

Determination of herbage samples obtained from oesophagus fistulae

Oesophagus fistulae are used in grazing studies to obtain representative herbage samples of the ingested diet (Grimes and Watkin, 1965; McManus et al., 1968 ; Wallace and Van Dyne, 1970; Milne et al., 1979; Lascano and Thomas, 1988; Taylor and Kothman, 1990; Common et al., 1997; Schlegel et al., 2000a). Wallace and Van Dyne (1970) even used collected extrusa from oesophagus fistulae on cattle in in vivo digestion trials with sheep to compare this measured digestibility with results from two indirect measurements of the digestibility of the grazed diet of the cattle. According to Mayes and Dove (2000) the assumption that extrusa samples from oesophagus fistulae represent the diet of non-fistulated grazing animals are questionable for the following reasons:

(1) Extrusa samples are collected over a limited time, whereas animals may be grazing or browsing an area for days or weeks.
(2) The diet selected by oesophageal-fistulated animals may differ from non-fistulated animals due to surgical preparation or different handling of the in the collection period, which influences the grazing behaviour.
(3) The composition of extrusa may differ from that of the plant material ingested, due to addition of saliva and the possibility of plant soluble and small fragments bypassing the fistula

Van Dyne and Torell (1964) reported that the time needed to get appropriate amounts of extrusa from small ruminants depends on their intake rate and can increase to four hours at pastures of low herbage allowance. Alder (1969) found in individual stall-feeding experiments a mean recovery value of 99.7% for the herbage extruded trough the fistulae in relation to herbage eaten by sampling extrusa for 2 h per day.
Woji and Iji (1996) wrote that sheep and goats were able to graze immediately after surgery and recovered completely within four weeks. However, fully recovered animals could be disturbed in their grazing behaviour indirectly by the sampling of the extrusa. Forbes and Beattie (1987) did not observe differences in grazing behaviour of fistulated and non-fistulated cows and sheep.
Van Dyne and Torell (1964) discussed in their review the contamination of the feed in the collected extrusa with salivary. Salivary contains up to 1 % ash and can lead to an increase of the ash content of the extrusa dry matter. They recommended therefore to relate the data on an ash-free basis. Furthermore, salivary can have an influence on the in vitro digestibility of the extrusa. Pinchak et al. (1990) found significant additions of Na, P and Ca in extrusa collected from oesophageal fistulae compared with the diet fed.
Hirschfeld et al. (1996), McCollum and Gillen (1998) as well as Ramsey et al. (1998) used rumen fistulae instead of oesophagus fistulae to obtain representative herbage samples from grazing ruminants. The expected contamination with salivary as well as with rumen liquid is higher for this sampling than for the sampling by oesophagus fistulae. Especially the influence on the in vitro digestibility is assumed to be higher. Lesperance et al. (1960) compared oesophageal and rumen fistula sampling and found more nitrogen-free extract in the samples obtained from the oesophagus fistulae than in the samples obtained from the rumen fistulae.

Fecal crude protein method

Numerous studies (Wallace and Van Dyne, 1970; Bartiaux-Thill and Oger, 1986; Schmidt, 1993; Boval et al., 2003 ; Lukas et al., 2005; Schlecht et al., 2006; Wang et al.; 2007) have shown the validity of the organic matter digestibility estimation by the fecal crude protein concentration in ruminants. Figure 1.12 shows the basic principals of the relationship between organic matter digestibility and fecal crude protein concentration. The estimation is due to a decrease in fecal output at increasing digestibility and an increasing excretion of indigestible microbial protein. The basis of the estimation is the high proportion of microbial protein in the total fecal protein of ruminants. Mason (1969) reported proportions of 71 to 97 %. He concluded that most of the non-dietary nitrogen in the feces originates from microbial protein generated in the rumen.

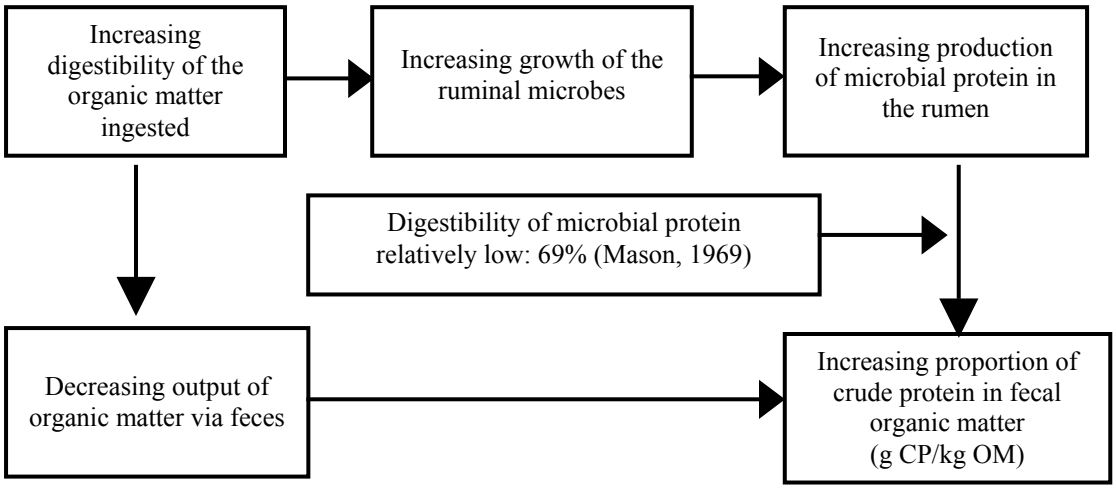

Figure 1.12. The principle of the relation between organic matter digestibility of the diet and fecal crude protein content in ruminants

The fecal crude protein method can lead to an overestimation of the diet digestibility caused by high contents of indigestible protein in the diet. Schlecht and Susenbeth (2006) reported that the approach might overestimate diet digestibility, if anti-nutritional dietary factors such as tannins increase fecal crude protein from feed or endogenous origin. Lukas et al. (2005) and Wang (2007) developed general regression equations for the estimation of organic matter digestibility by the fecal crude protein content for cattle and sheep, respectively. The data and equations are shown in Figure 1.13 and 1.14. Wang (2007) used a data base from 170

digestion trials with n = 750 individual observations including data from the Institute of Animal Nutrition of the Agricultural Research Centre, Braunschweig (Germany) and from own digestibility trials conducted in Inner Mongolia. Both authors examined, if the correction by the acid detergent-insoluble fecal crude protein, which represents the indigestible dietary crude protein leads to a more close relationship and improves the estimation of the diet digestibility. Lukas et al. (2005) found no improvement and concluded that the proportion of acid detergent-insoluble crude protein in the diets was too low to show a significant effect. Wang (2007) found an small improvement of the estimation, which did not justify the higher effort in laboratory work. Both authors found small but significant influences of the diet on the fecal crude protein content. However, the general equations showed a sufficient accuracy in either studies.

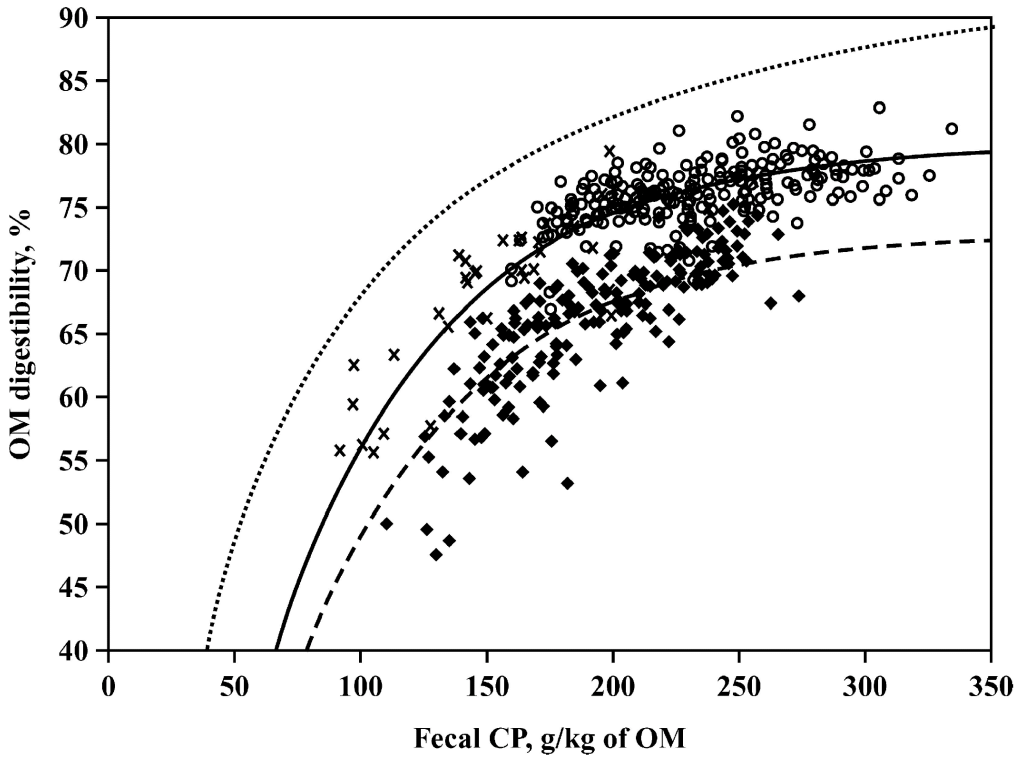

Figure 1.13. Relationship between fecal CP concentration and diet OM digestibility (DOM) in cattle derived from Equation DOM = ai − 107.7e(−0.01515 × CP) with a_1 = 79.76 (——), using data from Braunschweig (o) and Hohenheim (×), and a_2 = 72.86 (— —), using data from Gumpenstein (◆); and estimated from the theoretical Equation: DOM = ([28.08 × CP + 13.7 × diet OM (% of DM)])/(100 −CP) (- - - - -); (Lukas et al., 2005)

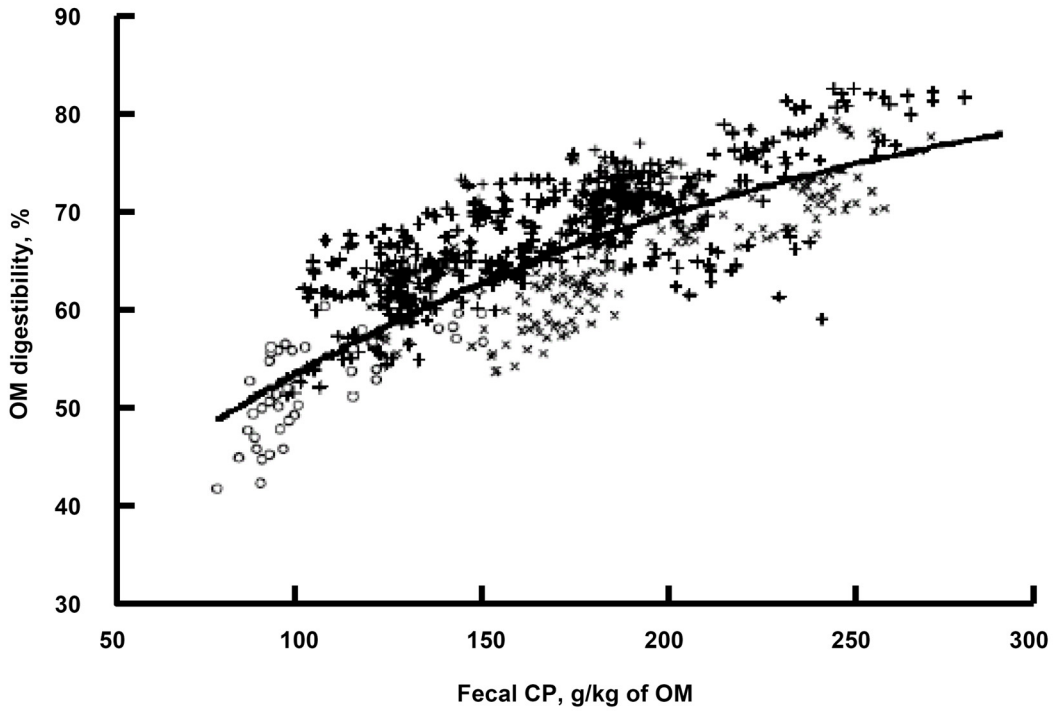

Figure 1.14. Relationship between concentration of CP in fecal OM and OM digestibility (DOM) in sheep according to the regression equation (DOM = 89.9 – 64.4 × exp(-0.5774 × CP [g/kg OM] / 100)) using data of grass (✚) and legume (✖) from Braunschweig and data from Inner Mongolia (**o**); (Wang, 2007)

Lukas (2002) conducted a grazing experiment with lactating grazing cows on two different swards and determined in vitro organic matter digestibilities of herbage samples obtained by cutting representative areas of the pastures. She compared those with the organic matter digestibility estimated by the fecal nitrogen method. Feces samples were obtained twice daily from the rectum in the morning and in the afternoon over five days and pooled afterwards according to daytime and animal. The differences in sward quality was significantly reflected by crude protein concentration in feces. The organic matter digestibility estimates of the fecal crude protein concentration did not differ ($P < 0.1$) between samples taken either in the morning or in the afternoon. Lukas (2002) therefore concluded that time of feces sampling does not affect the result and that one grab sample per day, which is pooled over five days is sufficient to get reliable estimates of diet digestibility.

1.4.6 Comparison of methods

Macoon et al. (2003) and Smit et al. (2005) compared different methods to estimate the herbage intake of dairy cows on pasture. Macoon et al. (2003) compared the herbage intake estimation by the energy requirement method, the sward disappearance method, and the combination of fecal output and prediction of digestibility. For the latter method they used pulse-dosed chromium mordanted fibre for estimating fecal output and the sward cutting method (hand-plucking) for estimating diet digestibility. As shown in Figure 1.15 herbage intake estimates of the pulse-dose marker method were generally higher, less rationally and showed more variability than the other two methods. Further no correlation were found between the results of the pulse-dose marker method and the other two methods, whereas the herbage disappearance method and the estimation of herbage intake by energy requirement of the animals showed a relatively high correlation. Macoon et al. (2003) concluded that the latter two methods may be useful and less costly alternatives to the pulse-dose marker method. However, it is doubtable to generalize this conclusion due to the fact that no direct measurement of the herbage intake is available to validate the three methods. Furthermore the estimation of diet digestibility by hand-plucking is questionable and chromium mordanted fibre is not a common inert marker for fecal output estimation in grazing experiments.

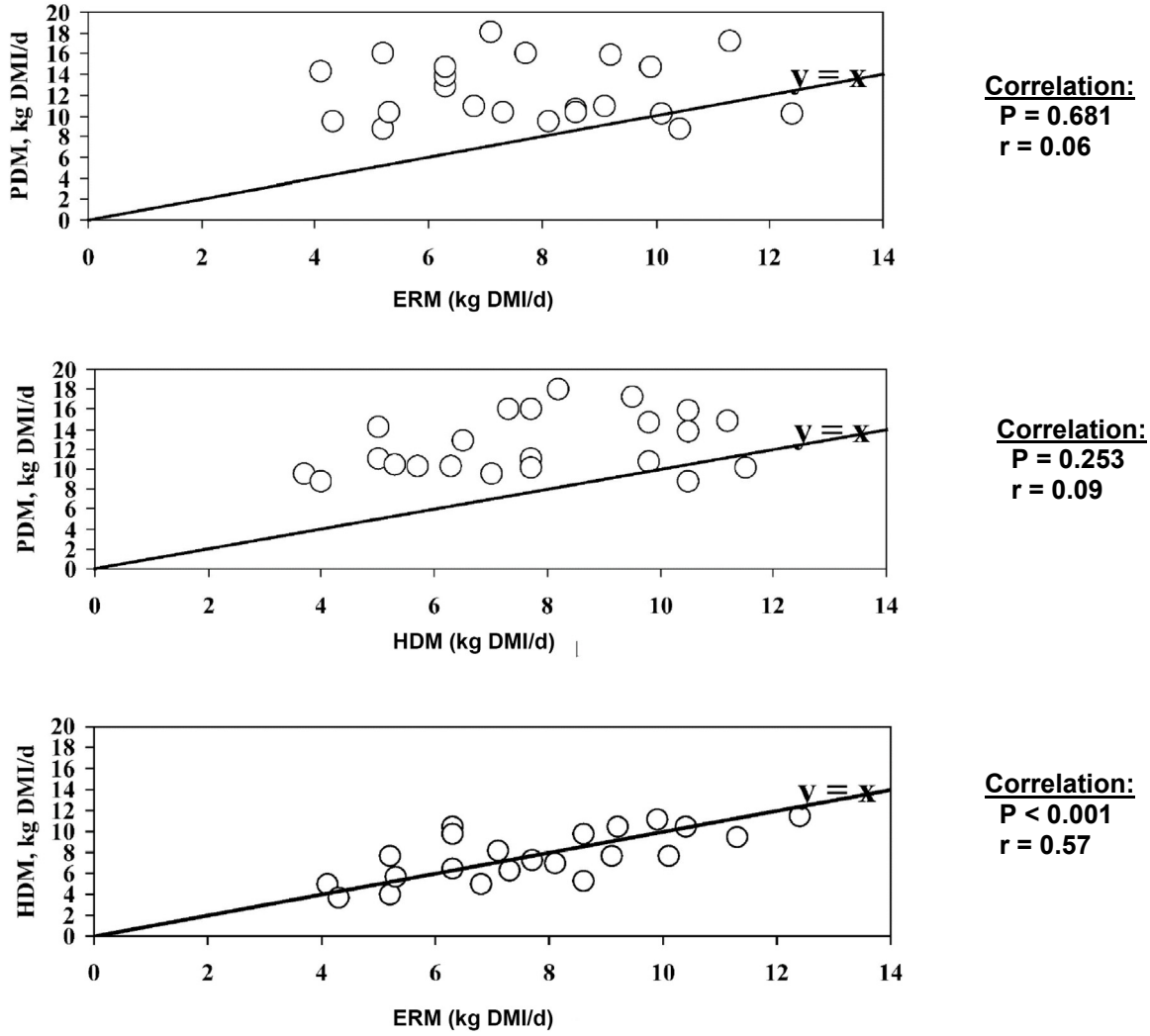

Figure 1.15. Relationships between dry matter intake estimates (DMI) by the pulse-dose marker method (PDM), the energy requirement method (ERM), and the herbage disappearance method (HDM), (Macoon et al., 2003)

Smit et al. (2005) compared the herbage intake estimation by the herbage disappearance method, the energy requirement method and the inert marker technique using internal and external n-alkanes. They found a high variation of the herbage disappearance method and generally higher estimates of the inert marker technique compared with the energy requirement method. Therefore, they concluded that the use of n-alkanes is the best of the three methods to estimate herbage intake of grazing dairy cows and that the herbage disappearance method should not be used.

1.5 External markers for fecal output estimation

Although several external markers are used to estimate fecal output of grazing ruminants for example granulated polyamide by Mahler et al. (1997) or chromium mordanted neutral detergent residue by Ruiz et al. (2001), only the three most important external markers n-alkanes, Cr_2O_3, and TiO_2 are described and discussed in this Chapter. The following criteria are used for their evaluation:

(1) Fecal recovery of the marker;
(2) time needed to achieve equilibrium of intake and excretion;
(3) diurnal excretion pattern of the marker;
(4) fecal output estimation by the inert marker.

Fecal recovery of an inert marker is the proportion of the excreted amount of its intake. To determine fecal recovery total collection of feces after achievement of equilibrium (see next paragraph) is needed. According to the properties of an ideal marker described by Owens and Hanson (1992) recovery should be close to 100%. If the fecal recovery of an marker is different from 100% it also can be used, when the difference is constant.

The equilibrium in intake and fecal excretion of a marker is determined by total fecal collection or grab sampling in time intervals starting at the first administration of the marker. It is achieved, when the concentration does not alter anymore. The time needed to reach the equilibrium determines the necessary length of the preliminary period after that feces samples can be taken. According to Owens and Hanson (1992) marker usually yields fecal concentration plateau after 5 to 7 days in cattle with constant feed intake.

The fecal marker concentration can still vary, when the marker is in equilibrium. The reason can be that the marker does not flow parallel with the digesta due to inhomogeneous blending of the marker in the forage ingested, which can be affected by the method of marker administration. High variations in fecal marker concentration require an increased grab sampling frequency to determine the mean fecal marker concentration over the day. Since increased grab sampling frequency means more costs, work, and stress for the animals, it is an objective to achieve low variation in the fecal marker concentration. The ideal situation – the marker is blended homogeneously in the forage fed – is not possible for external markers in grazing experiments. Controlled release devices for marker located in the rumen represents an approximation to this situation. Owens and Hanson (1992) reported that irregular dosing

and eating pattern cause a diurnal variation of the fecal marker concentration. For pulse-dosing increased administration frequency of the marker can lower the diurnal variation of fecal marker concentration. However, an increase of the dosing frequency causes more stress for the animals and can therefore alter their grazing behaviour.

1.5.1 N-alkanes

The surface wax of most higher plants contains a mixture of saturated straight-chain hydrocarbons (n-alkanes), which consist of 21 to 35 carbon atoms. N-alkanes with odd-numbered carbon chains predominate (>90%). Different plant species have different patterns of individual n-alkanes, with most herbage species tending to have mainly C_{29}- to C_{33}-alkanes. Dove and Mayes (1991) discussed in their review the use of n-alkanes for estimation of herbage intake in grazing experiments and noted that n-alkanes are indigestible and useful as inert markers. Since n-alkanes with even-numbered carbon chains do not occur naturally in the plants a direct herbage intake estimation of grazing ruminants by using odd-numbered n-alkanes as internal and even-numbered as external markers is possible:

$$\text{Herbage intake (g DM/day)} = \left[\frac{F_i \times D_e}{F_e}\right] \Big/ \left[H_i - \frac{F_i \times H_i}{F_e}\right] \qquad [6]$$

with:

F = Fecal concentration of the internal (i) or external (e) n-alkane

H = Concentration of the internal (i) n-alkane in the herbage ingested

D = Daily intake of the external n-alkane (e)

The equation comprises the fecal output estimation by the external n-alkanes and the estimation of the diet digestibility by the internal n-alkanes. However, the concentration of the internal n-alkanes in the herbage ingested has to be known. Dove and Mayes (1991) stated that sward sampling methods (e.g. hand-plucking) are not accurate enough and suggested the use of oesophagus fistulated animals to obtain representative samples of the herbage ingested. The external n-alkanes can be administered to the animals as pulse doses or by controlled release devices.

Fecal recovery

Fecal recoveries of n-alkanes are often less than 100%. Lippke (2002) reported that the recovery of n-alkanes in sheep increase with carbon chain length (Figure 1.16). Dove and Mayes (1991) suggested that n-alkanes are not affected by microorganism of the rumen but partially absorbed in the intestine.

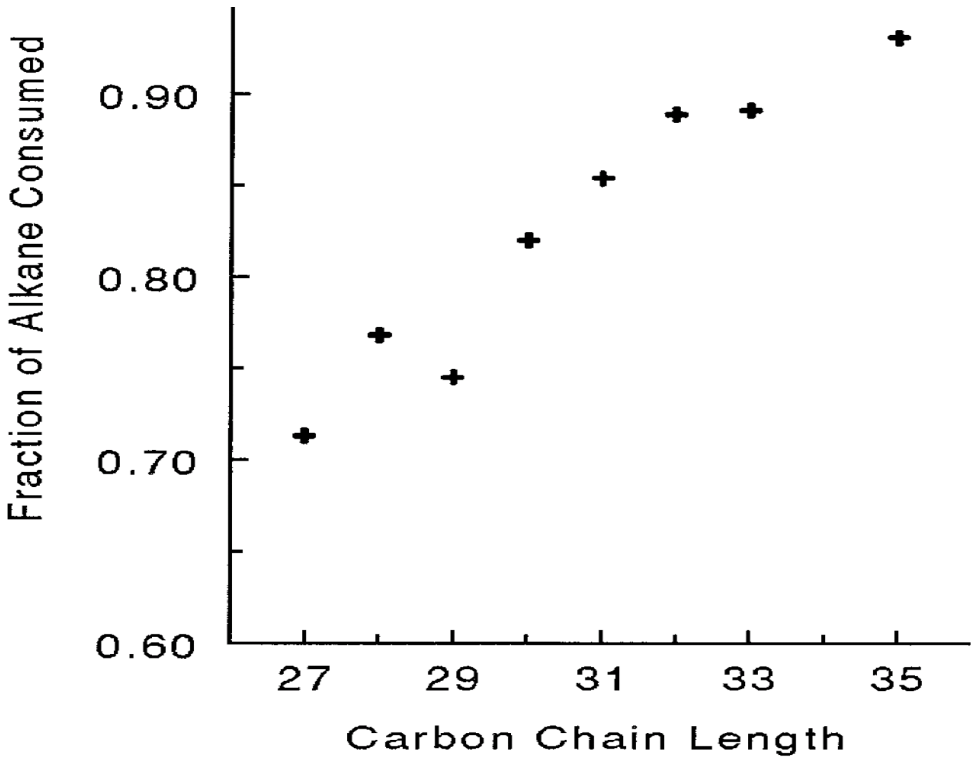

Figure 1.16. Recovery rates of various carbon chain length alkanes (Lippke, 2002)

Ouellet et al. (2004) determined fecal recoveries of n-alkanes administered by controlled release capsules to lactating dairy cows fed a diet with a low (30%) and a high proportion of concentrate (60%). They found higher fecal recoveries of internal (C_{27}, C_{29} and C_{33}) and external n-alkanes (C_{32} and C_{36}) in the low concentrate diet. Ohajuruka and Palmquist (1991) concluded from their study that the fecal recovery of C_{31}-alkanes decreases with increasing intake of this n-alkane. However, Dillon (1993) concluded from his results that fecal recovery is not affected by feeding level, concentrate supplementation, stage of lactation or feeding frequency. Piasentier et al. (1995) did not observe an influence of the diet of grazing ewes as well.

Equilibrium

Dillon (1993) assessed the time required to get a steady outflow of n-alkanes in late lactating dairy cows. External n-alkanes were administered as a daily pulse dose. The results showed that the markers reached maximum concentration at around 102 hours after the start of dosing. Ouellet et al. (2004) waited for eleven days after the administration of a controlled release capsule to lactating dairy cows to keep the recommendations of the manufacturer. On the other hand Berry et al. (2000) and Garcia et al. (2000) used also controlled release capsules but conducted only a preliminary period of seven days before they start to collect feces. However, in none of these studies the time required to get a steady outflow of the marker in the feces was determined.

Excretion pattern

Dove and Mayes (2005) reported in their literature review that diurnal variations in fecal n-alkane excretion pattern are not uniform. They stated that diurnal variation in the fecal n-alkane concentration are observed for the external and not for the internal n-alkanes. For pulse dosed n-alkanes Dillon (1993) found diurnal variation in the fecal n-alkane concentrations (Figure 1.17). He suggested the use of the C_{33}/C_{32}-alkane ratio and a twice daily dosing as well as twice daily fecal grab sampling to minimize the bias caused by diurnal variations in the fecal n-alkane concentration. Berry et al. (2000) compared the direct measured herbage intake of dairy cows with the herbage intake predicted by internal and controlled released external n-alkanes at three times of the day. They found more accurate estimates based on samples received in the morning than at midday and evening. Diurnal variation of fecal concentration in dairy cows were observed by Ouellet et al. (2004) for internal and controlled released external n-alkanes as well.

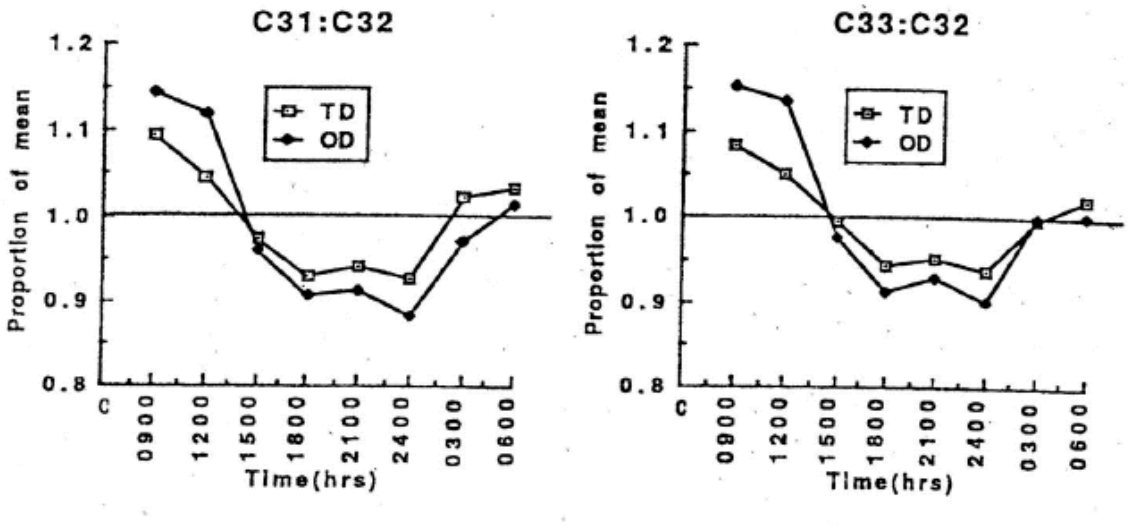

Figure 1.17. Mean variation throughout the day in ratios of herbage n-alkanes to dosed n-alkanes concentration in feces expressed as proportions of the mean value in dairy cows administered with one pulse dose of C_{32}-alkanes at 9 h (OD) or two pulse doses of C_{32}-alkanes at 9h and 16.5h (TD) per day, (Dillon, 1993)

Validation of herbage intake estimation

Dove and Mayes (1991) and Smit et al. (2005) reported that the use of the C_{32}/C_{33}-alkane ratio is most accurate to estimate herbage intake. Therefore, only the estimates of this ratio are discussed. As shown in Table 1.3 the estimates of herbage intake show a high accuracy.

Table 1.3. Estimation of herbage intake of sheep and cattle by dosed C_{32}-n-alkanes and herbage C_{33}-alkane (Dove and Mayes, 1991; modified)

Species	**Intake (kg DM/day)**	**Measured intake – estimated intake**		**Source**
		kg/day	**%**	
sheep	0.58	Nil	Nil	Mayes et al. (1986a)
sheep	0.11-0.27	Nil	Nil	Mayes et al. (1986b)
beef cattle	4.00	0.07	-1.7	Mayes et al. (1986c)
dairy cattle	10.8-14.6	0.405	-3.1	Dillon (1993)
dairy cattle	12.70	0.03	-0.2	Berry et al. (2000)

1.5.2 Chromic oxide (Cr_2O_3)

According to Titgemeyer (1997) Cr_2O_3 is the most widely used digesta marker because it is inexpensive, can easily be added to diets, and analysed accurately. He reviewed 124 studies using markers published in the Journal of Animal Science and found Cr_2O_3 in 90 of these studies used.

Fecal recovery

Cr_2O_3 can be administered to grazing ruminants as controlled release capsules or as pulse doses. In stall-feeding experiments the marker can also be added to the diet. For controlled release capsules fecal recovery can not be determined, because the exact release rate is not known. Therefore controlled release capsules are validated by determining the release rate, which is generally given by the manufacturer assuming a fecal recovery of 100%. Titgemeyer (1997) stated that fecal recovery of Cr_2O_3 often deviates from 100% especially in grazing studies. He calculated from literature a mean fecal recovery of 94% with a large variation among animals. The survey of studies given in Table 1.4 shows corresponding results.

Table 1.4. Fecal recoveries of Cr_2O_3 in different animal species and studies

Species	Fecal recovery (%)	Source
rat	96.2 – 100.1	Krawielitzki et al. (1987)
camel	82.5	Abdouli et al. (1992)
pig	74.6 – 79.7	Jagger et al. (1992)
cattle	89.2 – 96.4	Dillon (1993)
sheep	93.0 – 98.0	Piasentier et al. (1995)
sheep	92.0 – 107.9	Ferret et al. (1999)
pig	96.0	Kavanagh et al. (2001)
cattle	98.0 –112.0	Titgemeyer et al. (2001)

Ferret et al. (1999) measured the fecal recovery of Cr_2O_3 in digestion trials with sheep and found significant influences of the diet. The addition of concentrate to alfalfa hay increased the mean fecal recovery from 101.7 to 107.9%, the latter being significantly different from 100%. Ferret et al. (1999) found an influence of the diet on the fecal recovery: 96.6% for ryegrass and 104.8% for alfalfa diets. In contrast Piasentier et al. (1995) and Titgemeyer (2001) did not observe an influence of diet on fecal recovery of Cr_2O_3. Moreover, Dillon (1993) examined the influence of dose level (5 g versus 10 g per day) and administration frequency (1 versus 2 pulse doses per day) on the recovery in cattle and found no significant differences.

The use of controlled release capsules requires an uniform release rate of the marker. The manufacturer generally delivers a value for the release rate. However, concerns existing that release rate varies with diet and is not uniform. Ferreira et al. (2004) determined in their feeding trials with cattle that the used controlled release capsules provided a uniform marker release, which corresponded with the manufacturers value. However, they suggested it might be better to measure release rates within the particular experiment to obtain accurate estimates. Brandyberry et al. (1991), Momont et al. (1994), Santos and Petit (1996), and Williamson et al. (2000) found a release rate different from the value given by the manufacturer. Momont et al. (1994) supposed an influence of the H_2O kinetics on the release rate and Williamson et al. (2000) reported an influence of the animal in trials with steers. Hatfield et al. (1991) observed a significant effect of the diet on the release rate but no effect of grazing intensity.

Equilibrium

Titgemeyer et al. (2001) determined the fecal recoveries of day 2 to 6, 7 to 11, and 12 to 16 in steers after initiation of marker administration by daily pulse doses and found a less recovery in the first period compared to the other two periods. No difference could be found between the second and the third period. Dillon (1993) concluded from his study with grazing dairy cattle administered with daily pulse doses that an preliminary period of 5 days is adequate to reach equilibrium, which is similar to the observation of Ferret et al. (1999) who reported that equilibrium was obtained after six days in dairy ewes administered with daily pulse doses.

Luginbuhl et al. (1994) found a constant fecal Cr_2O_3 concentration in stall-fed lambs administered with controlled release capsules after 8 days in average with a range of 5 to 13 (Figure 1.18)

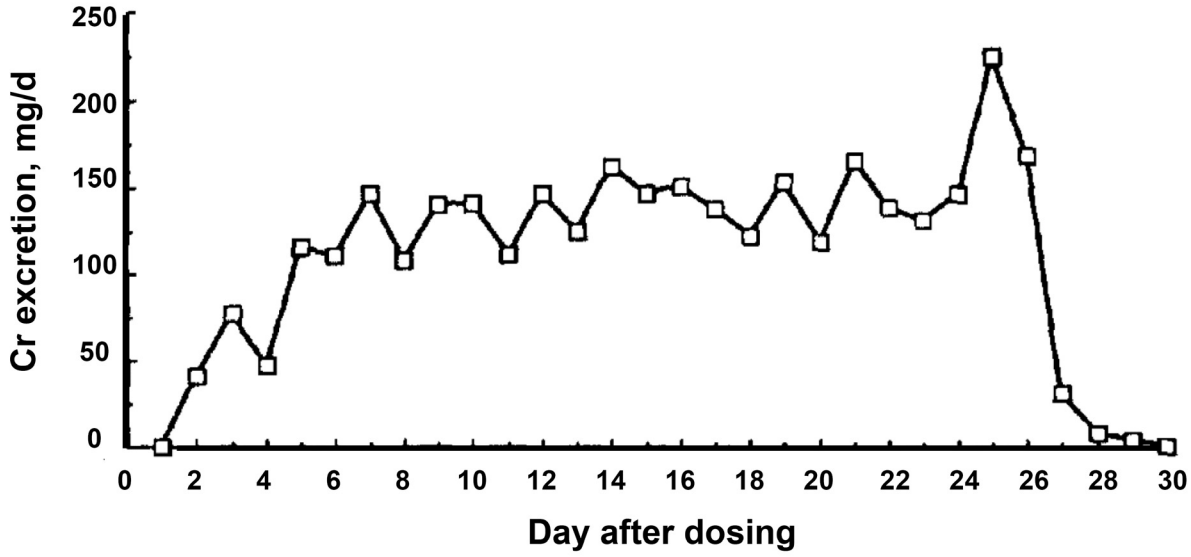

Figure 1.18. Fecal Cr_2O_3 excretion curve from one wether lamb fed alfalfa hay indoors in an individual pen and dosed with a controlled release capsule (Luginbuhl et al. 1994)

Excretion pattern

As shown in Figure 1.19 Myers et al. (2006) detected diurnal variation in the fecal marker concentration for Cr_2O_3 and TiO_2 pulse dosed twice per day at feeding times (6h, 18h). The marker concentration in the digesta at the duodenum increased after administration and reached a maximum after 2 to 4 hours. This indicates that the diurnal variation in the feces concentration is due to inhomogeneous blending of the marker with the forage in the rumen. Dillon (1993) found diurnal variations in fecal Cr_2O_3 concentration as well, which was not influenced by frequency of administration (1 versus 2 pulse doses per day) and marker intake level. Abdouli et al. (1992) reported different concentrations of Cr_2O_3 in feces between days in camels fed hay.

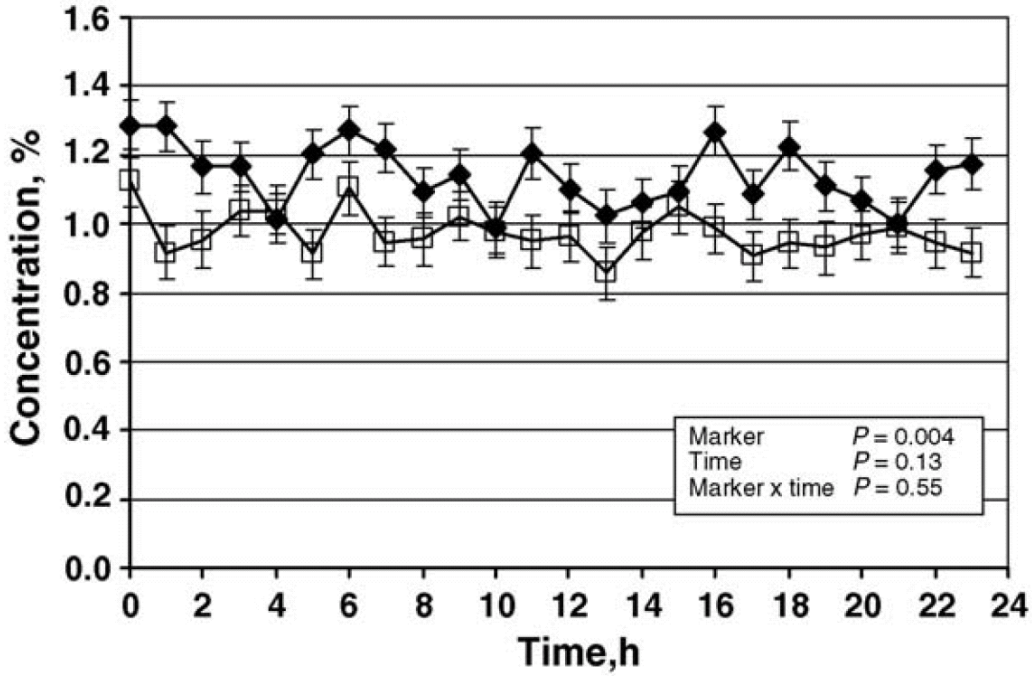

Figure 1.19. Concentrations of TiO_2 (■) and Cr_2O_3 (□) in fecal grab samples from ewes fed a forage diet (Myers et al., 2006)

For Cr_2O_3 administered by controlled release capsules the results about diurnal variations of fecal marker concentration reported in literature are different. Brandyberry et al. (1991) and Ferreira et al. (2004) found no difference in fecal marker concentration between grab samples obtained in the morning and in the evening. Momont et al. (1994) observed a slight improvement in accuracy of fecal output estimation, if grab sampling frequency was increased from once to twice a day. However, they concluded that this improvement did not justify the higher effort. In contrast Santos and Petit (1996) as well as Williamson et al. (2000) determined distinct diurnal variation in the fecal Cr_2O_3 concentration using controlled release capsules.

Validation of fecal output estimation

In Figure 1.20 the relationship between fecal output directly measured and fecal output estimation by Cr_2O_3 in dairy ewes (Ferret et al., 1999) indicates a high accuracy. Prigge et al. (1981) showed a high accuracy of the fecal output estimation as well, if the marker was administered in two pulse doses per day.

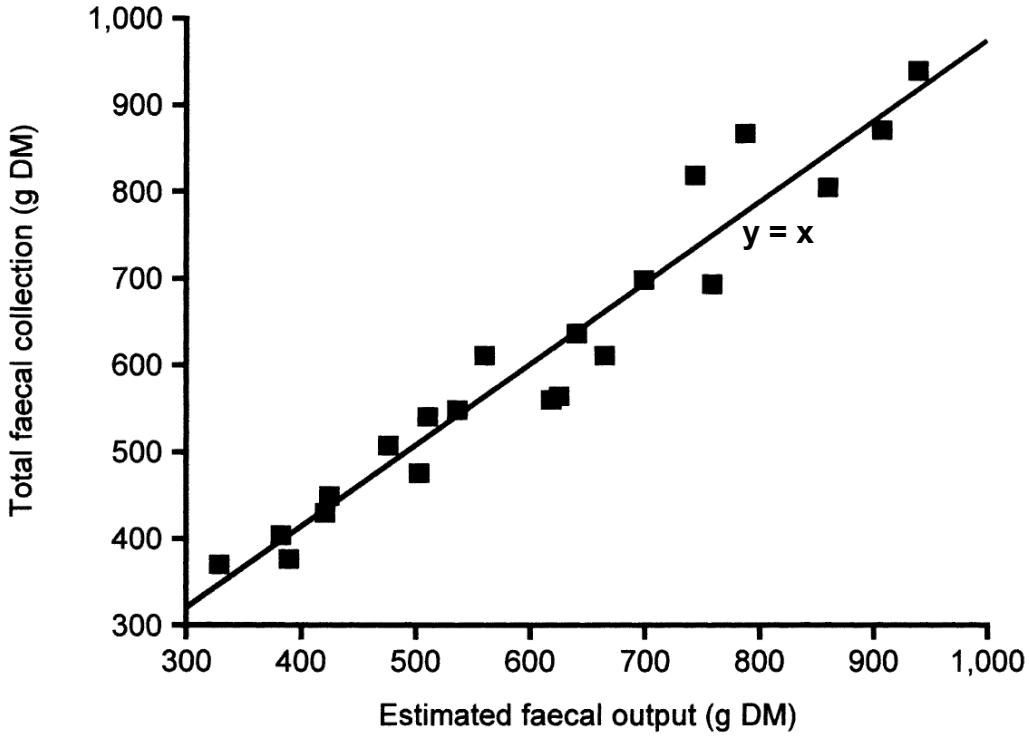

Figure 1.20. Relationship between fecal output directly measured and fecal output estimation by Cr_2O_3 administered by two pulse doses per day and analysed in pooled grab samples obtained on two times per day during two days in dairy ewes (Ferret et al., 1999)

Hatfield et al. (1991) observed that feed intake and supplementation with barley affect accuracy of fecal output estimation in wethers administered with controlled release capsules probably due to changes in the release rate of Cr_2O_3, which was assumed to be constant. An influence of diet was determined by Luginbuhl et al. (1994) as well. Several authors (Brandyberry et al., 1991; Buntinx et al., 1992; and Williamson et al., 2000) observed an overestimation of fecal output by using controlled released Cr_2O_3, which is probably caused by release rates differing from the manufacturers values. Therefore, Buntinx et al. (1992) concluded that controlled released Cr_2O_3 is not appropriate for fecal output estimation without validation of the release rate.

1.5.3 Titanium dioxide (TiO_2)

TiO_2 was less frequently used in the past for fecal output estimation than Cr_2O_3. However, according to Titgemeyer et al. (2001) the meaning of TiO_2 is increasing. Since no controlled release devices are existing for TiO_2, the marker is administered in pulse doses to grazing ruminants.

Fecal recovery

Recoveries of TiO_2 reported in different species are given in Table 1.5. in most of the studies mean recovery is below 100%. Njaa (1961) discussed possible reasons for incomplete fecal recovery of TiO_2 in rats.

(1) Marker accumulation in the caecum,
(2) losses of TiO_2 during administration or total fecal collection,
(3) inaccurate analysis of TiO_2 in feces, and
(4) losses during the preparation of the fecal samples (grinding).

Titgemeyer et al. (2001) added TiO_2 to forage and feces samples and found a an analytical recovery of 100.7%. However, Myers et al. (2004) spiked three sources of organic matter (a forage sample, a bovine fecal sample without Cr_2O_3 and a bovine feces sample containing Cr_2O_3) with different amounts of TiO_2 and found analytical recoveries of 96.7, 97.5, and 98.5% for the three organic matter sources, respectively. This supports to some extent assumption (3).

Table 1.5. Fecal recoveries of TiO_2 in different studies with different animal species

Species	Fecal recovery (%)	Source
rats	91.9 – 97.0	Njaa (1961)
rats	96.5 – 100.6	Krawielitzki et al. (1987)
chicken	98.7 – 99.7	Short et al. (1996)
pigs	96.9 – 98.3	Jagger et al. (1992)
cattle	95.5 – 101.5	Hafez et al. (1988)
cattle	90.0 – 95.0	Titgemeyer et al. (2001)
cattle	94.0 – 100.0	Brandt et al. (1987)
sheep	96.0 – 99.0	Brandt et al. (1987)

Equilibrium

Südekum et al. (1995) showed that a single pulse dose of TiO_2 is excreted more or less completely after 120 hours by steers and wethers fed 1.2 to 1.3 times of maintenance (Figure 1.21), which was confirmed in the study of Rothfuß (1996) with steers fed 1.5 times maintenance. This indicates that equilibrium in ingestion and excretion of TiO_2 is achieved within this time period. Titgemeyer et al. (2001) determined fecal recovery of TiO_2 within three periods after initiation of daily marker administration: day 2 to 6, 7 to 11, and 12 to 16. They found a significant lower recovery in the first period compared to the later periods.

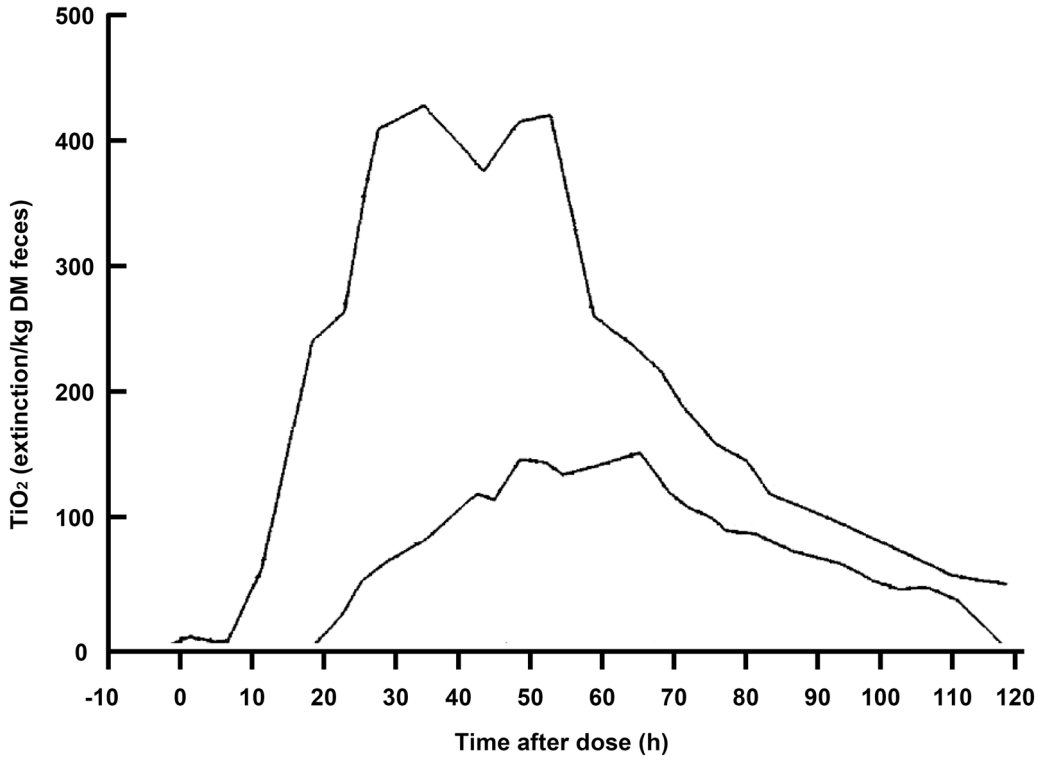

Figure 1.21. TiO_2 concentration (extinction/g DM) in the feces of two wethers fed one dose of TiO_2 concurrently with one single meal of whole plant maize silage preceded and followed by meals of whole-plant barley silage once daily at 7h (Südekum et al., 1995)

Excretion pattern

Myers et al. (2006) examined excretion pattern of TiO_2 administered to sheep in two pulse doses per day and found a diurnal variation in the fecal TiO_2 concentration (Figure 1.19) but not affected by the diet. TiO_2 concentration in the digesta of the duodenum increased after feeding time reaching maximum values after 2 to 4 hours. This variations indicate inhomogeneous blending of the marker with the forage in the rumen. Thus, it can be expected that diurnal variation in the fecal TiO_2 concentration occurs, if the marker is administered to the animals as pulse doses. Hafez et al. (1988) administered TiO_2 to cattle as a component of the concentrate supplement and detected differences in the TiO_2 concentrations of the grab samples obtained in the morning and the evening. However, no studies are available, which provide information about the effect of marker administration frequency. Jagger et al. (1992) observed no diurnal variation in the fecal TiO_2 concentration in feeding experiments with

pigs. However, the marker was blended homogeneously in the diet, which might be responsible for this observation.

Validation of fecal output estimation

In literature no study dealing with validation of fecal output estimation by TiO_2 was found. However, Hafez et al. (1988) and Titgemeyer et al. (2001) validated the digestibility estimation of OM and DM, respectively by the ratio method in cattle. Titgemeyer et al. (2001) found the digestibilities calculated by the ratio method significantly lower (1.6 - 4.3%) than measured by total feces collection. They suggested that a fecal recovery less than 100% was responsible for the lower digestibilities Hafez et al. (1988) observed an underestimation of the digestibility as well, when pooled grab samples obtained in the evening were taken. However, the estimation of digestibility by fecal TiO_2 concentration of grab samples obtained in the morning corresponded with the results of total feces collection. They assumed that more frequent grab sampling may improve the accuracy of digestibility estimates.

1.5.4 Comparisons of the external inert marker

Since it is our aim to conduct a grazing experiment with a large number of animals, we have to find a compromise between accuracy and practicability. The marker used should give reliable results and not cause too much field and laboratory work.

N-alkanes are able to estimate herbage intake accurately. However, the variation of internal n-alkane contents in forage specie may be problematic. Lin et al. (2006) examined n-alkane pattern of five dominant forage species in the typical steppe of Inner Mongolia. They found differences between species and an influence of growing season time on the concentration of internal n-alkanes in herbage. Thus, the ratio between the internal and external marker varies, which is used for herbage intake estimation. Since we expect differences in botanical composition of the diet between grazing intensities due to herbage selection, these problems require current calibration of the internal n-alkane pattern in the diet ingested. Because sward cutting methods are inaccurate to measure internal n-alkane concentration of a grazing animal's diet Dove and Mayes (1991) suggested the use of oesophageal fistulated animals. This additional effort would be very problematical for our large grazing experiment.

TiO_2 and Cr_2O_3 are external marker with similar characteristics as shown by Titgemeyer et al. (2001) and Myers et al. (2006). For Cr_2O_3 controlled release capsules existing, which would decrease the field work for administration of the marker and probably the variation of the fecal marker concentration as well. However, as shown in Chapter 1.5.2 the release rate can be influenced by diet and herbage. Consequently it needs calibration. To avoid this additional experimental work we decided to administer the external marker as pulse doses. As reported by Titgemeyer et al. (2001) and Myers et al. (2006) concerns are existing that Cr_2O_3 can cause health injuries. Furthermore, Jagger et al. (1992) and Myers et al. (2006) stated that TiO_2 is an appropriate alternative for the more common external marker Cr_2O_3.

The decision for the use of TiO_2 as an external inert marker for the fecal output estimation of grazing sheep was accompanied by the decision for the use of the fecal crude protein method to estimate the digestibility of the grazed diet. Since both methods need grab sampling and a Kjeldahl extraction in the analysis, the effort in field and laboratory work is at a low level and allows the examination of a large number of grazing animals. Thus, it is possible to compensate eventual high variation by enlarging the number of examined animals.

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2 Evaluation of titanium dioxide as an inert marker for estimation of fecal output in grazing sheep

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2.1 Abstract

The herbage intake of grazing ruminants is a crucial information for the evaluation of grazing strategies. However, direct measurement of herbage intake is not practical. Therefore, it is often derived from estimations of fecal output and digestibility of the herbage ingested. The aim of this study was to evaluate the inert marker TiO_2 administered to sheep orally by daily gelatine capsules for estimating fecal output by marker concentration of fecal grab samples of grazing sheep at the Inner Mongolian steppe. Indoor feeding experiments and grazing experiments were conducted to determine fecal recovery, time to reach equilibrium in intake and excretion of TiO_2 after initial dosing, diurnal variation in fecal marker concentration, and to validate fecal output estimation with TiO_2. Furthermore, frequency of TiO_2 administration and grab sampling was examined. In the indoor feeding experiments, fecal recovery of TiO_2 was lower ($P < 0.001$) in hay+concentrate diets than in hay diets with 98.9% and 108.0%, respectively. Furthermore, fecal recovery was higher ($P = 0.014$) in grazing intensity 5.0 sheep per ha compared to 2.0 sheep per ha with 107.0% and 100.4%, respectively. The significantly higher than 100% fecal recoveries of the hay diets and the high grazing recoveries could be caused by increased ingestion of soil, which contains 2.2 mg/g DM of TiO_2. However, the difference in fecal crude ash content between the grazing intensities was small, and therefore did not explain the higher fecal recovery in the high grazing intensity. The equilibrium in intake and excretion of TiO_2 was reached five days after initial TiO_2 dosing, which is therefore the minimum preliminary period before fecal sample collection. Diurnal variation in fecal TiO_2 concentrations was found in a grazing experiment, when fecal grab samples were collected on three different times of the day. The variation in fecal TiO_2 concentration was smaller with two times dosing compared with one time dosing of TiO_2 per day. This result was confirmed by the comparison of measured fecal output with estimated fecal output by TiO_2 concentration in fecal grab samples in an indoor feeding experiment. The estimation of fecal output was more accurate with two time dosing than one time dosing per day. Furthermore, the increase in frequency of grab sampling from one to two per day, improved the accuracy of fecal output estimation. In conclusion, these experiments showed that TiO_2 is a reliable marker for estimation of fecal output in grazing sheep.

2.2 Introduction

Herbage intake is a crucial information needed for evaluating the nutritional status of grazing ruminants in different grassland management systems. Garcia et al. (2003) showed that animal performance is more related to herbage intake than quality of diet ingested due to the tendency of grazing ruminants to maintain their diet quality rather than herbage intake in increasing grazing intensities by herbage selection. However, direct measurement of herbage intake of grazing ruminants is not practical. Herbage intake can be estimated indirectly by dividing total fecal output by indigestibility of the diet (Dove and Mayes, 1991). However, Mayes and Dove (2000) were concerned that the direct measurement of fecal output by feces bags attached to the animals with harnesses may lead to feces losses and may influence grazing behaviour of the animals. Thus, indirect methods for measuring fecal output were developed. The most common indirect method is the use of orally administered inert markers, where marker concentration is determined in fecal grab samples for estimating fecal output. Schneider and Flatt (1975) as well as Owens and Hanson (1992) stated that an ideal inert marker should have the following properties: the marker should (1) not be absorbed or be affected by the digestive tract, its microbial population or by the digesta, (2) flow parallel with the digesta, (3) not have toxic, laxative, costive or other physiological effects to the experimental animal and (4) be easily to analyse in the laboratory. In general, a crucial property of an inert marker is a high and constant recovery in feces.

In the past, chromic oxide (Cr_2O_3) was one of the most commonly used inert markers to predict fecal output in grazing ruminants. However, Cr_2O_3 recovery deviated from 100% in many and varies greatly among animals as reported by Titgemeyer et al. (2001). Moreover, Myers et al. (2006) wrote that concerns do exist about carcinogenic properties of Cr_2O_3 and health hazards, when the marker is inhaled. For the marker titanium dioxide (TiO_2) no negative health properties are not expected. Direct comparisons of Cr_2O_3 and TiO_2 in pigs (Jagger et al., 1992), in cattle (Titgemeyer et al., 2001), and recently in sheep (Myers et al., 2006) showed that TiO_2 is an appropriate alternative to Cr_2O_3.

The objective of this study was to evaluate TiO_2 as a marker for the estimation of fecal output in grazing sheep, when it is orally administered daily to sheep by gelatine capsules. The analysis of TiO_2 in feces as developed by Brandt and Allam (1987) was validated and the recovery under grazing and stall-feeding conditions was determined. The time to reach the equilibrium between TiO_2 intake and excretion was determined by measuring the daily fecal excretion of TiO_2 after the first day of administration to the animals. Furthermore, the effects

of frequency and time of marker administration and of grab sampling on excretion pattern of TiO_2 were determined, and estimations of fecal output were validated.

2.3 Materials and methods

To evaluate the validity of TiO_2 for estimating the amount of feces excreted, six experiments were conducted in 2005 and 2006 at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), the Institute of Botany of the Chinese Academy of Sciences, Beijing. The research station is located in the Xilin River Basin, Inner Mongolia Autonomous Region, China (116°42’ E, 43°38’ N). The experiments were conducted with sheep of the local fat-tailed breed of Inner Mongolia. Table 2.1 gives an overview about the design and objectives of the experiments.

Table 2.1. Objectives and design of the six experiments.

experiment	type	group	animals (number)	diet/ grazing intensity	TiO_2 administration (amount/time)	objectives
1	Indoor	1	8	hay 1 +concentrate 1	2.5 g / 7h	TiO_2 recovery, total feces collection on day 8-14
		2	8	hay 1 + concentrate 2		
		3	7	hay 2		
2	indoor	1	8	hay 3	2.5g / 7h	TiO_2 recovery, total fecal collection on day 8-14 and from day 1-13 daily
3	indoor	1	8	hay 4	2.5g / 7h	TiO_2 recovery, total collection on day 8-14, fecal output estimation by grab samples, obtained at 7h and 19h on day 8-14, grab samples of the 7 days pooled by animal and daytime: A = 7h, B = 19h and AB = (7h + 19h) / 2 [1)]
		2	8	hay 4	1.25g / 7h, 19h	
4	grazing	1	10	2 sheep/ha	2.5g / 9h	TiO_2 recovery, total feces collection on day 8-14
		2	10	5 sheep/ha		
5	grazing	1	6	4.5 sheep/ha	2.5g / 9h	excretion pattern of TiO_2; grab sampling on day 8-12 on 9h, 13h, 17h, grab samples not pooled
6	grazing	1	5	7.5 sheep/ha	2.5g / 9h	excretion pattern of TiO_2, grab sampling on day 8-11 on 9h, 13h, and 17h, grab samples pooled by animal for day 8-9 and 10-11
		2	5		1.25g / 9h, 17h	
		3	5		2.5g / 17h	

1) TiO_2 concentration of the pooled grab samples obtained at 7h (A = morning), at 19h (B = evening) and the mean of 7h and 19h (AB)

Experiments 1 to 3

Experiments 1 to 3 were stall feeding experiments with wethers (Table 2.1). All three experiments were divided into three periods of one week: (1) preliminary feeding of the diet in groups, (2) individual feeding in metabolic cages with daily preliminary administration of TiO_2 (day 1 to 7), and (3) individual feeding in metabolic cages with daily administration of TiO_2 and collection of total feces excretion in all three experiments (day 8 to 14). In experiment 2, total fecal collection started on day 1 until day 14, and in experiment 3, additionally grab samples of feces were taken on day 8 to 14.

The total feces excretion were collected by feces bags, which were fixed on the sheep by harnesses. The daily obtained feces were frozen until the end of the experiment and afterwards blended to one sample per animal. The total feces of the seven days of each animal was weighed, mixed and a representative sample was taken. Further, grab samples of experiment 3 were pooled by daytime and animal. All feces samples were dried for 36 h in an air-dry oven at 60 °C, ground through a 1 mm screen, and analysed for TiO_2.

The fecal output of experiment 3 was estimated by the TiO_2 concentration of the grab samples. Estimations were made by four measured or calculated fecal TiO_2 concentrations: grab samples obtained at 7h (A), grab samples obtained at 19h (B), mean of A and B (AB), and AB corrected by the mean fecal TiO_2 recovery in experiment 3.

Experiment 4

A grazing experiment of four weeks was conducted with two groups of 10 wethers on a 5 ha plot and a 2 ha plot, resulting in a grazing intensity of 2 and 5 sheep per ha, respectively. Table 2.1 displays the design and the objectives of experiment 4.

The offered herbage mass was 83 g DM/m² on the 5 ha plot and 35 g DM/m² on the 2 ha plot at the beginning of the 4^{th} week. During the 3^{rd} and 4^{th} week, daily TiO_2 was administered orally, and in the 4^{th} week total feces were collected by feces bags attached to the sheep by harnesses. The obtained total feces were treated as in experiment 1 to 3. The digestibility of organic matter ingested was estimated by the fecal crude protein concentration according to Wang (2007). The organic matter intake of the sheep was calculated by the estimated digestibility of organic matter ingested and the measured fecal output.

Experiment 5 to 6

The design and objectives of experiment 5 and 6 are given in Table 2.1. Experiments 5 and 6 were conducted as grazing experiments with growing female sheep (non-pregnant, non-lactating). The average body mass ± standard deviation was 39.9 ± 3.6 kg and 31.8 ± 3.6 kg for experiment 5 and 6, respectively. The experiments lasted four weeks, and during the last two weeks daily TiO_2 was administered orally. In the last week, fecal grab samples were taken at three times of the day (9, 13, and 17h) for five days and four days in experiment 5 and 6, respectively. In experiment 6 grab samples were pooled by animal and daytime for two days each, whereas in experiment 5 no grab samples were pooled. Feces samples were prepared as described in experiment 1 to 3. The offered herbage mass was 37 g/m² in experiment 5 and 52 g/m² in experiment at the beginning of the fecal collection period.

Analysis of TiO_2 in feces

The method to analyse the concentration of TiO_2 in feces is based on that of Brandt and Allam (1987) with minor modifications. In the first step, TiO_2 is extracted by the Kjeldahl procedure for three hours in 96% sulphuric acid. In the second step, 35% hydrogen peroxide (H_2O_2) is added to the filtered TiO_2 solution to form a yellow complex. In the third step colour intensity is measured in a Spectrophotometer (Jenway 6300) at a wavelength of 405 nm.

To validate the accuracy of the TiO_2-analysis, an experiment was conducted in which TiO_2 was added in eight different amounts to feces obtained from sheep, which did not receive any TiO_2. The concentration of TiO_2 was analysed by the method described above, and compared with the gravimetrically calculated concentration in a linear regression analysis. The results are shown in Figure 2.1. Further the feces without addition of TiO_2 was analysed, to determine the natural occurrence of TiO_2 and the need for correction.

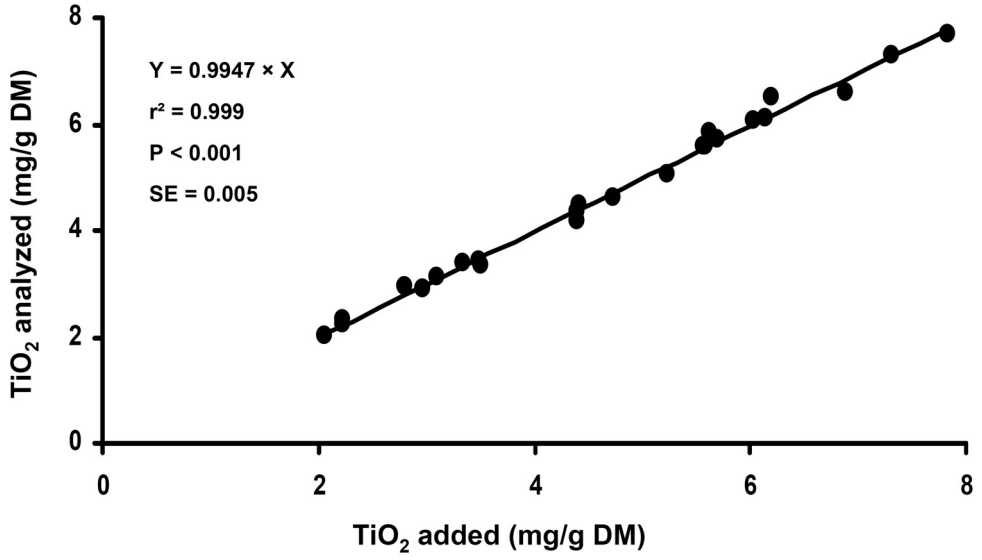

Figure 2.1: Relationship between TiO_2 added to feces and TiO_2 analysed in feces

The analysis detected a mean natural content of TiO_2 in the feces used of 0.2372 mg/g DM. However, according to Brandt and Allam (1987) the analysis is not valid for such minor contents. Furthermore, it is questionable, if really TiO_2 is responsible for this slight coloration of the solution. A correction of the analysed TiO_2 content of the sample by the natural occurrence did not lead to an improvement in accuracy of the analysis ($Y = 0.9494 \times X$, $r^2 = 0.9992$, $P < 0.0001$, $SE = 0.0056$) compared to the uncorrected analysis shown in Figure 2.1.

Statistical analysis

The statistical calculations were carried out by SAS (1988) with the procedures MIXED, GLM, TTEST and REG.

Fecal recovery of TiO_2

The fecal recovery of TiO_2 as determined in experiments 1 to 4 were analysed in two ANOVA. In the first ANOVA, recovery of TiO_2 in the indoor experiments (1 to 3) were analysed using the MIXED procedure with diet as fixed effect (hay + concentrate and hay) and group as random effect in the model. In the second ANOVA, recovery of TiO_2 in the grazing experiment 4 was analysed, using the GLM procedure, with grazing intensity as a

fixed effect in the model. Further, a t-test was used for each group in experiments 1 to 4, to test the null hypothesis that the mean recovery of one group is not equal to 100%.

Daily fecal recovery of TiO_2 after the start of TiO_2 application

The daily fecal TiO_2 recoveries from day 1 to 13 after the beginning of the TiO_2 administration to the sheep of experiment 2 were analysed by an ANOVA using the MIXED procedure with day as fixed effect and sheep as random effect in the model. The daily recoveries of each sheep were treated as repeated measurements. The best fit covariance structure was compound symmetry. To test for differences between the mean daily recoveries a multiple comparison was carried out. Further, all mean daily recoveries were tested for the null hypothesis that they were not equal to 100 % with the TTEST procedure.

Excretion pattern of TiO_2

The fecal TiO_2 concentration of the grab samples of experiment 5 and 6 were evaluated separately by an ANOVA conducted with the MIXED procedure. For experiment 5, the model includes day and daytime as fixed effects and sheep as random effect. In experiment 6, additionally administration group as fixed effect was included in the model. The fecal TiO_2 concentrations of each sheep were treated as repeated measurements to consider the correlation between the fecal TiO_2 concentrations of the grab samples of one sheep. The best fit covariance structure was “compound symmetry”. Further, a multiple comparison between the means of the daytime was carried out.

Estimation of fecal dry matter output by grab samples

The linear relationships between the fecal dry matter output estimated by the TiO_2 concentrations in the different grab samples A, B, AB, and ABR and the directly measured fecal output were determined using the REG procedure.

2.4 Results

Fecal recovery of TiO_2

Table 2.2 summarises the results of the t-Test for the null hypothesis that the determined recovery is different from 100% ($h_0 \neq 100\%$). The mean fecal recoveries of all individual experimental groups ranged from 95.9% to 109.5%. All recoveries of the hay diets were significantly higher than 100%.

In the indoor experiments, diet had a significant effect ($P < 0.001$) on fecal recovery, with 98.9% for the hay+concentrate and 108.0% for the hay diets. In grazing experiment 4, mean fecal recovery of TiO_2 was significantly lower ($P = 0.014$) in the grazing intensity of 2 sheep per ha than of 5 sheep per ha (100.4% and 107.0%, respectively).

Table 2.2. Animal weight, diets fed and diet composition, digestibility, and fecal TiO_2 recovery in experiments 1 to 4

experiment	**1**					**2**	**3**	**4**	
diet	**1**		**2**		**3**			**1**	**2**
body mass (kg)	33.3±2.6		36.5±1.6		37.4±2.6	39.8±3.4	40.3±3.6	56.0	55.3
diet	hay 1	conc. 1	hay 1	conc. 2	hay 2	hay 3	hay 4	grazing	grazing
OMI[1)] (g/d)	650	358	750	385	1065	1019	1175	1607	1502
CA[2)]	53	70	53	135	61	50	73	-	-
CP[2)]	92	184	92	166	75	77	85	-	-
CL[2)]	16	32	16	22	17	20	25	-	-
NDF[2)]	762	194	762	359	783	771	672	-	-
ADF[2)]	404	80	404	214	394	401	397	-	-
Lignin (sa)[2)]	65	24	65	49	48	48	58	-	-
dOM[3)]	0.592		0.548		0.534	0.565	0.471	0.579	0.559
recovery (%)	102.0		95.9		107.9	106.3	108.8	100.4	107.0
SE	1.13		2.35		2.40	2.57	1.11	1.83	1.58
P[4)] ($\neq 100$)	0.120		0.121		0.013	0.049	<0.001	0.814	0.002

1) mean organic matter intake of the sheep (g/day), in experiment 4 calculated by the digestibility of organic matter and the fecal organic matter excretion
2) concentration of crude ash (CA), crude protein CP), crude lipid (CL), neutral detergent fibre (NDF),acid detergent fibre (ADF),and acid detergent lignin (Lignin (sa)) in g per kg DM
3) digestibility of organic matter, in experiment estimated by the fecal crude protein content according to Wang (2007)
4) Probability of the t-test with the null hypothesis that mean recovery is different from 100% (α=0.05)

Daily fecal recovery of TiO_2 after the start of TiO_2 administration

The mean daily fecal TiO_2 recoveries of the days after first application of TiO_2 are given in Table 2.3. The mean recovery increased from 31.9% on day 1 to 98.5% on day 5. Significant differences existed between the first four days and the last nine days. However, no significant differences were observed between days 4 to 13, although recovery tended to be lower than 100% on day 4 (P = 0.073) and tended to be higher on day 10 (P = 0.062).

Table 2.3. Mean fecal TiO_2 recovery of the days after starting TiO_2 administration

day	recovery (%)	SE[1)]	range (%)	P ($h_0 \neq 100$)[2)]
1	31.9[a]	5.98	10.9 – 60.6	<0.001
2	78.2[b]	4.29	65.8 – 105.5	0.001
3	89.9[bc]	5.67	69.0 – 113.4	0.118
4	92.1[cd]	3.74	75.7 – 110.3	0.073
5	98.5[cd]	4.44	82.1 – 120.1	0.746
6	97.5[cd]	4.22	70.9 – 110.3	0.593
7	104.7[d]	3.12	89.9 – 119.7	0.172
8	101.2[cd]	4.24	86.7 – 125.1	0.787
9	99.9[cd]	2.18	85.3 – 104.6	0.956
10	107.3[d]	3.30	93.3 – 118.8	0.062
11	104.8[d]	4.87	81.7 – 118.1	0.353
12	97.3[cd]	2.73	83.9 – 107.7	0.353
13	105.1[d]	2.82	93.9 – 117.6	0.113

1) Standard error

2) Probability of the null hypothesis, that the mean recovery is not equal to100%

Within a column mean values with a common superscript are not significantly different at $\alpha = 0.05$

Excretion pattern of TiO_2

The results of experiments 5 and 6 are shown in Table 2.4. In both experiments, a significant effect of sampling time on fecal TiO_2 concentration was found ($P < 0.001$ and $P = 0.023$, respectively). Moreover, in experiment 6 the interaction between application group and daytime, in which the grab samples were obtained from the rectum, was significant ($P = 0.006$). However, no significant effects were observed for the administration group ($P = 0.170$), the day ($P = 0.626$), and for the interactions group×day ($P = 0.966$) and day×time ($P = 0.335$). In experiments 5 and 6 for all groups the TiO_2 concentration in the feces decreased significantly from 9h to 17h.

Significant differences between the fecal concentrations of the different sampling times in the application groups in experiment 6 were only determined in the group, in which the sheep received in the evening 2.5g TiO_2. The TiO_2 concentration was significantly lower in the fecal grab samples obtained at 17h than at 9h and 13h. In the other two application groups no

significant differences were found. However, in the administration group with one pulse dose of 2.5 g TiO_2 in the morning, the TiO_2 concentration in the grab samples obtained at 13h tended to be lower than obtained at 17h (P = 0.055).

Table 2.4. Mean fecal TiO_2 concentration (mg/g DM) of the fecal grab samples, obtained at different times of the day

Experiment	Daily administration of TiO_2 (amount/time)	Fecal TiO_2 concentration (mg/g DM) at different times of grab sampling			SEM
		9 h	13 h	5 h	
5	2.5g/ 9h	4,36[a]	4,03[a]	3,62[b]	0,205
6	all groups	6.41[a]	5.55[b]	5.37[b]	0.608
	2.5g/ 9h	4,73[a]	4,03[a]	5,38[a]	1,053
	1.25g/ 9h, 17h	5,95[a]	4,82[a]	4,96[a]	1,053
	2.5g/ 17h	8,57[a]	7,80[a]	5,77[b]	1,053

SEM = standard error of the means

Within a row mean values with a common superscript are not significantly different at $\alpha = 0.05$

Estimation of fecal dry matter output by grab samples

The equations of the linear regressions are shown in Table 2.5. In administration groups with 1 and 2 doses of TiO_2 per day the pooling of the grab samples A and B to AB improved the estimation of fecal output ($r^2 = 0.747$ and $r^2 = 0.966$, respectively). In the administration group with one pulse dose of TiO_2 in the morning the calculated linear regressions were not significant for the grab samples A and B, but for AB and ABR (P = 0.331, P = 0.079, P = 0.005 and P = 0.005, respectively). In the other administration group with two pulse doses of TiO_2 per day regressions of all samples were significant. The slopes of the regression equations for the AB samples in the administration groups of 1 and 2 pulse doses TiO_2 per day indicated that the estimation of the fecal output was underestimated (90.0 % and 95.2 %, respectively). The correction of the AB estimates by the mean fecal recovery of 108.8 % led to improved estimation values of 99.6% and 102.2% for administration group 1 and 2, respectively.

Table 2.5. Linear regressions between fecal output directly measured (X, independent) and estimated (Y, dependent) by the fecal TiO_2 concentration of different grab samples in two groups of application frequency

administration group	sample	linear regression	r^2	SE[5)]	P_{model}[6)]
2.5g TiO_2 at 7h	A[1)]	Y = 0.904 × X	0.150	94.37	0.331
	B[2)]	Y = 0.897 × X	0.397	78.55	0.079
	AB[3)]	Y = 0.900 × X	0.747	30.94	0.005
	ABR[4)]	Y = 0.996 × X	0.723	35.49	0.005
1.25g TiO_2 at 7h and 19h	A[1)]	Y = 0.988 × X	0.654	47.08	0.014
	B[2)]	Y = 0.915 × X	0.602	58.01	0.023
	AB[3)]	Y = 0.952 × X	0.966	12.88	<0.001
	ABR[4)]	Y = 1.022 × X	0.920	26.04	<0.001

1) pooled grab sample of 7 days obtained at 7h

2) pooled grab sample of 7 days obtained at 19h

3) pooled grab sample of grab samples A and B

4) AB corrected for the mean fecal recovery of the experiment

5) Standard error

6) Probability of the null hypothesis that the slope of the linear regression is different from 0.

2.5 Discussion

Fecal recovery

The mean fecal recoveries of TiO_2 in all diet groups in our study ranged from 95.9% to 108.8%. Mean fecal recoveries of TiO_2 ranged in chicken from 98.7 % to 99.7% (Short et al.,1996), in pigs from 96.9% to 98.3% (Jagger et al., 1992), in cattle from 95.5% to 101.5% (Hafez et al., 1988), or 90.0% to 95.0% (Titgemeyer et al., 2001), and in sheep from 96% to 99%. (Brandt et al., 1987). In these studies fecal TiO_2 recoveries were not significantly different from 100%. Njaa (1961) assumed that mean recoveries of less than 100% in rats are due to losses of TiO_2 during feeding or inaccuracy of the analysis. Contrary recoveries significantly higher than 100% were found in the hay diets and the high grazing intensity group of our study (Table 2.2). The supplementation of concentrates to hay diets seemed to decrease the recovery. Titgemeyer et al. (2001) found no differences in the recovery between

a hay diet and hay diets with different supplements. However, the statistical power of this study was low due to only two observations per diet.

The significant lower fecal recovery in the low grazing intensity of experiment 4 could be caused by an enhanced ingestion of TiO_2 contaminated soil in the high grazing intensity. We found in the soil of the study area an analytical TiO_2 concentrations of about 2.2 mg/g DM. Fries et al. (1982) reported that soil ingestion of grazing cattle can go up to 8% of dry matter intake. If we assume this very high value and consider the herbage intake of approximately 1500g DM/d of the sheep in the high grazing intensity, the mean daily soil ingestion would be $0.08 \times 1500g = 120$ g. This leads to an additional daily TiO_2 ingestion of 264 mg or 10.6% of the applied 2500 mg TiO_2 per day. An increased soil ingestion in high grazing intensities caused by low plant cover of the area, would be reflected in increased crude ash contents in feces. In the low grazing intensity group the fecal crude ash content tended with 129 g/kg DM to be lower ($P = 0.071$) than in the high grazing intensity with 141 g/kg DM at a similar fecal output level (776 g versus 772 g DM/day). However, the difference of the fecal crude ash means of the two groups were 1.2 percent units at a fecal output of 775 g DM per day, and a soil crude ash content of 700 g/kg DM. Thus, it would result in enhanced soil ingestion of $0.012 \times 775g / 0.7 = 13.3$ g soil per day in the sheep of the high grazing intensity compared to the sheep of the low grazing intensity. This justifies only an increase of 1.3 % in the fecal TiO_2 recovery. Furthermore, it does not explain the high fecal TiO_2 recoveries of the hay diets. Mayland et al. (1975) reported that TiO_2 is contained only in small quantities (less than 1 ppm) in plants not contaminated with soil. The crude ash content of the hay used in experiments 1, 2 and 3 differed between 51 and 72 g/kg DM, which does not indicate a considerable contamination with soil. The analysis of the hay for TiO_2 showed contents of 0.03 mg TiO_2 per g DM. This would increase the recovery for only 1.8 % at a feed intake of 1500 g DM/d and a daily administration of 2.5 g TiO_2. However, such small TiO_2 contents are under the accuracy level of the analysis. More research is needed to determine the reason for the high observed fecal recoveries. The natural occurrence of TiO_2 in feces should be determined with sensitive analysis in different grazing intensities to examine if an enhanced soil ingestion occurs and if it leads to higher contents of TiO_2 in feces, which would increase the fecal recovery with increasing grazing intensity.

Daily fecal recovery of TiO_2 after the start of TiO_2 administration

The mean daily fecal TiO_2 recoveries of 4 to 13 were not significantly different and did not deviate from 100%. However, day 4 tended to be lower than 100%. Therefore, an adaptation period of at least 5 days is recommended. Difficult to explain is the high variation between animals, which is persistent until the last days of measurement of daily recovery, and the high daily fecal recoveries of some animals on day 1 and 2 after first administration (60.6% and 105.5%, respectively). On day 10 a tendency was detected that the fecal recovery was higher than 100% ($P = 0.062$). This was probably due to the high recoveries discussed above.

Our results are in correspondence with Titgemeyer et al. (2001), who found a mean fecal recovery of TiO_2 in two steers of 79.4% on day 2 to 6, 94.3% on day 7 to 11, 91.2% on day 12 to 16, and 98.3% on day 17 to 21 after first TiO_2 administration. Only the mean recovery of the first period tended to be less than 100% ($P = 0.09$). Furthermore, a high variability of TiO_2 recoveries between the two animals was found. Rothfuß (1996) showed that a single pulse dose of TiO_2 given to a steer fed an energy level of 1.5 maintenance was completely excreted in feces within 120 hours and had a peak of excretion 24 hours after marker application. This corresponds with our results and supports the assumption that an adaptation period for TiO_2 of five days is sufficient for reliable measurements.

Excretion pattern of TiO_2

Dove and Mayes (1991) stated that after achieving an equilibrium in marker intake and excretion, the variation of fecal marker concentration within a day is the main problem for accurate estimation of fecal output by marker concentration in fecal grab samples. In experiments 5 and 6, a diurnal excretion pattern for TiO_2 was found. In both experiments, the grab sample obtained at 9h had a significantly higher TiO_2 concentration than the grab sample obtained at 17h. The explanation for this excretion pattern could be, that the sheep had a main grazing time in the morning. The marker flow out of the rumen would be high at this time and the remaining TiO_2 in the rumen is diluted by new herbage ingested. Thus, TiO_2 concentration in digesta would decrease at least until the next main grazing time after the hot temperatures at midday. These assumptions are supported by Myers et al. (2006), who observed an increase of TiO_2 concentration in duodenal digesta until a maximum at 2 to 4 hours after feeding and subsequent a decrease until the next feeding. However, it is difficult to explain why the administration of one TiO_2 pulse dose in the evening led to a similar excretion pattern than the administration of one pulse dose in the morning in experiment 6.

Myers et al. (2006) further assumed that a more frequent marker application may produce more constant diurnal fecal marker concentrations, because the marker is mixed more homogenously in the digesta. Our results support this assumption. In experiment 6 only the application group with two pulse doses per day showed no significant or tendentious differences in the fecal TiO_2 concentrations of 9h, 13h and 17h.

Estimation of fecal dry matter output by grab samples

Similar as in the excretion pattern experiments, the fecal output estimation in experiment 3 showed a positive effect of higher administration frequency of the marker. The regression slope of the fecal output estimations in the sheep, which received two pulse doses of TiO_2 per day, where significantly different from zero for all different types of pooled grab samples. The output estimations from the sheep, which received only one pulse dose per day, gave only reliable fecal output estimations for the pooled grab sample AB. In the administration group of two marker pulse doses per day, the grab sample AB gave the most accurate fecal output estimation. Both slopes of the AB samples in either administration groups underestimated fecal output. This could be due to the fecal TiO_2 recovery significantly higher than 100% in this experiment. This is confirmed by the better ABR estimates of the fecal output in both administration groups.

The results of the fecal output estimation by the fecal TiO_2 concentration in grab samples implicate that the increase of administration frequency from one to two pulse doses per day improves the accuracy of fecal output estimation. Moreover, the increase of collecting frequency from one to two grab samples per day, which are pooled to one is recommended to obtain reliable estimations. However, to realize this advice in grazing experiments, the higher effort in work and the enhanced stress for the animals, which could change the grazing behaviour should be considered. In experiment 3, a lower feed intake of 1135 g DM/d was detected in the sheep which received two pulse doses of TiO_2 per day compared to the sheep which received one pulse dose per day with 1286 g DM/d ($P = 0.034$).The reason for this difference may be more stress caused by administering two instead of one gelatine capsules per day to the sheep

2.6 Conclusions

The fecal recovery of TiO_2 was in some experiments significantly higher than 100%. This could be due to additional ingestion of TiO_2, which is abundant in the soil of the study area. Thus, an increase of soil ingestion with increasing grazing intensity could make a correction for TiO_2 related to grazing intensity necessary. However, more research is needed to determine the natural occurrence of TiO_2 in feces. After 5 days of TiO_2 application, an equilibrium in TiO_2 ingestion and excretion was achieved, and this is the minimum adaptation period.

In grazing experiments with administration of one TiO_2 pulse dose per day a diurnal excretion pattern was determined, which may be caused by inhomogeneous blending of the marker in digesta. Contrary, no diurnal pattern was found in sheep which received two pulse doses per day. Furthermore, the most reliable fecal output estimation was obtained with twice daily administration and fecal grab sampling. This leads to the recommendations for grazing experiments that the daily amount of TiO_2 should be administered to animals in two pulse doses per day and that two grab samples should be obtained per day.

In grazing experiments the animals should be stressed as little as possible. In our study, the effects of increasing the number of experimental treatments from one to two per day were not determined, but in a feeding trial a decreased feed intake was detected in the sheep, which received two instead of one capsules containing TiO_2 per day. At last the increase in work, time and costs by catching the grazing sheep two times per day should be considered, especially in large grazing experiments.

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3 Impact of grazing intensity on herbage mass, forage quality, live weight gain, and herbage intake of sheep on the Inner Mongolian steppe

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3.1 Abstract

The steppe of Inner Mongolia, China has severe ecological problems that are mainly caused by overgrazing. Within the Sino-German research collaboration "Matter Fluxes of Grasslands in Inner Mongolia as influenced by stocking rate" (MAGIM) a grazing experiment with six different grazing intensities of sheep (1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 sheep per ha) was conducted in the grazing season of 2005 in the Xilin River Basin. The objectives were to determine the effects of grazing intensity on herbage mass, forage quality, live weight gain, and herbage intake, and to derive an optimal grazing intensity, which realizes a high animal performance in a sustainable ecosystem. The live weight gain per sheep decreased significantly with increasing grazing intensity, whereas intake of organic matter and digestible organic matter per sheep tended to decrease ($P = 0.090$ and $P = 0.065$, respectively). The digestibility of organic matter ingested and offered were not found as influenced by grazing intensity ($P = 0.116$ and $P = 0.471$, respectively). Herbage mass decreased ($P = 0.035$) from 1500 kg DM/ha on the lowest grazing intensity to 600 kg DM/ha on the highest grazing intensity. The composition of the offered herbage was not affected by grazing intensity, except the ADL content ($P = 0.039$) which increased with grazing intensity. Significant relationships between ADL and digestibility of organic matter ingested as well as herbage intake indicate the high meaning of lignification of the fibre rich herbage (NDF = 726g/kg DM ± 7.1 SE). Herbage intake per ha increased significantly with grazing intensity ($P < 0.001$), whereas live weight gain per ha was significantly lowest at grazing intensity 1.5 sheep per ha and no differences were found among the other grazing intensities ($P = 0.049$). Therefore, it can be concluded that short term heavy grazing does not lead to a reduced animal performance per ha. However, long term heavy grazing is expected to reduce productivity of the grassland and consequently may reduce animal performance. Since in our one year study long term effects of grazing intensity could not be determined and grassland productivity varies greatly with precipitation in the Inner Mongolian steppe, no general recommendation for an optimal grazing intensity could be derived.

3.2 Introduction

The ecosystem of the Inner Mongolia Autonomous Region, China belongs to one of the largest grassland regions in the world. The farming consists mainly of grazing livestock production systems. In the last decades the natural grassland of Inner Mongolia was degraded by unsustainable grazing. Kawamura et al. (2005) reported an increase of livestock density in the Xilin River Basin from 0.49 sheep units per ha in 1983 when the ownership of the land altered from governmental to private to 0.76 sheep units per ha in 2001. According to Zhang et al. (2006) heavy grazing leads to a decreased plant cover and vegetation height. This causes an increase of wind erosion in the dry and windy winter of Inner Mongolia. The severe consequences are desertification and decreased productivity of the grassland and increased sand and dust storm frequencies. The sand and dust storms cause every year high economic damages in central China accompanied by health injuries of the population.

According to Garcia et al. (2003) increasing grazing intensity of sheep can lead to decreasing herbage intake of grazing sheep, when offered herbage mass is reduced and energy requirement for maintaining high herbage intakes are high. Spedding (1965) stated that sheep are grazing highly selective, which can lead to large differences between herbage quality offered and ingested. Moreover, increasing the grazing intensity can reduce the offered herbage mass and therefore limit herbage selection, which can lead to decreasing quality of herbage ingested (Animut et al., 2006). Consequently sheep grazing in high intensities could be limited in performance due to reduced herbage intake and quality of herbage ingested.The objectives of this study are to determine the effects of grazing intensity on herbage mass, forage quality, live weight gain, and herbage intake of sheep. Furthermore the optimal grazing intensity, which realizes high animal productivity under sustainable ecological conditions, is derived and used to give recommendations for sustainable land use in Inner Mongolia.

3.3 Materials and methods

Study area

The study area includes 28 ha within an experimental site of 200 ha in the Xilin River Basin, Inner Mongolia Autonomous Region, China (116°42' E, 43°38' N) and belongs to the Inner Mongolia Grassland Ecosystem Station (IMGERS), which is administered by the Institute of Botany, the Chinese Academy of Sciences, Beijing. According to the slope of the area it was divided into two blocks: "Flat" and "Slope". The pasture is dominated by two grass species: the perennial rhizome grass *Leymus chinensis* and the perennial bunchgrass *Stipa grandis*. The diversity of the plant species of the two different blocks is given in Table 3.1.

Table 3.1. Diversity of plant species in the flat and slope areas of the study area (mean of green above ground dry matter (DM) biomass in percent ± standard deviation, determined in the beginning of July 2005)

Plant species	**"Flat"**	**"Slope"**
Leymus chinensis	45.0 ± 10.8	30.6 ± 11.0
Stipa grandis	24.8 ± 8.9	28.7 ± 13.1
Agropyron michnoi	8.1 ± 7.5	15.6 ± 6.3
Carex korshinskyi	7.6 ± 3.2	11.1 ± 5.0
Cleistogenes squarossa	7.6 ± 1.7	5.9 ± 2.9
Achnatherum sibiricum	2.2 ± 2.1	3.9 ± 3.3
others	4.8 ± 2.3	4.2 ± 2.7

The soil of the study area was determined as a calcic chernozem (IUSS Working Group WRB, 2006). The mean precipitation and temperature from 1982 to 2003, measured at a weather station near IMGERS were 343 mm and 0.7 °C, respectively. The precipitation of the grazing season 2005 was very low, as shown in Figure 3.1.

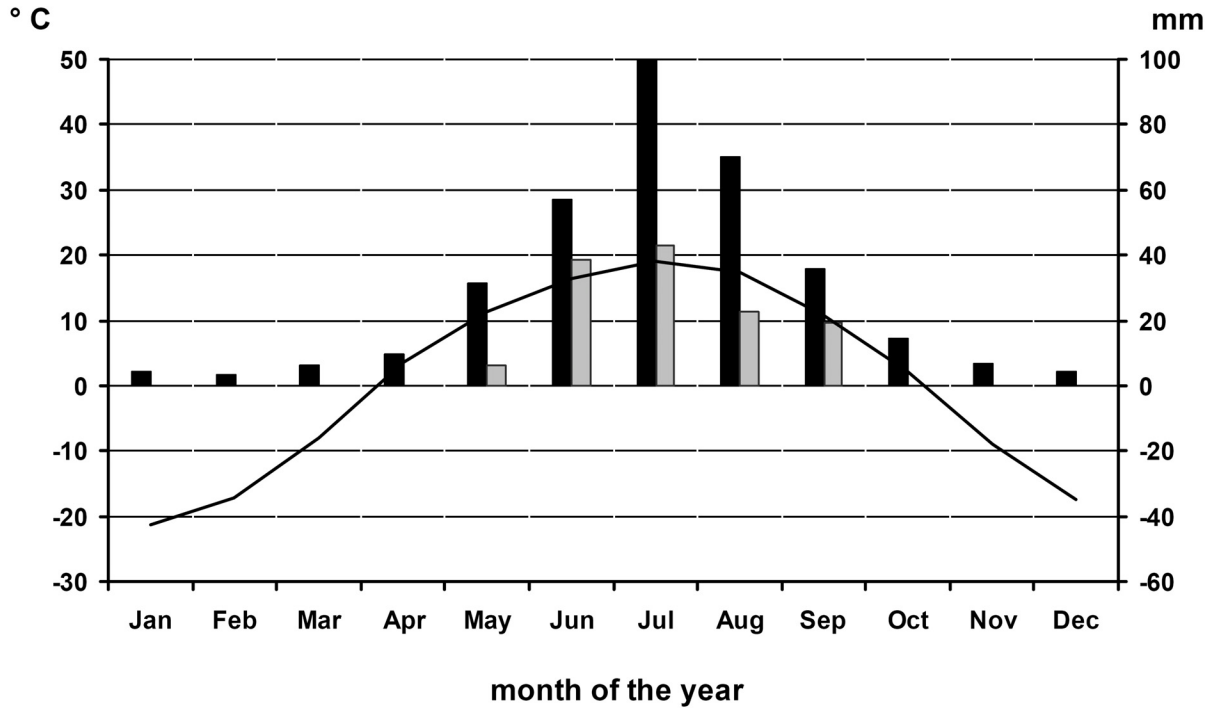


Figure 3.1. Average air temperature (°C, line) and precipitation (mm, columns) of the months from 1982 to 2003 (dark columns) and in the grazing season 2005 (bright columns) at IMGERS.

Animals

132 non-pregnant and non-lactating female sheep from the local fat-tailed breed were used. The sheep were born in spring 2004, and were at the beginning of the grazing experiment in June 2005 approximately 15 months old with an average live weight of 31.6 kg ± 4.8 kg. During the grazing experiment the sheep had free access to water and minerals in lick stones. In July the animals were treated against endoparasites.

Design of the grazing experiment

The grazing experiment was conducted in the growing season of 2005. The sheep were driven to the grazing area on June 10 and removed on September 16. Thus the grazing season lasted 98 days. Six different grazing intensities were installed: 1.5, 3.0, 4.5, 6.0, 7.5 and 9.0 sheep per ha. Except the lowest grazing intensity the size of all experimental plots were 2 ha. The number of animals were adjusted accordingly. In the lowest grazing intensity with 1.5 sheep per ha the size of the plot were 4 ha to achieve 6 sheep per plot. Each grazing intensity had a

replication in the flat and slope area. In every experimental plot 6 sheep were used for measurements of feed intake and digestibility of herbage ingested. The scheme of the grazing experiment is shown in Table 2.

Table 3.2. Scheme of the grazing experiment

Grazing intensity (sheep/ha)	**1.5**	**3.0**	**4.5**	**6.0**	**7.5**	**9.0**
area per plot (ha)	4	2	2	2	2	2
sheep per plot	6	6	9	12	15	18
sheep used for measurements	6	6	6	6	6	6

Herbage measurements

The samples of the measurements for offered herbage mass (HM) and herbage quality were obtained at one day of the fecal collection period (see below) by manual sward cutting three 0.25 m × 2 m transects, representatively selected for each plot. Before the standing herbage at assumed grazing height of 1cm was cut, the litter was combed out. The collected herbage material was pooled by plot, weighed and dried in a 60°C oven for 24 h. The content of organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid deteregent fibre (ADF) and acid deteregent lignin (ADL) were determined in the herbage samples by the NIRS system after calibration. The calibration was carried out by laboratory analysis of sub-sets of herbage samples, which were selected randomly. Dry matter content was determined by drying at 105°C until a constant dry weight was reached. OM content was calculated as the difference between the dry sample and the residue (ash) after incineration of the dry sample at 550°C over night. The CP content was calculated from the nitrogen (N) content (CP = N× 6.25), which was analyzed by a C/N-Analyzer (vario Max CN, Elementar Analysensysteme, Hanau, Germany) which is based upon the DUMAS combustion method. NDF, ADF, and ADL were analyzed sequentially by an ANKOM 200 Fiber Analyzer (Ankom Technology, Macedon, NY, USA) according to the procedures described by Van Soest and Wine (1967). In vitro digestibility of organic matter was determined with the cellulase technique according to De Boever (1993).

Measurements on animals

Live weight gain

The live weight of six animals per plot was measured at day 0 (“start”), at day 49 (“middle”) and at day 98 (“end”) of the grazing season. Daily and total live weight gain was calculated for the periods from day 0 to 49, day 49 to 98 and day 0 to 98.

Digestibility of organic matter ingested

The digestibility of organic matter ingested (DOM) was determined by the fecal crude protein method using the regression equation of Wang (2007), which was fit on digestibility data from sheep in Germany and Inner Mongolia. For measuring the CP and OM content of the feces, one grab sample per day was obtained from six sheep per plot on five following days in three periods: July 11 – 15 (“July”), August 8 - 12 (“August”) and September 12-16 (“September”). Because of the two plot replications twelve sheep per grazing intensity were examined. Daily grab samples were pooled by animal and period and analyzed for concentrations of DM, OM, CP and titanium dioxide (TiO_2).

Herbage intake

The daily organic matter intake (OMI) and digestible organic matter intake (DOMI) per sheep was estimated in vivo in two and three steps respectively. In the first step the daily fecal organic matter output (OMO) was calculated by the fecal concentration of the inert marker TiO_2, which is assumed to be inert in the digestive tract and to be distributed equally in the digesta. TiO_2 is commonly used in digestibility studies to avoid total collection of feces by grab sampling and measuring the passage of digesta as shown by Jagger et al. (1992), Südekum et al. (1995), Short et al. (1996), Titgemeyer et al. (2001), and Myers et al. (2006). The marker was administered orally to the sheep as a daily pulse dose of 2.5g by a gelatin capsule at three ten days periods: July 6 - 15 (“July”), August 3 - 12 (“August”) and September 7 - 16 (“September”). On the last five days of these periods, grab samples of feces were obtained daily from the rectum correspondingly to the sampling for measuring of the fecal nitrogen content.

The concentration of TiO_2 was measured photometrically after extraction by the Kjeldahl method as described in Chapter 2. OMO was calculated by dividing the daily intake of TiO_2

(mg) by the fecal concentration of TiO_2 (mg/g DM), assuming a fecal recovery of TiO_2 of 100%. In the second step, OMI was calculated with the estimation of DOM by equation of Wang et al. (2007) and OMO as follows:

$$\text{OMI [g/day]} = \text{OMO [g/day]}/ (100\text{-DOM}) \times 100$$

For calculating the daily intake of digestible organic matter the calculated OMI was multiplied by DOM.

Statistical analysis

Analysis of variance

An analysis of variance was conducted as a mixed model with block, grazing intensity, and period as fixed effects as well as sheep and block × intensity as random effects using the MIXED procedure of SAS (1988). The measurements obtained on one sheep at different periods were treated as repeated measurements with sheep as subject. The best fit covariance structure was compound symmetry. The data obtained from the animals were analyzed with the subsequent model:

$$Y_{ijkl} = \mu + B_i + GI_{ij} + S_{ijk} + B \times GI_{ij} + P_{ijkl} + B \times P_{ijkl} + GI \times P$$

With:

B_i: fixed effect of the area (i = flat or slope).

GI_{ij}: fixed effect of the grazing intensity (j = 1…6) within block i

S_{ijk}: random effect of sheep k (1…6) within block i and grazing intensity j

P_{ijkl}: fixed effect of the period l within block i, grazing intensity j and sheep k

$B \times GI_{ij}$: random effect of the interaction of block i and grazing intensity j

$B \times P_{ijkl}$: fixed effect of the interaction of block i and period l

$GI \times P_{ijkl}$: fixed effect of the interaction of grazing intensity j and period l

For the analysis of the data obtained from the herbage samples, a similar model was used, but plot instead of sheep is the random effect. The best fit covariance structure was compound symmetry as well.

Relationships between herbage parameters and quality of herbage ingested (DOM) and organic matter intake

Linear regression equations between herbage parameters (HM, CP, NDF, ADF, ADL) ad the independent variables and DOM as well as OMI per sheep ad dependent variables were calculated with the REG procedure of SAS (1988).

3.4 Results

Analysis of variance

The probabilities of the effects of block, grazing intensity, measuring period are given in Table 3.3. Tables 3.4 and 3.5 show the means of the examined parameters due to the effects of measuring period and grazing intensity, respectively.

Table 3.3. Probability values of the effects for the different parameters. Herbage mass (HM), digestibility of organic matter offered (IVDOM), digestibility of organic matter ingested (DOM), organic matter intake (OMI), digestible organic matter intake (DOMI), average daily gain (ADG)

Parameter	**block**	**GI[1)]**	**period**	**block × period**	**period × GI[1)]**
HM (kg DM/ha)	0. 35	0.035	0.013	0.607	0.574
CP (g/ kg DM)	0.350	0.788	< 0.001	0.013	0.701
NDF (g/kg DM)	0.239	0.684	0.012	0.059	0.026
ADF (g/kg DM)	0.076	0.386	0.005	0.180	0.732
ADL (g/kg DM)	0.757	0.039	< 0.001	0.006	0.152
IVDOM (g/g)	0.034	0.471	< 0.001	0.300	< 0.001
DOM (g/g)	0.163	0.116	< 0.001	0.002	0.087
OMI (g /sheep/d)	0.153	0.090	0.011	0.608	0.001
DOMI (g/sheep/d)	0.295	0.065	< 0.001	0.321	0.001
OMI (kg/ha/d)	0.386	<0.001	0.085	0.799	0.338
DOMI (kg/ha/d)	0.618	<0.001	0.017	0.674	0.257
ADG (g/sheep/d)	0.372	0.018	<0.001	0.879	<0.001
ADG (g/ha/d)	0.296	0.049	0.002	0.542	0.035

1) GI = grazing intensity (sheep/ha)

The block did not affect the parameters measured. Only IVDOM was significantly (P = 0.034) lower in the flat area compared to the slope area with 0.559 and 0.581, respectively. Furthermore the ADF content of the herbage offered tended (P = 0.076) to be lower in the slope area than in the flat area with 342 g/kg DM and 352 g/kg DM, respectively.

Table 3.4. Mean values of herbage mass (HM), digestibility of organic matter offered (IVDOM), digestibility of organic matter ingested (DOM), organic matter intake (OMI), and digestible organic matter intake (DOMI) in three periods of the grazing season.

Parameter	Period			Standard error
	July	August	September	
Herbage				
HM (kg DM/ha)	1042[a]	1004[a]	711[b]	120.1
CP (g/kg DM)	98.1[a]	88.4[b]	76.8[c]	0.63
NDF (g/kg DM)	730[a]	726[ab]	723[b]	0.3
ADF (g/kg DM)	340[a]	348[b]	353[b]	0.3
ADL (g/kg DM)	40.9[a]	49.5[b]	54.1[c]	0.53
IVDOM (g/g)	0.588[a]	0.570[b]	0.551[c]	0.0041
Animals				
DOM (g/g)	0.565[a]	0.556[b]	0.538[c]	0.0033
OMI (g/sheep/d)	1263[a]	1148[b]	1144[b]	28.3
DOMI (g/sheep/d)	689[a]	639[b]	617[b]	16.9
OMI (kg/ha/d)	6292	5810	5742	207.2
DOMI (kg/ha/d)	3529[a]	3196[b]	3051[b]	121.3

Within a row means with a common superscript are not significantly different at $\alpha = 0.05$

Herbage mass, chemical composition and in vitro digestibility of herbage decreased significantly from July to September (Table 3.4). Correspondingly DOM and ADG per sheep, ADG per ha, OMI per sheep, DOMI per sheep, and DOMI per ha decreased significantly with proceeding grazing season.

Table 3.5. Means of herbage mass (HM), composition of herbage offered (CP, NDF, ADF, ADL), and in vitro digestibility of organic matter offered (IVDOM) in three periods of the grazing season as influenced by grazing intensity

Parameter	Period	Grazing intensity (sheep/ha)						SEM[1)]	P_{slice}[2)]
		1.5	3.0	4.5	6.0	7.5	9.0		
HM	July	1455	1206	999	1109	588	894	294.3	-
(kg DM/ ha)	August	1696	1257	716	1086	659	609	294.3	-
	September	1554	1067	499	488	355	301	294.3	-
	mean	1568	1177	738	894	534	601	259.7	-
CP	July	87	101	101	102	100	98	6.8	-
(g/kg DM)	August	83	93	87	89	87	92	6.8	-
	September	68	81	82	77	77	76	6.8	-
	mean	79	92	90	89	88	89	6.3	-
NDF	July	737	728	725	725	731	733	7.6	0.829
(g/kg DM)	August	723	723	725	721	733	733	7.6	0.763
	September	708	714	722	721	732	738	7.6	0.147
	mean	723	722	724	722	732	735	7.1	-
ADF	July	349	331	336	344	341	342	6.5	-
(g/kg DM)	August	348	342	332	352	357	347	6.5	-
	September	357	347	341	353	357	361	6.5	-
	mean	351	340	339	350	352	350	5.0	-
ADL	July	41.2	39.7	40.9	42.2	42.1	39.8	1.82	-
(g/kg DM)	August	45.7	49.0	49.6	49.4	53.0	50.2	1.82	-
	September	52.1	54.1	50.4	53.2	57.3	57.8	1.82	-
	mean	46.3^{a}	47.6^{ac}	47.0^{ac}	48.3^{abc}	$50,8^{b}$	49.3^{bc}	0.43	-
IVDOM	July	0.567	0.603	0.600	0.584	0.586	0.590	1.264	0.216
(g/g)	August	0.569	0.579	0.578	0.570	0.556	0.571	0.961	0.609
	September	0.561	0.561	0.563	0.559	0.534	0.525	0.636	0.085
	mean	0.566	0.581	0.0.580	0.571	0.559	0.562	0.379	-

Within a row means with a common superscript are not significantly different at $\alpha = 0.05$

1) Standard error of the means

2) Probability of the test that the grazing intensity has an effect within the respective period

Table 3.6. Means of digestibility of organic matter ingested (DOM), organic matter intake (OMI), digestible organic matter intake (DOMI), average daily gain (ADG) and live weight (LW) in three periods of the grazing season as influenced by grazing intensity

Parameter	Period	Grazing intensity (sheep/ha)						SEM[1)]	P_{slice}[2)]
		1.5	3.0	4.5	6.0	7.5	9.0		
DOM	July	0.580	0.582	0.565	0.558	0.549	0.559	0.0080	-
(g/g)	August	0.584	0.568	0.553	0.554	0.547	0.542	0.0080	-
	September	0.564	0.546	0.540	0.531	0.530	0.519	0.0080	-
	mean	0.573	0.565	0.552	0.547	0.542	0.540	0.0073	-
OMI	July	1234^{ac}	1441^{b}	1115^{a}	1140^{a}	1093^{a}	1281^{c}	69.0	0.004
(g/sheep/d)	August	1203	1187	1220	1194	957	1129	69.0	0.087
	September	1324^{a}	1278^{ac}	1128^{c}	1111^{c}	889^{b}	1135^{ac}	69.0	<0.001
	mean	1254	1302	1154	1148	980	1182	48.2	-
DOMI	July	715^{a}	838^{c}	629^{ab}	638^{ab}	599^{b}	716^{a}	41.3	0.001
(g/sheep/d)	August	692^{a}	673^{a}	676^{a}	659^{a}	522^{b}	612^{ab}	41.3	0.049
	September	747^{a}	699^{ab}	608^{b}	589^{b}	469^{c}	589^{b}	41.3	<0.001
	mean	718	737	638	629	530	639	35.1	-
ADG	day 1-49	91.6^{a}	93.7^{a}	96.7^{a}	76.5^{ab}	60.7^{b}	62.7^{b}	9.46	0.004
(g/sheep/d)	day 50-98	75.6^{a}	89.3^{a}	34.0^{b}	13.8^{b}	29.7^{b}	11.8^{b}	9.46	<0.001
	mean	83.6^{a}	91.5^{a}	65.3^{b}	45.1^{b}	45.2^{b}	37.2^{b}	7.79	-
OMI	July	1.85	4.32	5.01	6.84	8.20	11.53	0.508	-
(kg/ha/d)	August	1.82	3.56	5.49	7.16	6.66	10.17	0.508	-
	September	1.99	3.84	5.08	6.66	6.67	10.22	0.508	-
	mean	1.89^{a}	3.91^{b}	5.19^{b}	6.89^{c}	7.18^{c}	10.64^{d}	0.388	-
DOMI	July	1.07	2.52	2.83	3.83	4.49	6.44	0.297	-
(kg/ha/d)	August	1.04	2.02	3.04	3.95	3.60	5.51	0.297	-
	September	1.12	2.10	2.73	3.54	3.52	5.30	0.297	-
	mean	1.08^{a}	2.21^{b}	2.87^{b}	3.77^{c}	3.87^{c}	5.75^{d}	0.229	-
ADG	day 1-49	137	281	435	459	455	564	59.2	0.029
(g/ha/day)	day 50 - 98	113	268	153	83	222	106	59.2	0.329
	mean	125	275	294	271	339	335	46.2	-
LW	day 1	30.1	29.4	32.5	32.5	32.1	34.6	-	-
(kg)	day 49	34.6	34.0	37.2	36.2	35.1	37.7	-	-
	day 98	38.3	38.4	38.9	36.9	36.5	38.3	-	-

Within a row means with a common superscript are not significantly different at $\alpha = 0.05$

1) Standard error of the means

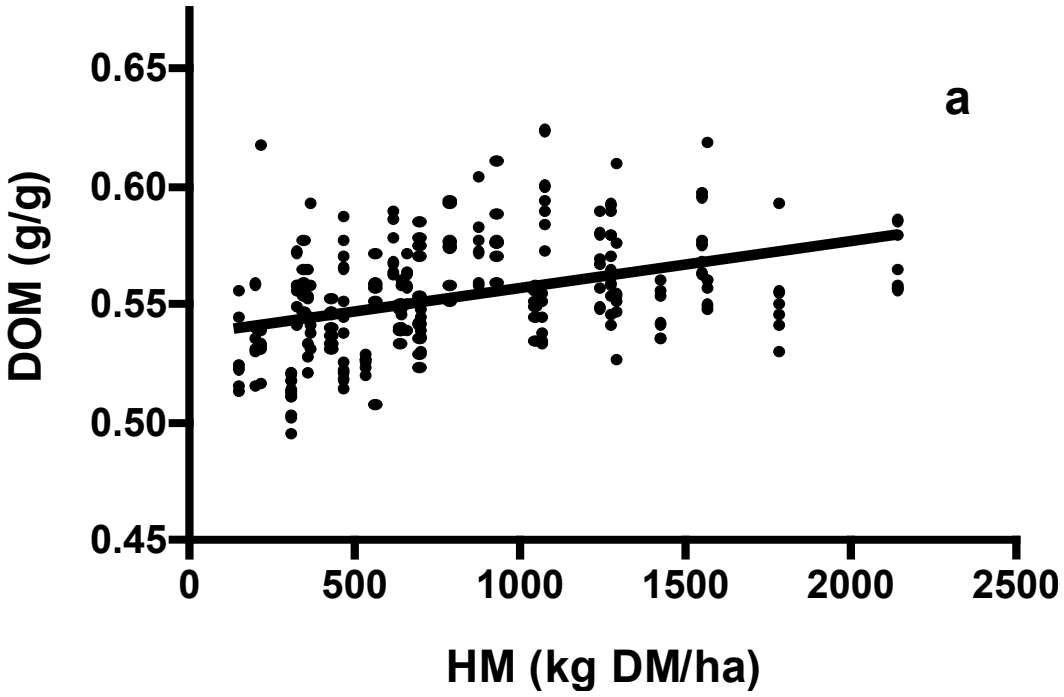
2) Probability of the test that the grazing intensity has an effect within the according period

The means of the parameters for the grazing intensities are shown in Table 3.5 and 3.6. The grazing intensity showed no significant effect for the parameters measured on the animals in unit per sheep and day, except for HM and ADG per sheep ($P = 0.035$ and $P = 0.007$, respectively). However, both herbage intake parameters OMI per sheep and DOMI per sheep showed a tendency ($P = 0.090$ and $P = 0.065$, respectively) to decrease with grazing intensity. In contradiction to this, OMI and DOMI per ha increased with grazing intensity ($P < 0.001$), and ADG per ha was lowest at the lowest grazing intensity ($P = 0.049$). The other five intensities did not differ in ADG per ha significantly.

The interaction between grazing intensity and period was significant for NDF content and IVDOM of herbage mass offered ($P = 0.026$ and $P < 0.001$, respectively). For parameters measured on animals the interaction was significant for OMI and DOMI per sheep as well as for ADG per sheep ($P = 0.001$, $P = 0.001$ and $P = 0.035$, respectively). Further, it tended to affect IVDOM and ADG per ha ($P = 0.087$ and $P = 0.077$, respectively). However, within each period grazing intensity did not affect NDF and IVDOM. In September, IVDOM was in a small range from 0.559 to 0.563 g/g in grazing intensities 1.5 to 6.0 sheep per ha and tended to be lower ($P = 0.085$) on the 7.5 and 9.0 sheep per ha intensities with 0.534 and 0.525 g/g, respectively. For OMI, DOMI and ADG per sheep, grazing intensity had a significant effect in all three periods, except for DOMI per sheep in August, where only a tendency could be found. The means and probabilities are given in Table 3.6.

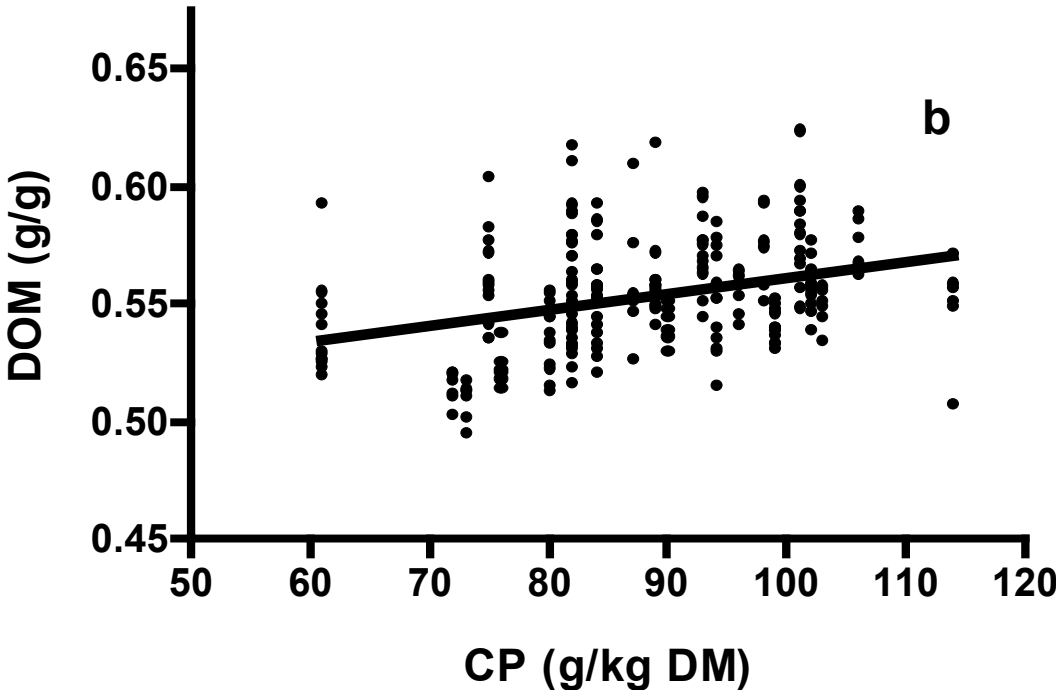
Relationships between herbage parameters and quality of herbage ingested (DOM) and organic matter intake

Figure 3.2 shows the relationships between herbage parameters and DOM as well as OMI per sheep. Between DOM and all measured herbage parameters HM, CP, NDF, ADF, ADL and IVDOM significant relationships were found. The closest relationship to DOM had ADL and IVDOM, whereas the NDF content of herbage offered showed only a slight influence on DOM. For OMI per sheep only a significant influence of HM and ADL could be found.



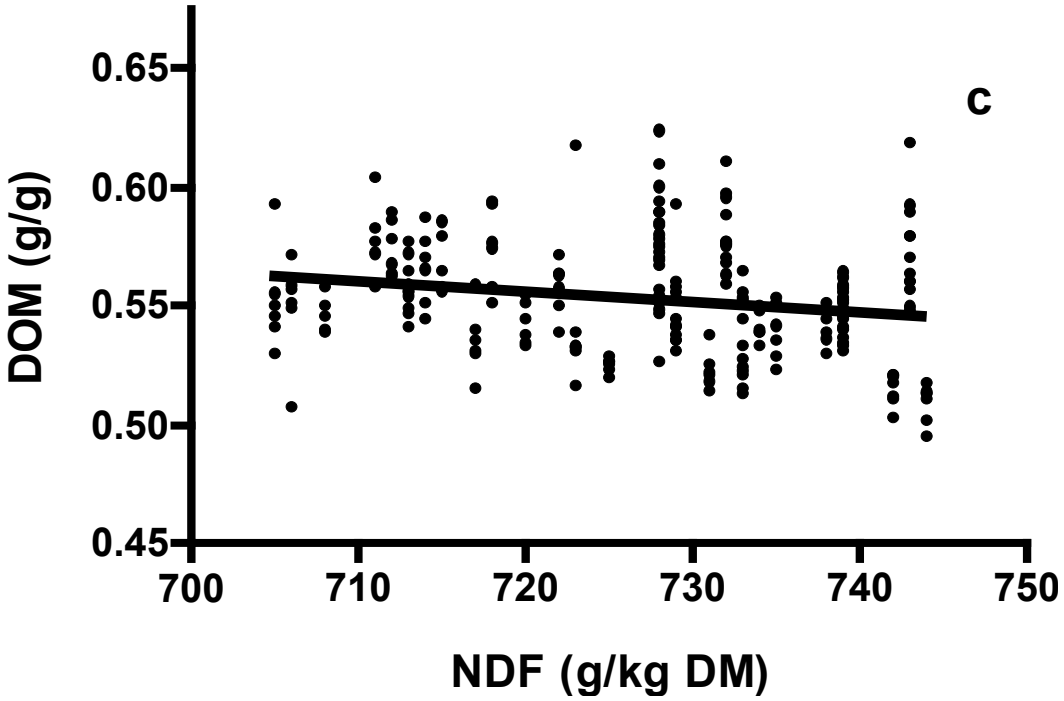
$DOM = 0.537 + 0.0000202 \times HM$, $r^2=0.165$,

$Sy.x = 0.0221$, $P < 0.001$



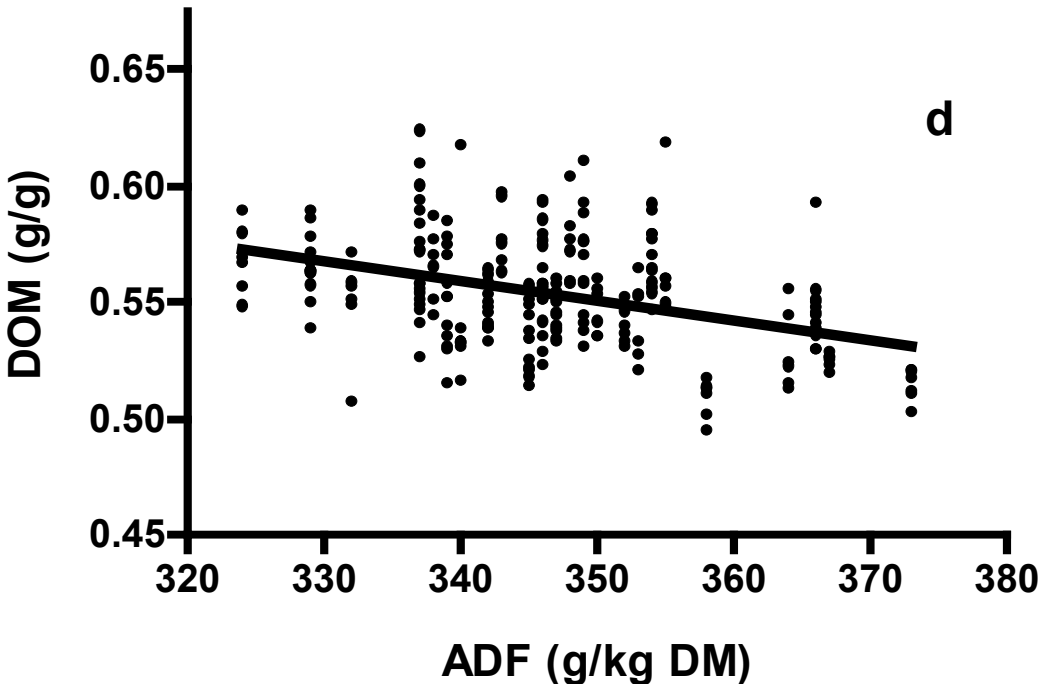
$DOM = 0.494 + 0.000673 \times CP$, $r^2=0.1143$,

$Sy.x = 0.0221$, $P < 0.001$



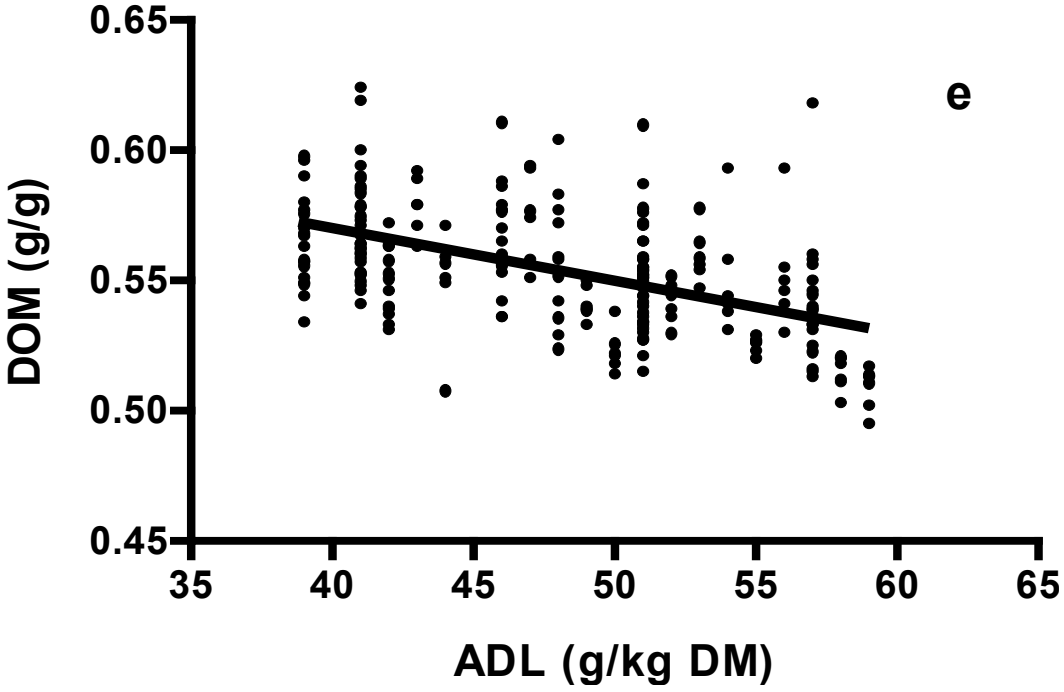
DOM = 0.884 - 0.000455×NDF, r^2=0.046,

Sy.x = 0.0236, P = 0.002

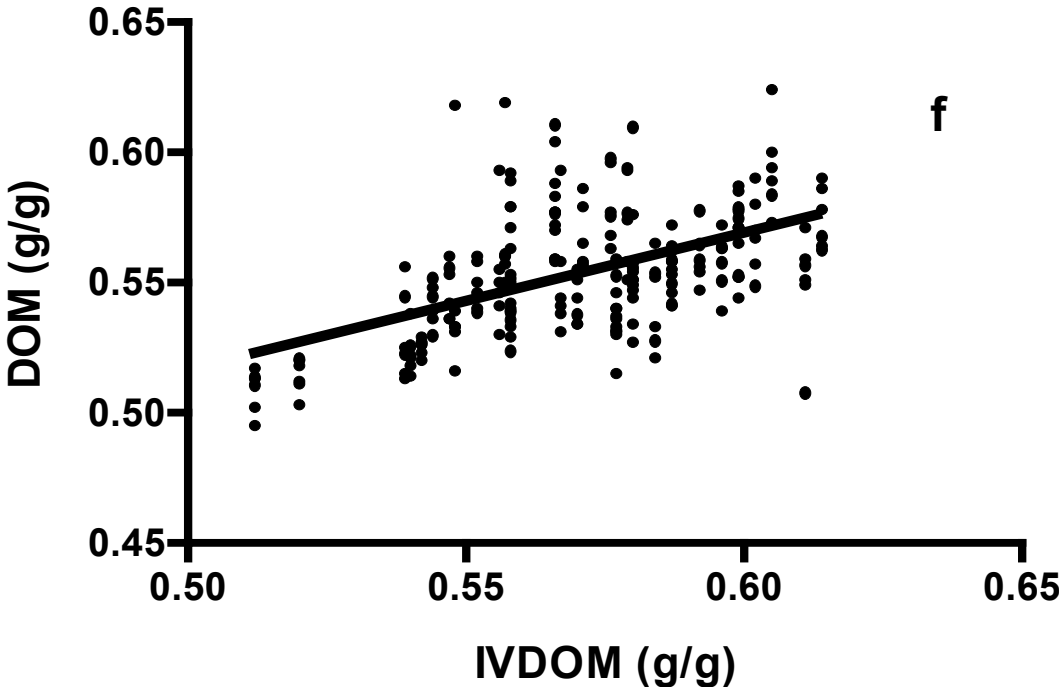


DOM = 0.845 + 0.000841×ADF, r^2=0.152,

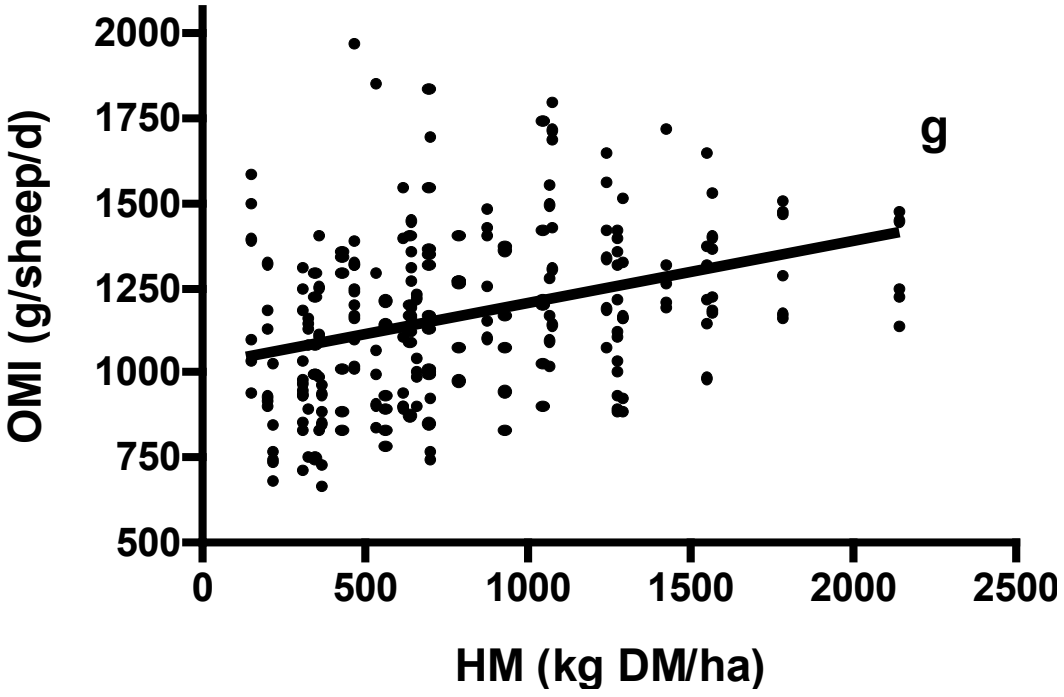
Sy.x = 0.0223, P < 0.001



DOM = 0.651- 0.00203×ADL, r^2=0.261,
Sy.x = 0.0208, P < 0.001

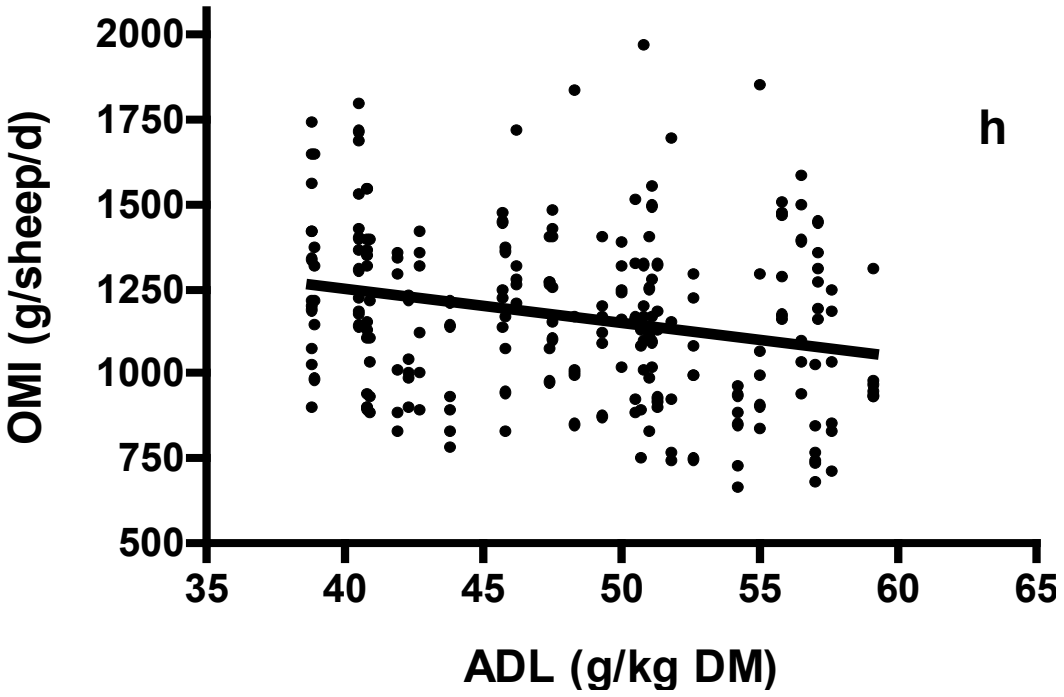


DOM = 0.253 + 0.526×IVDOM, r^2=0.282,
Sy.x = 0.0205, P < 0.001



OMI = 1022 + 0.01854×HM, r^2=0.127,

Sy.x = 233.7, P < 0.001



OMI = 1648 - 9.93×ADL, r^2=0.060,

Sy.x = 242.6, P < 0.001

Figure 3.2. Linear regressions equations between digestible organic matter (DOM) and herbage mass (HM, a), crude protein content of herbage offered (CP, b), neutral detergent fiber content of herbage offered (NDF, c), acid detergent fiber content of herbage offered (ADF, d), acid detergent lignin content of herbage offered (ADL, e), in vitro digestibility of herbage offered (IVDOM, f) and between OM intake (OMI) per sheep and herbage mass (HM, g) and acid detergent lignin content of herbage offered (ADL, h)

3.5 Discussion

Effect of grazing season

The quantity and the digestibility of the offered herbage decreased significantly from July to September. Consequently, DOM, OMI, DOMI, and ADG per sheep decreased during grazing season. The relationships between herbage parameter and DOMI as well as OMI per sheep confirm that the decrease of herbage mass and quality during the grazing season is responsible for the decrease in individual animal performance. The decrease of herbage quality from July to September could be caused by maturing of the herbage during the grazing season. The significant increase in ADL content of the herbage offered confirms this assumption. According to Bai et al. (2004) senescence of plant species in the Inner Mongolian steppe starts early in the growing season. This could have intensified the maturing process together with the extreme low precipitation in the vegetation period 2005, which caused a low regrowth of the sward of 687 kg DM/ha ± 124 kg DM/ha (mean ± standard error) compared to the long term mean of 1925 kg DM/ha reported by Bai et al. (2004).

Effects of grazing intensity on herbage mass and quality

Grazing intensity did not effect herbage parameters except the ADL content, which increased with grazing intensity and the HM, which decreased with increasing grazing intensity. Due to the poor regrowth of the sward caused by the extreme low precipitation in the grazing season 2005 a decrease of HM with increasing grazing intensity is expected, which was found in our results by the decreasing HM with increasing grazing intensity.

Effects of grazing intensity on quality of herbage ingested

No significant effect of grazing intensity was found on DOM. O'Reagain and McMeniman (2002) wrote that sheep grazing on rangelands are highly selective. According to Spedding (1965) herbage selection can lead to large differences between quality of herbage ingested and herbage offered. However, Garcia et al. (2003) stated that sheep prefer to maintain diet quality rather than herbage intake, when grazing intensity increases and herbage availability decreases. This could have led in our study to maintained DOM in the high grazing intensities by herbage selection. Animut et al. (2006) found that high grazing intensities decrease HM on offer, and therefore limit the potential for forage selection. This corresponds with our results that HM decreases with increasing grazing intensity from 1568 to 601 kg DM/ha in grazing intensity 1.5 and 9.0 sheep per ha, respectively. Thus, a reduced potential for herbage selection can be assumed. Furthermore, the significant relation between HM and DOM indicate that herbage selection occurred. The often in temperate grasslands observed increase of quality of herbage offered in high grazing intensities, e.g. by Kristensen (1988) and Schlegel et al. (2000), can be excluded in our study due to the results of IVDOM, which was not influenced by grazing intensity in July and August and tended to decrease with increasing grazing intensity in September. The small range of mean IVDOM values (0.525 to 0.603) indicate a general low variability of the digestibility in the offered herbage. This could be an explanation for a low meaning of herbage selection by the animals. This assumption is supported by the results of Schiborra et al. (2007) who found only minor digestibility differences in the vertical structure of the sward on an area within our study area. However, this measurement was conducted in an ungrazed area and the horizontal structure was not examined, which can be very heterogeneous in swards grazed by sheep due to their behavior of grazing in small patches, when herbage mass on offer is not limited. The relative strong relation between IVDOM and DOM also indicates a low level of selection. Additional to the small range of digestibility values a high variation between animals in DOM was found, which makes it difficult to determine the effect of grazing intensity on DOM as significant. IVDOM gave higher values than DOM, which contradicts the assumption that selection of herbage occurs. However, the reason for higher in vitro digestibilities of the offered herbage compared to estimated digestibilities of herbage ingested could be the difference between the in vitro and the in vivo method, according to Schiborra et al. (2007). In general, the cell wall contents of the herbage were high with a high lignification (ADL in NDF was 6.6%). Thus, the lignification (ADL) of the herbage seems to be a very important quality parameter of the herbage of our study area, which is reflected by the relative strong influence of ADL on

DOM. Furthermore, ADL increased significantly with increasing grazing intensity. This indicates a decreasing DOM with increasing grazing intensity not caused by herbage selection of sheep but decreasing quality of herbage offered. However, the reason for the result that DOM was not significantly affected by grazing intensity might be the high variation of the result and therefore a not sufficient number of replications.

Effect of grazing intensity on herbage intake

The OMI and DOMI per sheep tended to decrease with increasing grazing intensity. This corresponds with Garcia et al. (2003), who found that sheep reduce herbage intake and maintain quality of herbage ingested, when HM is limiting. This is further confirmed by the significant influence of HM on OMI per sheep found in our study and the decrease of HM with increasing grazing intensity. Moreover, with similar HM, OMI per sheep increased significantly from 7.5 sheep per ha to 9.0 sheep per ha in July and September. This increase of OMI and DOMI was caused by a higher estimated fecal output with TiO_2 in the highest grazing intensity. A methodical bias by the marker TiO_2 can therefore not be excluded, as discussed in Chapter 2.5. A further explanation could be a lower lignification of the offered herbage in 9.0 sheep per ha grazing intensity compared to 7.5 sheep per ha. However, this difference was not significant. When the highest grazing intensity is omitted in the model, the influence of grazing intensity on OMI and DOMI per sheep was stronger over all periods ($P = 0.058$ and $P = 0.033$, respectively). It is also possible that the herbage intake of grazing intensity 7.5 sheep per ha is underestimated by our measurement. Perhaps the sheep of grazing intensity 7.5 sheep per ha were more stressed by our measurements than sheep of other grazing intensities. This could have led to a decreased herbage intake in the measuring period. The contradiction between ADG and OMI per sheep confirms this assumption. However, no objective measurements for stress were conducted and the lower mean herbage intake in grazing intensity 7.5 sheep per ha compared to 9.0 sheep per ha was observed in the flat as well as in the slope area in all three periods, which makes a random influence of stress unlikely.

The significant negative effect of ADL on OMI per sheep underlines the high meaning of the cell wall content and its lignification in herbage of this region. Furthermore, the significant increase of ADL with increasing grazing intensity indicate a negative effect of increasing grazing intensities on herbage intake, since lignification of herbage decreases herbage intake of ruminants (Van Soest, 1994). In general, a high OMI with 3.4% of BW was found for the

sheep in this grazing experiment. Especially the high NDF and ADL contents in the herbage lead to the expectation that the herbage intake is low due to slow passage rates of the feed. Schlecht et al. (1999) reported that ruminants of arid to semi-arid regions are adapted to low quality herbage by a relatively high herbage intake. The volume capacity of the rumen can show high levels, which enables the animals to a high herbage intake even with low passage rates of the forage in the rumen.

OMI and DOMI per ha increased significantly with grazing intensity in our study. Thus, the tendency of increasing OMI and DOMI per sheep with decreasing grazing intensity (except grazing intensity 9.0 sheep per ha) could not compensate the increasing number of animals in the high grazing intensities. This leads to the conclusion that in a short term view the herbage yield of the Inner Mongolian steppe is used to a higher extent by grazing sheep in high grazing intensities.

Effect of grazing intensity on animal performance

ADG per sheep decreased significantly with increasing grazing intensity. The maximum was found in the grazing intensity of 3.0 sheep per ha, which corresponds with the maximum of DOMI per sheep. Due to the fact that DOMI per sheep only tended to increase with decreasing grazing intensity the question arises, why the ADG per sheep increased distinctly. An answer could give the study of Lachia and Aguilera (2005), who reported that sheep increase grazing time and therefore walking in pastures of low herbage allowance, which can be caused by high grazing intensities. This could have led in our study to increasing energy costs with increasing grazing intensity. Therefore, the ADG per sheep could have decreased with increasing grazing intensity although energy intake did not decrease.

The mean ADG per ha was affected significantly by grazing intensity. However, only a significant difference could be found between 1.5 sheep per ha and all other grazing intensities. The maximal ADG per sheep was obtained at 3.0 sheep/ha and the maximal ADG per hectare at 7.5 sheep/ha. This indicates that an optimal GI depends on the parameter, and can not be derived based on a one-year experiment. Han et al. (2000) found a decreased ADG per sheep and an increased ADG per ha with increasing grazing intensity in a grazing experiment conducted in Inner Mongolia over one grazing season, which confirms our results. The results of ADG per ha indicate that short term heavy grazing has no negative effect on animal performance per ha. This could be a reason for the occurrence of overgrazing in the Inner Mongolian steppe by farmers, which have no long term interest on pastures due to the

system of ownership. However, general recommendations for land use by the results observed in our one year study are not reliable because long term effects of grazing intensity are not regarded and the variability of the grassland productivity between years is high as reported by Yu et al. (2004).

3.6 Conclusions

HM, IVDOM, and DOM were not found significantly affected by grazing intensity in our study. However, herbage intake per sheep tended to decrease and ADG per sheep decreased significantly with increasing grazing intensity. The results confirm the assumption that herbage intake is more sensitive to grazing intensity than of herbage digestibility. Besides the lower DOMI higher energy requirements for grazing activities in the high grazing intensities may have led to the distinct reaction of ADG per sheep to grazing intensity. The calculated linear regressions between herbage variables and quality of herbage ingested as well as individual herbage intake showed that quality of herbage ingested depends on quality of herbage offered. Especially the lignification of cell wall contents in the fiber-rich herbage of the Inner Mongolian grassland showed a strong relationship to quality of herbage ingested and herbage intake of sheep. Although ADG per sheep decreased with increasing grazing intensity the ADG per ha was compensated by the increase in number of animals. Thus, high live weight gain per ha can be achieved in high grazing intensities on the short term.

3.7 References

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4 General discussion

4.1 The use of TiO_2 in grazing experiments with sheep

In this Chapter the results of the methodical Chapter 2 will be evaluated also in regard of the results of the grazing experiment described in Chapter 3. Chapter 2 shows that TiO_2 is an appropriate marker for measuring fecal output in grazing sheep. The fecal recovery is close to 100% and relatively constant. The equilibrium of intake and excretion is achieved within five days and therefore a preliminary period, in which the marker is administered but no fecal samples are obtained, of five days is sufficient. The fecal recovery was significantly higher than 100% in hay diets and increased from 100.4% in a low grazing intensity to 107.0% in a high grazing intensity. It can not be excluded that the fecal TiO_2 recovery is increased by increased ingestion of soil containing TiO_2 in high grazing intensities. However, no influence of grazing intensity was observed in the grazing experiment described in Chapter 3 on crude ash content in feces ($P = 0.900$), which indicates no increased soil ingestion with increasing grazing intensity. The determined high herbage intake per sheep in the highest grazing intensity of 9.0 sheep per ha was caused by a high fecal output due to a low concentration of TiO_2 in feces. A dilution of the administered TiO_2 by high intakes of indigestible soil, containing lower concentrations of TiO_2 than feces, must also be reflected by higher contents of ash in feces. This was not found as described above. However, experiments are planed to examine the natural occurrence of TiO_2 in feces as influenced by grazing intensity on our study area to exclude this factor. Furthermore, the fecal TiO_2 recovery in different grazing intensities will be examined more detailed.

In the experiments 5 and 6 of Chapter 2 diurnal variations of fecal marker concentration were found. This can lead to high differences in fecal output estimation. In Experiment 5 for example, the mean fecal output per sheep estimated with TiO_2-dosing fecal sampling at 9h would be significantly lower than dosing and sampling at 17h with 573.4 and 690.6 g DM/day, respectively. The diurnal variation of the fecal TiO_2 concentration was less in animals administered with two instead of one TiO_2 pulse dose per day. In experiment 3 the fecal output estimation was improved by administering two pulse doses TiO_2 and collecting two grab samples per day. Therefore, the question arises why we administered only one pulse dose and collected one grab sample per day in the grazing experiment, as described in Chapter 3. The first reason was the high effort in work. Together with not presented additional treatments in the grazing experiment of chapter 3 we examined 120 sheep grazing on 20 plots, which were spread over an area of approximately 200 ha. The handling of all sheep with once

daily dosing and sampling needed a whole day. The second reason is the increase in stress for the animals through catching and treating them two times per day. In the first experimental period in July, we needed to adapt the animals to catching and had the impression, that they were highly stressed in the first days. However, no objective measurements for determining stress were made. Furthermore, the significantly decreased feed intake of the sheep in experiment 3 of chapter 2, which received two pulse doses per day, confirms that two times dosing and sampling may increase stress and hence reduce herbage intake, which is one of our main measurements to evaluate effects of grazing intensity, as described in Chapter 3. If diurnal variation of the fecal TiO_2 concentration occurs in the grazing experiment, a correction is not possible due to the time schedule of the experiment. Every day in the ten day administration period the twenty plots were treated at the same time. Thus, the capsules were given in every plot at approximately the same time of the day. Since this time sequence of treating plots did not follow the order of grazing intensity but was due to the random distribution of the plots at the area (shortest distance to walk), a systematic bias of dosing and sampling on the evaluation of the effects of grazing intensity is not to be expected.

In the grazing experiment of Chapter 3 herbage mass offered affected the fecal output of the sheep significantly (Figure 4.1). Although the regression coefficient was very small, it indicates that the marker TiO_2 was able to reflect a relationship between this two parameters, which supports the concluded reliability of TiO_2 as a marker for estimating fecal output in grazing sheep.

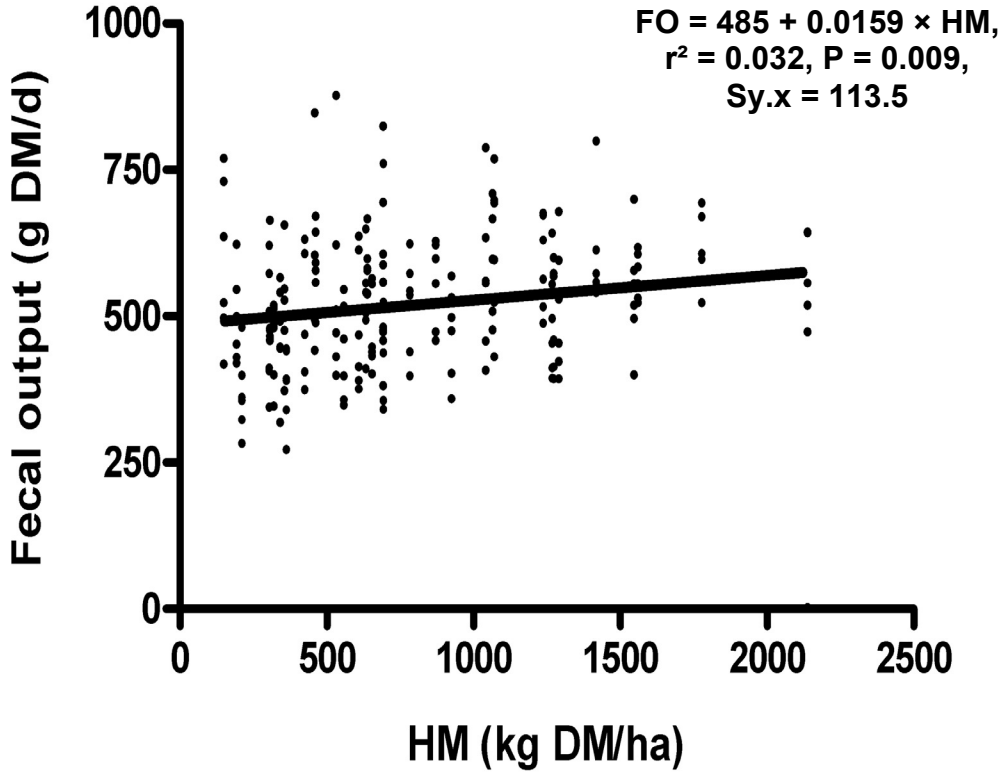

Figure 4.1. Linear regression between fecal output per sheep (FO) and herbage mass (HM)

4.2 Implications of the results of the grazing experiment

In the grazing experiment the live weight gain per sheep decreased significantly with increasing grazing intensity. However, the intake of digestible organic matter showed only a tendency. This difference could be caused by different energy requirements for grazing activity in the different grazing intensities, as discussed in Chapter 3.5. In the following the results of energy requirements of the sheep calculated by the equations of SCA (1990) and ARC (1980) as described by Corbett and Ball (2002) will be presented and discussed. As shown by the equations [7] and [8] beside the energy requirement for maintenance ($ME_{maintenance}$) the additional requirement of metabolizable energy for grazing rather than eating from a trough ($ME_{grazing}$) was determined by body weight, dry matter intake, and digestibility of dry matter ingested. Furthermore, the energy requirement for walking ($ME_{walking}$) was derived from body weight, the relief of the grassland (flat or steep ground), and the quantity of herbage mass available (kg DM per ha).

$$ME_{grazing} = \frac{LW \times 0.05 \times DMI \times (0.9 \times D)}{k_m} \quad [7]$$

$$ME_{walking} = \frac{LW \times 0.05 \times T \times (0.9 \times D)}{(GF + 3) \times k_m} \quad [8]$$

where:

DMI = dry matter intake (kg/day)

D = digestibility of dry matter

T = a value varying with terrain from 1.0 for level ground to 2.0 for steep, hilly ground

GF = the quantity of green forage available

k_m = efficiency of energy for maintenance

To calculate the metabolizable energy available for growth per sheep (MEgrowth = MEI-$ME_{maintenance}$ - $ME_{grazing}$ - $ME_{walking}$) the metabolizable energy intake (MEI) was calculated by the equation 0.15 + 0.1557 × DOM – 0.013 × CA according to Menke and Steingass (1987). All calculated results were analysed by the MIXED procedure of SAS (1988) in an ANOVA with the fixed effects block, grazing intensity, and period and with the random effects sheep and block × intensity. The model is described more detailed in Chapter 3.3. The results are shown in Tables 4.1, 4.2, and 4.3.

Table 4.1. Probability values of the effects for the different parameters: average daily gain (ADG), ME available for growth (ME_{growth}), ME expended for walking ($ME_{walking}$), ME expended for grazing ($ME_{grazing}$), ME expended for maintenance ($ME_{maintenance}$), and ME intake (MEI)

Parameter	**block**	**GI[1)]**	**period**	**block × period**	**period × GI[1)]**
ADG (g/sheep/d)	0.372	0.018	<0.001	0.879	<0.001
ME_{growth} (MJ/sheep/d)	0.151	0.072	<0.001	0.147	0.001
$ME_{walking}$ (MJ/sheep/d)	0.007	0.242	<0.001	<0.001	<0.001
$ME_{grazing}$ (MJ/sheep/d)	0.270	0.434	<0.001	0.800	<0.001
$ME_{maintenance}$ (MJ/sheep/d)	0.925	0.790	<0.001	0.780	<0.001
MEI (MJ/sheep/d)	0.307	0.063	<0.001	0.305	0.001

1) GI = grazing intensity (sheep/ha)

Table 4.2. Means of average daily gain (ADG), ME available for performance (ME_{growth}), ME expended for walking ($ME_{walking}$), ME expended for grazing ($ME_{grazing}$), ME expended for maintenance ($ME_{maintenance}$), and ME intake (MEI) in three periods of the grazing season as influenced by grazing intensity

Parameter	Period	Grazing intensity (sheep/ha)						SEM[1)]	P_{slice}[2)]
		1.5	3.0	4.5	6.0	7.5	9.0		
MEI	July	11.05^{a}	12.96^{b}	9.71^{ac}	9.84^{ac}	9.22^{c}	11.04^{ac}	0.641	0.001
(MJ/sheep/d)	August	10.69^{ac}	10.40^{ac}	10.42^{ac}	10.16^{ac}	8.03^{b}	9.42^{c}	0.641	0.047
	September	11.52^{a}	10.76^{ac}	9.35^{ac}	9.06^{ac}	7.20^{b}	9.04^{c}	0.641	<0.001
	mean	11.09	11.37	9.83	9.68	8.15	9.83	0.545	-
$ME_{maintenance}$	July	6.14	6.27	6.05	6.31	6.17	6.61	0.217	0.543
(MJ/sheep/d)	August	6.38	6.31	6.35	6.48	6.21	6.62	0.217	0.823
	September	6.81	6.79	6.43	6.49	6.30	6.69	0.217	0.473
	mean	6.44	6.44	6.44	6.28	6.42	6.23	0.215	-
$ME_{grazing}$	July	1.13	1.30	1.08	1.17	1.15	1.40	0.105	0.258
(MJ/sheep/d)	August	1.19	1.22	1.30	1.29	1.06	1.33	0.105	0.481
	September	1.48	1.52	1.28	1.30	1.07	1.45	0.105	0.129
	mean	1.27	1.35	1.22	1.25	1.09	1.40	0.097	-
$ME_{walking}$	July	0.56	0.72	0.83	0.85	0.90	0.89	0.108	0.198
(MJ/sheep/d)	August	0.59	0.80	0.95	0.93	0.95	1.02	0.108	0.066
	September	0.67^{a}	0.93^{ab}	1.03^{b}	1.06^{b}	1.06^{b}	1.13^{b}	0.108	0.045
	mean	0.61	0.82	0.94	0.95	0.97	1.02	0.108	-
ME_{growth}	July	3.22^{ab}	4.73^{b}	1.75^{ac}	1.52^{ac}	1.00^{c}	2.13^{ac}	0.628	<0.001
(MJ/sheep/d)	August	2.53^{a}	2.08^{ac}	1.83^{ac}	1.45^{ab}	-0.16^{b}	0.45^{bc}	0.628	0.027
	September	2.56^{a}	1.52^{ab}	0.61^{b}	0.21^{bc}	-1.22^{c}	-0.23^{bc}	0.628	0.001
	mean	2.77	2.77	1.40	1.06	-0.13	0.78	0.562	-
ADG	day 1-49	91.6^{a}	93.7^{a}	96.7^{a}	76.5^{ab}	60.7^{b}	62.7^{b}	9.46	0.004
(g/sheep/d)	day 50-98	75.6^{a}	89.3^{a}	34.0^{b}	13.8^{b}	29.7^{b}	11.8^{b}	9.46	<0.001
	mean	83.6^{a}	91.5^{a}	65.3^{b}	45.1^{b}	45.2^{b}	37.2^{b}	7.79	-

Within a row means with a common superscript are not significantly different at $\alpha = 0.05$

1) Standard error of the means

2) Probability of the test that the grazing intensity has an effect within the according period

Table 4.3. Mean values of ME available for performance (ME_{growth}), ME expended for walking ($ME_{walking}$), ME expended for grazing ($ME_{grazing}$), ME expended for maintenance ($ME_{maintenance}$), and ME intake (MEI) in three periods of the grazing season.

Parameter	Period			Standard error
	July	August	September	
MEI (MJ/sheep/d)	10.64[a]	9.85[b]	9.49[b]	0.261
$ME_{maintenance}$ (MJ/sheep/d)	6.25[a]	6.39[b]	6.59[c]	0.089
$ME_{grazing}$ (MJ/sheep/d)	1.21[a]	1.23[a]	1.35[b]	0.043
$ME_{walking}$ (MJ/sheep/d)	0.72[a]	0.87[b]	0.98[c]	0.044
ME_{growth} (MJ/sheep/d)	2.39[a]	1.36[b]	0.57[c]	0.257

The MEI and consequently ME_{growth} tended to be affected by grazing intensity ($P = 0.063$ and $P = 0.072$, respectively). The mean ME_{growth} decreased with increasing grazing intensity, whereas MEI was high in the low grazing intensities 1.5 and 3.0 sheep per ha and seemed to decrease to a plateau from 4.5 to 9.0 sheep per ha. However, the lowest MEI was found in grazing intensity 7.5 sheep/ha. The requirements of the sheep ($ME_{maintenance}$, $ME_{grazing}$, and $ME_{walking}$) increased from July to September, whereas MEI and consequently ME_{growth} decreased from July to September. The interaction of grazing intensity and period were significant for all determined energy parameters. The $ME_{walking}$ did not differ among grazing intensities in the first period of the grazing season with similar herbage mass, but tended to increase in August and increased significantly in September with increasing grazing intensity ($P = 0.066$ and $P = 0.045$, respectively).

The results show the reason for the low performance of the Inner Mongolian sheep compared to sheep in temperate regions. Although the sheep realize a high organic matter intake of 3.3% of the body weight (8.1% of metabolizable body weight) the low digestibility of the diet and the high energy requirements for physical activity ($ME_{grazing} + ME_{walking}$) activity lead to daily live weight gain less than 100g. The NRC (1981) stated that grazing goats can have an energy requirement for physical activity from 25% in a flat sward with high herbage allowance to 75% of energy requirement for maintenance in a steep sward with low herbage allowance. Our results show a mean energy requirement for the physical activity of the sheep ($[ME_{walking}+ME_{grazing}]/ME_{maintenance}$) of 29 % and 39% of $ME_{maintenance}$ in the grazing intensities 1.5 and 9.0 sheep per ha, respectively. Animut et al. (2005) observed in a grazing experiment with three grazing intensities of sheep an increasing energy expenditure with increasing

grazing intensities and concluded that this increased energy requirement was caused by an increased number of steps and grazing time per day. This is also confirmed by our results of $ME_{walking}$, which increased significantly with increasing grazing intensity in September, when the differences between swards of the grazing intensities were highest due to the poor regrowth of the sward in 2005. Thus, the sheep compensated low herbage availability by increasing the grazing time to prevent a high decrease of herbage intake.

Besides an increased energy requirement for walking with increasing grazing intensity, the energy requirement for chewing and ruminating could also be increased as the digestibility of herbage is decreased by increasing grazing intensity. Susenbeth et al. (1998) reported that the energy requirement for chewing and ruminating in cattle increased from high to low digestible forage from 10% to 30% of the ME contained in the forage. However, due to the small range in the digestibility of organic matter ingested (0.52-0.58)in our study, as shown by Table 3.6 in Chapter 3.4. great differences of the energy requirement for chewing and ruminating between grazing intensities can not be expected. However, the lignification (ADL content) of the herbage offered increased with grazing intensity. This could lead to an increasing requirement for ruminating with increasing grazing intensity. The lignification of the herbage was not included in the equation for $ME_{grazing}$ and no influence of grazing intensity was found for $ME_{grazing}$. Furthermore, it is possible, that the equation for $ME_{grazing}$ underestimates the energy requirement for ruminating because the very high NDF content of the forage in our study is not regarded as well and it is questionable if the equation of Corbett and Ball (2002) is calibrated for forages with NDF contens higher than 70%.

Table 4.4 shows the mean ME_{growth} expended for 1g live weight gain and the calculated energy content of 1g live weight gain of the sheep in the different grazing intensities. The sheep of grazing intensity 7.5 sheep per ha showed a negative value, which is biologically not possible. This result supports the hypothesis discussed in Chapter 3.5 that the herbage intake of the sheep of grazing intensity 7.5 sheep per ha is underestimated. According to ARC (1980) the efficiency of live weight gain for growing sheep is $k_g = 0.0435 \times M/D$ (MJ ME/kg DM). M/D is the energy content of the herbage ingested. We calculated a mean M/D of 8.1 MJ/kg DM, which results in $k_g = 0.35$. The efficiency for MEI per g ADG shows a decrease with increasing grazing intensity. This supports the assumption that sheep grazing in high grazing intensities need more energy for grazing activity. The high energy contents per g live weight gain in grazing intensities 1.5 and 3.0 could be associated with a higher content of fat. The small differences between grazing intensities 4.5 to 9.0 sheep per ha, could be due to the circumstance that the potential for protein retention is not completely achieved even in

grazing intensity 4.5 sheep per ha. Thus, the composition of live weight gain did not alter in the grazing intensities equal or higher than 4.5 sheep per ha.

Table 4.4. Average daily gain (ADG), efficiency of MEI and ME_{growth} per g ADG, and energy content of body mass gain of sheep in the different grazing intensities

	Grazing intensity (sheep/ha)					
	1.5	**3.0**	**4.5**	**6.0**	**7.5**	**9.0**
MEI (kJ ME/g ADG)	137.7	124.3	150.5	214.6	180.3	264.2
ME_{growth} (kJ ME/g ADG)	33.1	30.3	21.4	23.5	(-2.9)	21.0
Body mass (kJ /g ADG)	11.7	10.7	7.5	8.3	-	7.4
ADG (g/sheep/d)	83.6	91.5	65.3	45.1	45.2	37.2

The described grazing experiment will be conducted at least until 2008. The examinations will be extended for measurements of grazing activity by a GPS-system and of chewing activity by a chewing counter. This will give more precise information about the energy requirements/expenditure of sheep in different grazing intensities.

The main objective of the grazing experiment conducted in 2005 was to give recommendations for animal performance with sustainable land use in the Inner Mongolian steppe. Therefore, the aim of the experiment was to find an optimal grazing intensity, which realizes a high animal performance in a long term sustainable ecosystem. To determine this optimal grazing intensity a long term representative data basis of sheep grazing on the Inner Mongolian steppe is needed, which is not given by our results in 2005. As shown in Chapter 1.2.1 (Figure 1.3), first the variability in precipitation and therefore in herbage yield of the grassland is very high between years and second in 2005 the precipitation was the lowest since 1982. This confirms the request for a long term data basis to give reliable recommendations for land use in Inner Mongolia. This is supported by Zhang (1998), who found no differences in herbage parameter between a grazed area and a non-grazed area in the Xilin River Basin and stated that the measurement period of three years was too short to overcome the differences in weather among years. Therefore, continuation of the grazing experiment until at least 2008 is necessary to obtain a reliable database for determining an optimal grazing intensity. Furthermore, no significant influence of grazing intensity could be found for the offered herbage mass, digestibility of organic matter offered, digestibility of

organic matter ingested and herbage intake per sheep in 2005. Since the variation was high in herbage mass and herbage intake and the study area was in a good condition at the beginning of the grazing experiment a possible effect of grazing intensity is difficult to determine statistically. The evaluation of data from several years enables the consideration of long term effects of different grazing intensity on for example a change in biodiversity, soil composition and water availability, which contribute to the long term effect of grazing intensity. Furthermore, a high variation could be compensated statistically by gaining more "degrees of freedom" through collecting data for more than one year.

The highest mean live weight gain per ha was found in grazing intensity 7.5 sheep per ha and no significant differences could be found between grazing intensities higher than 1.5 sheep per ha. This indicates that short term heavy grazing does not lead to a reduced animal performance. This result is confirmed by the grazing experiment of Han et al. (2000), who observed in a one year grazing experiment a decreasing individual animal performance with increasing animal performance, but an increasing animal performance per ha. This could be an explanation why overgrazing in the Inner Mongolian steppe occurs. The farmers may have no long term interest in the productivity of the area, due to uncertain ownership of the land. Therefore they used the described positive short term effect of heavy grazing. Furthermore, our results show that DOMI per ha increased with increasing grazing intensity. Thus, the use of the herbage offered increases with increasing grazing intensities. A farmer, who is realizing heavy grazing is able to feed more sheep per ha at maintained animal performance per ha. However, this attitude in grazing management is not sustainable on the long term as shown by the degradation of the Inner Mongolian steppe by Tong et al. (2004). The grassland productivity and therefore animal performance per ha is expected to decrease by heavy grazing, as discussed in Chapter 1.2.3. In our one year experiment no long term effects can be determined. However, mean herbage mass at the end of the grazing experiment in September decreased from 1554 to 301 kg DM/ha in grazing intensities 1.5 and 9.0 sheep per ha, respectively. This indicates the beginning of grassland degradation by reducing the plant cover, which leads to high vulnerability of the area for wind erosion in the following winter. Furthermore, the soil is expected to degrade by compaction and consequently a reduced ability to store water. It is to expect that the areas of our high grazing intensities started to degrade in our grazing experiment.

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5 Summary

The present dissertation was conducted within the Sino-German research collaboration "Matter fluxes of grasslands in Inner Mongolia as influenced by stocking rate" (MAGIM), founded by the German research foundation (DFG). The objective of this research unit is the examination of different grazing management systems of sheep in the Inner Mongolian steppe to contribute to the reduce of the severe ecological problems of this region mainly caused by overgrazing. Therefore, a grazing experiment was conducted in the grazing season of 2005 in the Xilin River Basin. To assure accurate measurements of herbage intake of sheep, which is a crucial information for evaluating different grazing managements, the inert marker TiO_2 was evaluated for the estimation of fecal output in sheep grazing at the Inner Mongolian steppe. The determination of fecal output is used together with the digestibility of herbage ingested for the prediction of herbage intake, since a direct measurement is not practical.

For the evaluation of the inert marker TiO_2 administered to sheep orally by daily gelatine capsules for estimating fecal output by marker concentration of fecal grab samples indoor feeding experiments and grazing experiments were conducted to determine fecal recovery, time to reach equilibrium in intake and excretion of TiO_2 after initial dosing, diurnal variation in fecal marker concentration, and to validate fecal output estimation with TiO_2. Furthermore, frequency of TiO_2 administration and grab sampling was examined. In the indoor feeding experiments, fecal recovery of TiO_2 was lower ($P < 0.001$) in hay+concentrate diets than in hay diets with 98.9% and 108.0%, respectively. Furthermore, fecal recovery was higher ($P = 0.014$) in grazing intensity 5.0 sheep per ha compared to 2.0 sheep per ha with 107.0% and 100.4%, respectively. The significantly higher than 100% fecal recoveries of the hay diets and the high grazing recoveries could be caused by increased ingestion of soil, which contains 2.2 mg/g DM of TiO_2. However, the difference in fecal crude ash content between the grazing intensities was small, and therefore did not explain the higher fecal recovery in the high grazing intensity. The equilibrium in intake and excretion of TiO_2 was reached five days after initial TiO_2 dosing, which is therefore the minimum preliminary period before fecal sample collection. Diurnal variation in fecal TiO_2 concentrations was found in a grazing experiment, when fecal grab samples were collected on three different times of the day. The variation in fecal TiO_2 concentration was smaller with two times dosing compared with one time dosing of TiO_2 per day. This result was confirmed by the comparison of measured fecal output with estimated fecal output by TiO_2 concentration in fecal grab samples in an indoor feeding experiment. The estimation of fecal output was more accurate with two time dosing than one

time dosing per day. Furthermore, the increase in frequency of grab sampling from one to two per day, improved the accuracy of fecal output estimation. In conclusion, these experiments showed that TiO_2 is a reliable marker for estimation of fecal output in grazing sheep.

A grazing experiment with six different grazing intensities of sheep (1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 sheep per ha) was conducted in the grazing season of 2005 in the Xilin River Basin. The objectives were to determine the effects of grazing intensity on herbage mass, forage quality, live weight gain, and herbage intake, and to derive an optimal grazing intensity, which realizes a high animal performance in a sustainable ecosystem. The ADG per sheep decreased with increasing grazing intensity ($P = 0.018$), whereas intake of organic matter and digestible organic matter per sheep tended to decrease ($P = 0.090$ and $P = 0.065$, respectively). The assumption that not only DOMI per sheep is responsible for the increasing ADG per sheep with decreasing grazing intensity but also increasing energy requirement for physical activity with increasing grazing intensity caused by low herbage allowance was confirmed by calculations of energy requirements of the sheep. The digestibility of organic matter ingested and offered were not found as influenced by grazing intensity ($P = 0.116$ and $P = 0.471$, respectively). Herbage mass decreased from 1500kg DM/ha in grazing intensity 1.5 sheep per ha to 600kg in grazing intensity 9.0 sheep per ha ($P = 0.035$). The herbage composition was not affected by grazing intensity, except the ADL content ($P = 0.039$) which increased with grazing intensity. Significant relationships between ADL and digestibility of organic matter ingested as well as herbage intake indicate the high meaning of lignification of the fibre rich herbage (NDF = 726g/kg DM ± 7.1 SE). Herbage intake per ha increased with grazing intensity ($P < 0.001$), whereas live weight gain per ha was lowest at grazing intensity 1.5 sheep per ha and no differences were found among the other grazing intensities ($P = 0.049$). Therefore, it can be concluded that short term heavy grazing does not lead to a reduced animal performance per ha, but is even able to feed more animals per ha. However, long term heavy grazing is expected to reduce productivity of the grassland and consequently may reduce animal performance. Since in our one year study long term effects of grazing intensity could not be determined and grassland productivity varies greatly with precipitation in the Inner Mongolian steppe, no reliable recommendation for an optimal grazing intensity could be derived.

Zusammenfassung

Die vorliegende Dissertation wurde innerhalb der chinesisch-deutschen Forschergruppe der DFG “Matter fluxes of grasslands in Inner Mongolia as influenced by stocking rate” (MAGIM) angefertigt. Das Ziel dieser Forschergruppe ist es, verschiedene Beweidungsstrategien von Schafen in der Steppe der Inneren Mongolei, China zu untersuchen, um einen Beitrag zur Lösung der schweren ökologischen Probleme dieser Region beizutragen, welche hauptsächlich durch Überbeweidung verursacht werden. Aus diesem Grund wurde ein Weideexperiment in der Vegetationsperiode 2005 im Xilin River Basin durchgeführt. Um eine genaue Messung der Futteraufnahme von weidenden Tieren zu gewährleisten, welche eine wichtige Information zur Bewertung von Beweidungsstrategien liefert, wurde die Eignung des inerten Markers TiO_2 zur Schätzung der Kotausscheidung untersucht. Die Schätzung der Kotabgabe wird zusammen mit der Verdaulichkeit der Ration genutzt, um die Futteraufnahme auf der Weide indirekt zu schätzen, da die direkte Schätzung kaum möglich ist.

Zur Evaluation der Schätzung der Kotausscheidung über die fäkale Konzentration des inerten Markers TiO_2, welcher den Schafen täglich oral in Gelatinekapseln verabreicht wurde, wurden Fütterungs- und Weideversuche durchgeführt, mit dem Ziel die fäkale Wiederfindung, die Zeit bis zum Eintreten des Gleichgewichts in Aufnahme und Ausscheidung nach erster TiO_2-Gabe, die Variation der Kotkonzentration des Markers im Tagesverlauf und die Genauigkeit der Schätzung der Kotauscheidung zu bestimmen. Ferner wurden die Effekte der Frequenz der Sammlung von Kotproben und der TiO_2-Verabreichung untersucht. In den Stallfütterungsversuchen war die Wiederfindung in den mit Kraftfutter supplementierten Heurationen niedriger mit 98.9% als in den reinen Heurationen mit 108% ($P < 0.001$). Im Weideversuch wurde mit 107.0% eine höhere Wiederfindung in Schafen der Beweidungsintensität 5.0 Schafe pro ha gefunden als in den Schafen der Beweidungsintensität 2.0 Schafe pro ha ($P = 0.014$). Die signifikant höheren Wiederfindungen als 100% könnten durch die erhöhte Aufnahme von Erde, welche auf der Versuchsfläche im Mittel 2.2g TiO_2/kg Trockensubstanz enthielt. Der Rohaschegehalt im Kot der untersuchten Tiere, welcher mit erhöhter Erdaufnahme ansteigen müsste, unterstützt diese Theorie allerdings nicht. Das Gleichgewicht in Aufnahme und Ausscheidung des TiO_2 wurde nach fünf Tagen nach erster TiO_2-Gabe erreicht. Daraus resultiert eine notwendige Länge einer Vorperiode vor dem Beginn der Kotsammlung von mindestens fünf Tagen. Es wurde eine Variation der Markerkonzentration im Tagesverlauf bei weidenden Tieren festgestellt, denen an drei

Zeitpunkten des Tages Kotproben aus dem Rektum entnommen wurden. Diese war allerdings geringer und nicht mehr signifikant, wenn den Tieren zweimal am Tag statt einmal TiO_2 verabreicht wurde. Dieses Ergebnis wurde bestätigt durch den Vergleich zwischen der direkt gemessenen und der über das TiO_2 geschätzten Kotausscheidung in einem Stallversuch, in dem die Verabreichung von zwei statt einer TiO_2-Gabe pro Tag die Schätzgenauigkeit verbesserte. Ferner wirkte sich auch die Sammlung von zwei statt einer Kotprobe pro Tag, welche gepoolt wurden, positiv auf die Schätzgenauigkeit aus. In der Schlussfolgerung wurde das TiO_2 als ein geeigneter inerter Marker zur Schätzung der Kotausscheidung von weidenden Schafen befunden.

In der Vegetationsperiode 2005 wurde ein Weideexperiment mit sechs Beweidungsintensitäten (1.5, 3.0, 4.5, 6.0, 7.5 und 9.0 Schafen pro ha) durchgeführt. Die Ziele waren die Bestimmung der Effekte der Beweidungsintensität auf Futterangebot (HM), Futterqualität (IVDOM + DOM), Lebendgewichtszunahme (ADG) und Futteraufnahme (OMI + DOMI) sowie die Ableitung einer optimalen Beweidungsintensität, welche eine hohe Tierleistung gewährleistet unter nachhaltigen ökologischen Bedingungen. ADG pro Schaf sank mit steigender Beweidungsintensität ($P = 0.018$), während OMI und DOMI eine Tendenz zur Abnahme zeigten ($P = 0.090$ bzw. $P = 0.065$). Die Annahme, dass nicht nur eine geringere DOMI für die Abnahme der ADG mit steigender Beweidungsintensität verantwortlich ist, sondern auch ein steigender Energiebedarf für Weideaktivität durch sich verschlechternden Futterzugang wurde durch Bedarfskalkulationen bestärkt. Für DOM und IVDOM wurde kein Einfluss der Beweidungsintensität beobachtet ($P = 0.116$ bzw. $P = 0.471$) und mögliche Gründe diskutiert. Das Angebot grüner Biomasse sank von ca. 1500kg in Beweidungsintensität 1.5 Schafe pro ha auf ca. 600kg Trockensubstanz pro ha in Beweidungsintensität 9.0 Schafe pro ha ($P = 0.035$). Zwischen der Beweidungsintensität und der chemischen Zusammensetzung des angebotenen Futters konnten keine Beziehungen gefunden werden mit Ausnahme des ADL-Gehaltes, welcher mit steigender Beweidungsintensität stieg ($P = 0.039$). Gefundene signifikante negative Einflüsse des ADL-Gehaltes auf DOM und OMI unterstreichen die hohe Bedeutung der Lignifizierung dieses faserreichen Futters (NDF = 726g/kg TS ± 7.1 SE). Die Futteraufnahme pro ha (OMI, DOMI) stieg mit der Beweidungsintensität ($P < 0.001$), während ADG pro ha geringer war in der Beweidungsintensität mit 1.5 Schafen pro ha als in den höheren Beweidungsintensitäten ($P = 0.049$), welche untereinander keine signifikanten Unterschiede zeigten. Aus diesem Ergebnis kann gefolgert werden, dass kurzeitige starke Beweidung die Tierleistung pro ha

nicht reduziert und sogar das Futterangebot der Fläche besser nutzt durch Ernährung einer erhöhten Anzahl Tiere. Dies könnte eine Hauptursache der auftretenden Überbeweidung in der Steppe der Inneren Mongolei sein, da die Bauern kein langfristiges Interesse am Land zeigen aufgrund der unsicheren und ständig wechselnden Besitzverhältnisse. Allerdings ist zu erwarten, dass langfristige Überbeweidung die Produktivität des Weidelandes verringert. Da in unserer Studie keine langfristigen Effekte der Beweidungsintensität, z.B. auf Bodenparameter und Artenzusammensetzung des Grünlandes, berücksichtigt werden können, und die Weideerträge zwischen den Jahren mit den Niederschlagsmengen stark variieren, ist es nicht möglich, aus unseren Daten eine allgemeingültige optimale Beweidungsintensität abzuleiten.

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6 Appendix

Table 6.1. Mean values for the measured herbage mass parameters on the grazing plots

Period	Block	GI (sheep/ha)	HM (kg DM/ha)	NM (% HM)	OM (% DM)	CP (% DM)	NDF (% DM)	ADF (% DM)	ADL (% DM)	IVDOM (g/g)
July	flat	1.5	1592	2.6	950	93	732	343	39	0.576
July	flat	3.0	1101	2.3	947	101	728	337	41	0.605
July	flat	4.5	1323	3.9	944	96	739	342	41	0.587
July	flat	6.0	1609	2.9	940	89	743	355	41	0.557
July	flat	7.5	481	11.1	938	99	739	352	42	0.577
July	flat	9.0	1059	1.3	948	103	739	345	39	0.580
July	slope	1.5	1106	6.5	951	89	728	347	42	0.576
July	slope	3.0	1317	3.1	944	82	743	354	43	0.558
July	slope	4.5	1312	5.4	946	101	728	324	39	0.602
July	slope	6.0	674	9.1	948	106	712	329	41	0.614
July	slope	7.5	608	7.5	945	114	706	332	44	0.611
July	slope	9.0	695	5.3	952	102	722	329	42	0.596
August	flat	1.5	2312	7.4	950	84	715	346	46	0.571
August	flat	3.0	900	12.6	946	98	718	346	47	0.579
August	flat	4.5	851	18.3	948	82	735	346	48	0.558
August	flat	6.0	1680	15.4	952	75	729	350	46	0.547
August	flat	7.5	850	18.1	937	90	738	366	52	0.544
August	flat	9.0	706	9.8	948	99	734	342	49	0.558
August	slope	1.5	1572	11.1	950	68	695	341	41	0.586
August	slope	3.0	1080	14.0	953	82	732	349	46	0.566
August	slope	4.5	1614	19.8	955	87	728	337	51	0.580
August	slope	6.0	582	20.5	947	93	714	338	51	0.599
August	slope	7.5	492	29.8	942	102	713	354	53	0.592
August	slope	9.0	467	21.6	943	84	729	349	54	0.567
September	flat	1.5	2059	13.5	949	61	705	366	56	0.556
September	flat	3.0	738	12.9	943	82	708	347	57	0.552
September	flat	4.5	607	23.3	948	76	731	345	50	0.540
September	flat	6.0	707	24.3	951	61	725	367	55	0.542
September	flat	7.5	451	31.7	942	72	742	373	58	0.520
September	flat	9.0	382	18.8	947	73	744	358	59	0.512
September	slope	1.5	1354	16.9	945	62	682	343	51	0.577
September	slope	3.0	1049	16.6	953	75	711	348	48	0.566
September	slope	4.5	1396	23.4	945	80	720	347	51	0.570
September	slope	6.0	392	17.6	943	89	713	337	51	0.587
September	slope	7.5	268	26.6	942	94	717	339	51	0.577
September	slope	9.0	260	17.1	962	82	723	340	57	0.548

Table 6.2. Mean values of the parameters measured on animals for the grazing plots

Period	Block	GI (sheep/ha)	fecal output (g DM/day)	fecal DM (% FM)	fecal OM (% DM)	fecal CP (% DM)	DOM (g/g)	OMI (g OM/day)	DOMI (g OM/day)
Juli	flat	1.5	601.6	31.6	87.6	10.7	58.0	1252.7	725.8
Juli	flat	3.0	688.5	32.0	87.5	11.3	59.4	1479.4	875.9
Juli	flat	4.5	544.6	30.1	88.1	9.5	55.4	1076.2	595.4
Juli	flat	6.0	628.2	29.9	88.7	10.2	56.6	1289.9	732.6
Juli	flat	7.5	570.3	33.4	89.1	9.0	54.0	1102.1	593.8
Juli	flat	9.0	615.1	33.9	90.5	9.6	54.9	1234.3	677.3
Juli	slope	1.5	555.0	35.4	88.1	10.7	57.9	1162.0	673.2
Juli	slope	3.0	643.8	32.4	90.2	10.5	56.9	1351.0	770.5
Juli	slope	4.5	530.1	34.6	89.1	10.6	57.5	1112.1	639.9
Juli	slope	6.0	479.5	36.7	89.3	9.5	54.9	950.2	521.3
Juli	slope	7.5	517.4	38.0	89.4	9.8	55.8	1044.7	581.9
Juli	slope	9.0	625.1	33.7	89.1	10.3	56.9	1289.2	732.4
August	flat	1.5	634.9	32.7	87.6	10.1	56.9	1288.1	731.6
August	flat	3.0	583.3	33.7	87.3	10.4	57.5	1193.2	684.0
August	flat	4.5	584.7	32.9	89.2	9.0	53.9	1134.2	612.7
August	flat	6.0	682.4	30.0	87.3	9.1	54.7	1313.4	717.9
August	flat	7.5	545.0	33.2	87.2	8.9	54.2	867.8	472.4
August	flat	9.0	582.6	34.1	88.9	9.1	54.2	1129.9	612.4
August	slope	1.5	526.9	38.7	86.9	10.6	58.0	1097.9	639.5
August	slope	3.0	562.0	37.2	89.7	10.1	56.1	1149.7	645.4
August	slope	4.5	632.6	35.0	87.4	10.0	56.6	1276.9	723.5
August	slope	6.0	524.4	38.8	87.6	9.7	56.0	1044.0	583.9
August	slope	7.5	428.1	41.8	87.1	9.3	55.1	829.4	455.8
August	slope	9.0	577.8	40.4	86.7	8.9	54.2	1096.5	594.5
September	flat	1.5	676.6	35.7	87.6	9.4	55.3	1326.4	733.8
September	flat	3.0	662.2	36.1	87.4	9.2	54.9	1283.4	704.7
September	flat	4.5	655.7	34.9	88.5	8.3	52.3	1217.9	637.1
September	flat	6.0	633.3	34.7	86.4	8.1	52.5	1153.6	606.1
September	flat	7.5	540.8	39.4	87.4	7.8	51.4	971.6	498.9
September	flat	9.0	561.9	38.6	88.4	7.7	50.9	1010.3	513.3
September	slope	1.5	629.5	36.2	86.4	10.3	57.6	1282.5	737.5
September	slope	3.0	638.2	37.1	90.1	9.3	54.3	1260.2	685.0
September	slope	4.5	524.3	33.5	85.5	9.3	55.6	1011.7	563.3
September	slope	6.0	565.8	42.4	86.8	8.6	53.5	1057.5	566.8
September	slope	7.5	418.8	42.5	86.2	9.0	54.5	793.2	431.8
September	slope	9.0	693.5	42.0	84.8	8.2	53.0	1248.7	659.2

Table 6.3 Botanical composition of the herbage mass offered, measured in July

Block	**GI**	**botanical composition of the herbage mass offered (% of green DM)**							
	(sheep/ha)	**Stipa grandis**	**Leymus chinensis**	**Achna-therum**	**Agro-pyron**	**Carex**	**Cleistogenes squarrosa**	**Potentilla ac.**	**other species**
flat	1.5	22,1	56,9	1,7	0,9	6,0	7,3	0,6	4,5
flat	3.0	33,9	44,7	2,5	4,1	6,0	4,6	1,6	2,6
flat	4.5	31,6	34,3	0,0	15,7	3,8	8,6	1,8	4,2
flat	6.0	10,9	58,1	1,1	7,4	7,1	8,4	3,8	3,2
flat	7.5	19,2	32,5	1,5	18,8	12,6	9,5	3,0	2,9
flat	9.0	31,1	43,3	6,1	1,9	10,2	6,8	0,1	0,5
slope	1.5	22,1	44,1	1,4	10,7	4,4	9,1	1,5	6,7
slope	3.0	44,6	25,4	8,1	8,3	10,7	1,9	0,0	1,0
slope	4.5	23,4	27,2	2,0	23,7	10,7	7,0	3,1	2,9
slope	6.0	15,9	40,2	1,1	13,1	20,0	6,0	0,0	3,7
slope	7.5	46,1	13,6	2,8	15,4	9,7	8,4	0,0	4,0
slope	9.0	20,2	33,4	8,1	22,5	11,1	2,8	0,0	1,9

Table 6.4. Precipitation and temperature at the study area in May, June and July 2005

Day	May			June			July		
	p[1] (mm)	Ta[2] (°C)	Tc[3] (°C)	p[1] (mm)	Ta[2] (°C)	Tc[3] (°C)	p[1] (mm)	Ta[2] (°C)	Tc[3] (°C)
1	-	-	-	1.7	10.3	16.1	1.3	17.1	22.4
2	-	-	-	0.0	11.2	15.8	0.0	18.9	26.2
3	-	-	-	0.0	14.4	22.6	1.6	19.7	26.1
4	-	-	-	0.0	19.2	26.4	0.1	17.0	23.3
5	-	-	-	0.6	12.9	17.1	0.0	18.8	25.6
6	-	-	-	1.2	16.5	22.5	0.0	20.8	29.9
7	-	-	-	0.0	16.3	24.6	0.0	21.4	30.3
8	-	-	-	0.0	14.8	22.5	2.2	16.8	23.8
9	-	-	-	0.0	16.2	23.9	0.8	14.8	22.6
10	-	-	-	0.0	16.5	24.1	1.0	15.7	22.9
11	-	-	-	0.0	17.4	27.4	0.3	16.4	24.2
12	-	-	-	0.2	22.3	29.5	0.0	18.9	29.1
13	-	-	-	2.1	15.0	21.3	1.5	21.5	31.8
14	-	-	-	3.1	11.8	17.6	0.9	20.4	29.9
15	-	-	-	13.9	13.4	19.8	0.4	23.7	31.2
16	-	-	-	0.0	16.0	24.1	0.0	24.7	33.8
17	-	-	-	0.0	18.2	26.4	0.0	25.6	35.5
18	-	-	-	0.0	18.5	25.9	0.0	25.1	34.3
19	-	-	-	1.4	17.7	23.0	8.9	23.7	31.1
20	0.0	13.5	21.2	0.0	18.4	24.4	0.1	25.1	32.4
21	0.0	11.8	17.4	0.0	21.0	28.7	1.1	19.0	24.8
22	0.0	7.2	13.4	0.0	24.1	33.0	1.8	17.9	23.4
23	0.0	10.1	16.7	0.0	21.3	30.3	0.0	21.2	29.7
24	0.0	14.6	22.0	0.0	22.1	29.6	12.2	21.0	27.9
25	0.0	11.5	18.2	0.1	19.7	26.8	0.2	20.7	26.1
26	0.0	12.0	20.7	0.0	20.7	29.3	0.7	16.5	22.1
27	0.3	13.5	18.6	0.0	19.6	29.9	0.1	16.7	23.6
28	0.0	16.3	24.2	0.2	20.0	28.2	1.5	16.4	21.7
29	1.6	14.8	19.2	13.4	14.8	19.7	0.0	18.2	24.3
30	1.4	16.4	23.3	0.6	17.3	23.2	0.9	18.2	23.6
31	0.3	14.7	21.1				5.6	16.8	22.3
∑, mean	3.6	13.0	19.5	38.5	17.3	24.5	43.2	19.6	27.0
1982 - 2003	11.2	13.6	-	57.0	16.5	-	100.0	19.0	-

[1] precipitation

[2] air temperature (2.5 m)

[3] canopy radiative temperature

Table 6.5. Precipitation and temperature at the study area in August and September 2005

Day	August			September		
	p[1] (mm)	Ta[2] (°C)	Tc[3] (°C)	P[1] (mm)	Ta[2] (°C)	Tc[3] (°C)
1	0.3	16.2	21.8	0.5	8.6	14.8
2	0.9	18.1	24.8	0.0	10.9	17.2
3	0.0	18.3	25.0	0.0	13.4	20.1
4	0.0	17.9	26.1	0.0	14.4	21.6
5	0.0	17.4	24.1	0.0	16.9	24.0
6	0.0	19.1	28.3	0.0	16.9	24.0
7	0.0	20.6	29.0	0.0	17.5	24.3
8	0.0	21.9	30.8	0.0	18.7	25.8
9	0.0	20.5	28.3	0.0	19.6	25.6
10	0.0	23.0	31.4	1.4	16.0	22.8
11	0.0	25.7	34.1	0.3	17.1	20.7
12	9.1	20.2	25.9	0.0	12.3	18.8
13	0.0	22.0	29.1	0.0	13.8	21.1
14	0.2	24.1	30.9	0.0	15.3	21.7
15	9.4	15.8	21.7	0.0	14.1	19.4
16	0.0	13.7	19.1	0.2	16.5	23.5
17	0.0	14.0	20.7	-	-	-
18	0.0	15.8	22.1	-	-	-
19	0.0	16.3	24.0	-	-	-
20	0.0	18.1	24.8	-	-	-
21	0.1	16.6	23.0	-	-	-
22	0.0	17.4	26.1	-	-	-
23	0.0	17.9	25.3	-	-	-
24	0.0	17.1	25.5	-	-	-
25	0.0	16.8	25.9	-	-	-
26	0.0	18.6	26.2	-	-	-
27	0.0	19.5	27.6	-	-	-
28	0.0	20.8	27.2	-	-	-
29	0.0	19.2	25.9	-	-	-
30	0.0	18.3	25.7	-	-	-
31	2.6	18.7	23.1	-	-	-
∑, mean	22.6	18.7	25.9	2.4	15.1	21.6
1982 - 2003	69.8	17.4	-	22.3	12.0	-

[1] precipitation

[2] air temperature (2.5 m)

[3] canopy radiative temperature

LEBENSLAUF

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