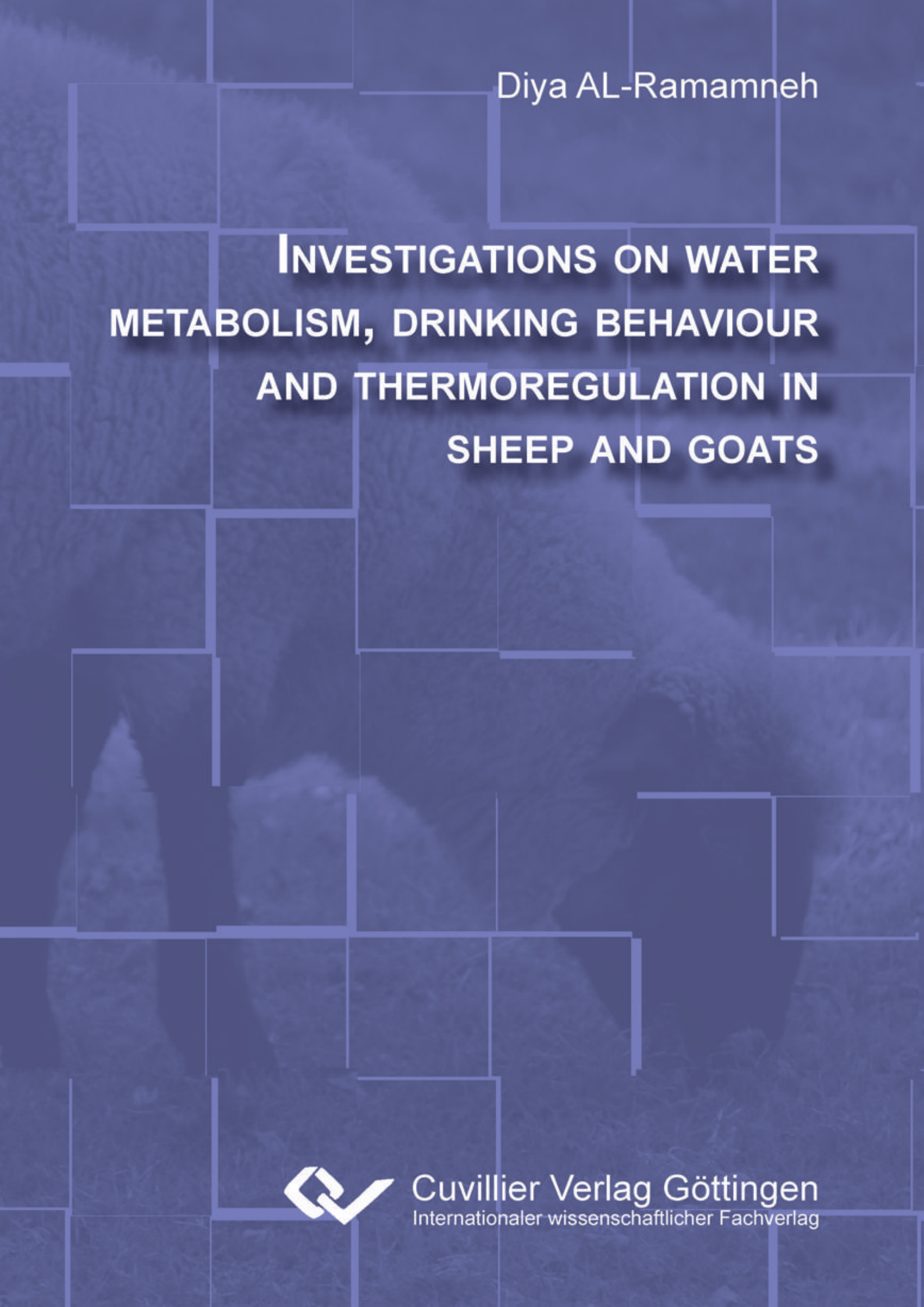
Diya AL-Ramamneh

Investigations on water metabolism, drinking behaviour and thermoregulation in sheep and goats



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Investigations on water metabolism, drinking behaviour and thermoregulation in sheep and goats

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Summary

Water availability is considered as one of the crucial factor in sustainable animal production, especially in arid and semi - arid areas. Water is an important component of animal nutrition because it fulfills a wide range of physiological and chemical functions inside the animal body for internal metabolism and heat dissipation.

The aim of this study was to evaluate the suitability of the deuterium (D_2O) dilution technique in estimating water intake and turnover rate in sheep and goats under different practical conditions (water restriction and wool shearing) and to compare it with direct water measurements. In addition, sheep and goats were compared in their water requirement and drinking behaviour. Thermoregulation in relation to water intake was studied in sheep before and after shearing by using infrared thermography.

Three experiments were conducted at the Department of Animal Sciences, Georg – August - University of Goettingen, Germany. In each trial, German blackhead mutton sheep and Boer goats were kept under controlled stable conditions. The first experiment was conducted to test wether the D_2O dilution technique accurately predicts water intake in sheep and goats and wether the species differ in their water intake and drinking behaviour. In this study, eight dry females from each species had access to rye grass hay and water *ad libitum*. Individual daily water intake was measured for two weeks by re - weighing water buckets and was also estimated from water kinetics using the D_2O dilution technique, respectively. A dose of 0.2 g D_2O / kg body mass was administered to each animal and blood samples were collected at 12, 24 h and 2, 4, 7, 9, 11 and 14 days after the application of D_2O. Prior to the isotope administration a base line blood sample was drawn to determine the background level of D_2O in each animal. In addition, individual drinking behaviour (frequency and duration) was observed during the experimental periods every two to three days for 24 hours by using a time - lapse (8 - folds) video recording system. Infrared lights were used to facilitate data recording during night. German

blackhead mutton sheep differed from Boer goats in their water and feed intakes and drinking behaviour, with higher feed and water intake in sheep (1.6 ± 0.3 kg / day and 4.7 ± 0.9 l / day) than in goats (1.3 ± 0.3 kg / day and 2.3 ± 0.4 l / day), respectively. The species differences in their water intake were also confirmed by using the D_2O dilution technique with higher total water intakes estimated for sheep than for goats. Furthermore, drinking duration confirmed the higher amount of water intake in sheep (4.06 ± 1.98 min / 24h) compared to goats (1.48 ± 0.53 min / 24h).

In the second experiment, the physiological responses of sheep and goats to water restriction using the D_2O dilution technique and drinking behaviour were assessed and compared to *ad libitum* water offerings. Ten dry females of both species were allocated into two treatment groups. The control groups (n = 5, for each species) received water *ad libitum*, while the treatment groups (n = 5, for each species) received water for 3 h / day during the first week and 6 h / 2 days in the second week of the experiment. Individual feed and water intake and respiratory rate were measured daily while rectal temperature and body mass were recorded once a week. In addition, D_2O was injected to estimate individual water intake for both species and drinking behaviour was recorded. Water restriction for 21 h / day or 42 h / 2 days caused no significant differences in water and feed intake or body mass in both species. This absence of differences between species in their water intakes were also confirmed by using the D_2O dilution technique. When water restriction was increased from 3 h / day to 6 h / 2 days, animals increased their drinking frequency, drinking duration and almost doubled their water intake during the hours of water availability. Furthermore, sheep had higher respiratory rates and rectal temperatures compared to goats under water restriction conditions that can be explained with higher drinking frequencies for evaporative cooling in sheep.

In the third experiment, the effect of shearing in German blackhead mutton sheep on water intake was investigated. Additionally physiological and behavioural responses

before and after shearing under temperate climatic conditions (18.3 - 21.4 ° C) were recorded. Fourteen dry ewes were divided into two groups based on wool length, a shorn control group (n = 7, average wool length 2.3 ± 0.8 cm) and an unshorn group (n = 7, average wool length 10.6 ± 1.2 cm). Two weeks into the experiment, the wooly group was shorn (average wool length 1.1 ± 0.2 cm). Body mass, rectal temperature and surface temperature were measured once a week by using infrared thermography to characterize surface temperature differences before and after shearing. Drinking behaviour was recorded every 2 to 3 days while respiratory rate, water and feed intake were measured daily. Deuterium was injected two times before and after shearing to estimate individual water intakes. The newly shorn ewes were more efficient in their thermoregulation compared with their unshorn status. Their enhanced thermoregulatory efficiency after shearing was demonstrated by reduced water consumption, respiratory rate, rectal temperature, and drinking frequency. The differences in water intake between shorn and unshorn ewes were also confirmed by using the D_2O dilution technique with higher water intake either measured or estimated in unshorn ewes. Infrared thermography showed that the surface temperature of the rump and leg increased after shearing to allow higher heat dissipation from the animal's body.

The present studies suggest that Boer goats have superior water management mechanisms compared to German blackhead mutton sheep even under temperate environments. Both species showed the capability to tolerate a moderate water shortage by activating several physiological mechanisms and behavioural strategies. Shorn sheep showed a better heat tolerance to temperate conditions compared to unshorn sheep. The D_2O dilution method gave accurate estimates of water intake in sheep and goats under different practical management practices. The application of D_2O dilution technique, video recordings, and infrared thermography in animal production offers useful techniques for the understanding of reactions in domestic animals to their environment under natural

conditions. Accordingly, selection of animal species that are more adapted to water shortages and higher ambient temperatures would be an effective way to maximize sustainable animal productivity in arid and semiarid areas.

Chapter 1

Introduction

Introduction

Importance of water

Water fulfills a wide range of physiological and chemical functions and plays an essential role in all life processes (King, 1983; Murphy, 1992). About 99 % of all molecules in the body are water, which forms about 70 % of the body mass of the animals (MacFarlane and Howard, 1972).

Water is used for two main functions: intermediary metabolism for normal rumen fermentation and metabolism, proper flow of feed through the digestive tract, nutrient absorption, and normal blood volume (King, 1983; Murphy, 1992). Another function of water is to dissipate internal or absorbed heat by evaporative cooling through sweating or panting (King, 1983; Murphy, 1992).

Water balance

The total body water is all the water in the animal body including that inside and outside of cells (MacFarlane and Howard, 1972), and must remain practically constant in the long term (King, 1983). Accordingly, all water loss (evaporative, urine, faeces and milk production) must be compensated by an equal intake (drinking water, water in feed and metabolic water generated by oxidation of organic compounds) by the animals (Maynard et al., 1981; King, 1983; Murphy, 1992; Freer et al., 2007).

Feeds contain variable amounts of water depending on the moisture content, which may range from as low as 5 % in dry feeds to as high as 90 % or more in juicy plants (Sirohi et al., 1997). Water consumption of animals tends to be higher on feeds that contain high protein or fiber content, because animals need a higher water turnover for the excretion of nitrogen in the urine (Ferreira et al., 2002).

Oxidation of organic compounds can be used to produce metabolic water by animals under harsh conditions such as heat when water is needed for heat dissipation by

vaporization (King, 1983). One gram of metabolized carbohydrates, fat and protein yield 0.56, 1.07 and 0.42 gram water, respectively (Maynard et al., 1981).

Faecal water represents a larger source of water loss than urine. Therefore, the ability to extract and reabsorb faecal water in the colon is important for the water balance and this ability is different between species with higher ability of goats to produce dried faeces than sheep or cattle (Silanikove, 2000).

Furthermore, livestock species vary in terms of their ability to concentrate urine and / or decrease renal urine flow and retain substances to the body fluids depending on the concentrating ability of the kidneys (King, 1983). Arid adapted species (sheep and goats), which produce urine that is more concentrated, have longer loops of Henle than do other species (e.g. cattle) with short loops (MacFarlane, 1968b; McNab, 2002). More details are presented in chapter 3. The amount of water saved by concentrating the urine is relatively small compared with that lost by evaporation. This pathway of water loss is the major mechanism for body temperature control. Cutaneous evaporation is dependent upon the activity level of the animal, ambient temperature, humidity, and wind speed (King, 1983).

Livestock that are well adapted to arid areas may be able to tolerate food and water shortage for several days depending on oxidation of their fat deposits (hump of the camels and fat tail of the sheep) and other physiological and behavioural mechanisms (Silanikove, 2000). MacFarlane and Howard (1972) found that camels who were dehydrated by 20 to 25 % replaced 60 % of the weight lost as water (80 – 100 l) in the first drink, while sheep and cattle replaced 75 % of the body weight. The animals replaced all the weight lost from dehydration in 1 or 2 days. In this context, Adolph (1982) divided mammals into two categories: those, which refill water lost rapidly, and those, which do so gradually. Silanikove (1989) reported that upon rehydration, cattle may drink up to 18 % of their body weight, sheep 20 %, camels 25 % and it may reach 40 % in the desert black Bedouin

goats, within 3 - 10 minutes. In general, ruminants can replace 15 - 20 % of their body weight at the first drinking and 20 – 25 % within 1 - 1.5 hour (King, 1983).

Factors influencing water balance

Several studies documented that livestock vary in terms of their water requirements (Table 1) depending on several factors that influence their water intake due to their genetic differences (Tajane et al., 1992), physiological status and age (Das et al., 1999), animal activity (Pond et al., 1995), body size (Daramola and Adeloye, 2009), species or breed (Aganga et al., 1989; Squires, 1993; Silanikove, 2000; Ferreira et al., 2002), environmental temperature and humidity (King, 1983; Murphy, 1992), dry matter intake (Nocek and Braun, 1985; Silanikove, 1992), feeding regime and food consumption (King, 1983; Sirohi et al., 1997; Salem et al., 2006), milk yield (Hamadeh, et al., 2006), water availability (Alamer, 2006), water temperature (Savage et al., 2008) and disease status (King, 1983; Murphy, 1992).

Marked differences appear between ruminants and monogastric animals in their water requirements and their tolerance of water shortage because ruminants have a large fluid reservoir (the rumen), which can store large volumes of water to be used under water shortage condition (Silanikove, 1992; Burgos et al., 2001).

Recommendations on water consumptions for livestock are summarized in Table 1. Water consumption varies widely among the different classes of livestock and is influenced by factors such as climate and type of feed being consumed.

Table 1: Recommendations of water requirements for livestock

Type of livestock		Average daily consumption (l / head)	References
Sheep	Feeder lamb (27 - 50 kg BW)	3.6 - 5.2	NRC, 1985
	Gestating meat ewe / ram (80 kg BW)	4.0 - 6.5	NRC, 1985
	Lactating meat ewes plus unweaned offspring (80 kg BW)	9.0 - 10.5	NRC, 1985
	Gestating dairy ewe / ram (90 kg BW)	4.4 - 7.1	NRC, 1985
	Lactating dairy ewe (90 kg BW)	9.4 - 11.4	NRC, 1985
	Lactating ewes on dry feed	9	ANZECC, 2000
	Mature sheep on dry pastures	7	ANZECC, 2000
	Mature sheep on green pastures	3.5	ANZECC, 2000
	Fattening lambs on dry pasture	2.2	ANZECC, 2000
	Fattening lambs on green pasture	1.1	ANZECC, 2000

Table 1 continued

Type of livestock		Average daily consumption (l / head)	References
Cattle	Dairy calves (1 - 4 months)	4.9 - 13.2	Adams et al., 1995; McFarland, 1998
	Dairy heifers (5 - 24 months)	14.4 - 36.3	Adams et al., 1995; McFarland, 1998
	Dairy cows in milking (13.6 l / d)	68 - 83	Adams et al., 1995; McFarland, 1998
	Dairy cows in milking (22.7 l / d)	87 - 102	Adams et al., 1995; McFarland, 1998
	Dairy cows in milking (36.3 l / d)	114 - 136	Adams et al., 1995; McFarland, 1998
	Dairy cows in milking (45.5 l / d)	132 - 155	Adams et al., 1995; McFarland, 1998
	Dry cows	34 - 49	Adams et al., 1995; McFarland, 1998
	Beef cattle (181 - 364 kg BW)	15 - 40	NRC, 2000
	Beef cattle (364 - 636 kg BW)	27 - 55	NRC, 2000
	Calves	22 - 25	Adams et al., 1995; McFarland, 1998
Horses	Small (500 lb)	13 - 20	NRC, 1989; Groenendyk et al., 1988
	Medium (1000 lb)	26 - 39	NRC, 1989; Groenendyk et al., 1988
	Large (1500 lb)	39 - 59	NRC, 1989; Groenendyk et al., 1988
	Working	55	ANZECC, 2000
	Grazing	35	ANZECC, 2000

Table 1 continued

Type of livestock		Average daily consumption (l / head)	References
Poultry (per 1000 birds)	Chicken broiler (1 - 4 week age) / 21 °C	50 - 260	North Mack and Bell Donald, 1990
	Chicken broiler (1 - 4 week age) / 32 °C	50 - 415	North Mack and Bell Donald, 1990
	Chicken broiler (5 - 8 week age) / 21 °C	345 - 470	North Mack and Bell Donald, 1990
	Chicken broiler (5 - 8 week age) / 32 °C	550 - 770	North Mack and Bell Donald, 1990
Poultry (per 100 birds)	Laying hen	32	ANZECC, 2000
	Non - laying hens	18	ANZECC, 2000
	Turkeys	55	ANZECC, 2000

Several studies have shown that goats require less water than sheep and cattle (King, 1983; Aganga et al., 1989; Murphy, 1992; Silanikove, 1992; Ferreira et al., 2002; Freer et al., 2007). This ability is related to their feeding behaviour, digestive function and physiological adaptation to utilize available feed and water (Silanikove, 2000; Daramola and Adeloye, 2009). Goats show a particular feeding behaviour by selecting palatable feed parts from plants with high fiber contents. They have greater rates of saliva secretions, and more tolerance to tannins in the plant parts (Devendra, 1990; Silanikove, 2000; Daramola and Adeloye, 2009). Silanikove (2000) analysed the physiological basis of the superiority of goats in economizing water resources by the role of their rumen as water reservoir that can be utilized during dehydration and rapid rehydration. In addition, the small body size, low metabolic requirements, efficient utilization of high fibre forage, the ability to minimize water losses via urine and faeces and to reduce nitrogen requirements via urea recycling and nitrogen conservation are the main adaptation strategies of goats to withstand food and water shortage (Silanikove, 2000; Daramola and Adeloye, 2009). In this context, it is of interest to note that this suggested superiority of goats for desert conditions was also found in the Boer goats originating from dry areas used in the first and the second study where animals were kept under temperate climatic conditions (further information is presented in chapter 2 and 3 in this thesis).

The water requirement of animals increases during the dry season, as consequence of the insensible water loss through respiration and evaporation (King, 1983; Freer et al., 2007; McKinley et al., 2009). Water and salt are lost in the urine, feces and sweat (Silanikove, 1992; Pennisi et al., 2004).

Heat is considered as a major restriction of animal productivity because it adversely affects animal homeostasis (Marai et al., 2007). Arid adapted species can tolerate scarcity of food and water, and high solar radiation better than less well adapted species (Silanikove, 2000; McKinley et al., 2009). According to Silanikove (1992, 2000) the general

response to thermal stress in mammals to maintain homeostasis includes increased respiration rates, panting, sweating, water intake and reduced heart rates, feed intake and reduced milk production (Murphy, 1992; Marai et al., 2007).

Thermoregulation in sheep is influenced by characteristics of their fleece (Pennisi, et al., 2004) which are related to breed, age, sex, and environmental condition like temperature, relative humidity, and wind (Sleiman et al, 1995; Pennisi, et al., 2004). Klemm (1962) described the Merino sheep coat as thick and tightly packed thus hindering the rate of evaporation of sweat from the animal. Klemm (1962) found that the Merino fleece is efficient in hot dry temperature as insulator while in hot humid temperature it is hindering water vaporization of the fleece. On the other hand, a shorn sheep is better able to adapt to a hot and humid environment (Klemm, 1962; Hatem et al., 2009; Gerken, 2010). In this context, infrared thermography (IR) could be a suitable technique for monitoring thermoregulative efficiency in animals under different environmental conditions. The IR is a modern and non - invasive technique of thermal profile visualization. Gerken and Barow (1998) used IR for measuring the skin temperature at different regions of the body in suckler cows. Consequently, IR can be used to detect changes in vascular circulation due to increase or decrease in the body temperature (Mccafferty, 2007). Maccafferty (2007) pointed out that infrared radiation released by bare - skinned animals is controlled by the skin surface temperature but the radiation released from most mammals may originate from both the skin or the coat cover. Accordingly, coat characteristics play a major role in determining solar heating of the surface. Dirty and compact or black coat colours have higher surface temperatures than shorn or white clean coats on sunny days compared to pale coat colours (Maccafferty, 2007). Bare skin has an emissivity of 0.98 and the emissivity of dry fur is relatively uniform in mammals, ranging between 0.98 to 1.0, but the emissivity of the coat can also be changed by dirt or other materials.

This non - invasive technique allows a very detailed evaluation of surface temperature that can be correlated with internal body temperature without the need of internal temperature loggers. Preliminary reports suggested that eye temperature recorded by IR could be used to determine rectal and vaginal measurements in domestic animals (Sykes et al., 2006; Maccafferty, 2007). If this method can be confirmed, then it may open a non - invasive technique for monitoring internal temperature under both laboratory conditions and field studies. Additional information exists in chapter 4 of this thesis.

Measurement of water intake and water turnover

Recent technical developments allow the direct measurement of individual water intakes under stable conditions, but require expensive technical devices for accurate measurement of the drinker's water flow and the animal identity (McAllister et al., 2000). Similar systems could be adapted to range conditions when no further water sources are available. However, water loss by evaporation and water spoilage cannot be accurately estimated. Furthermore, these devices are not available under extensive (e.g., nomadic) conditions. The measurement of water intake by weighing, e.g., water buckets before and after water administration is laborious and also imposes constants with regard to free movement of animals. Desiccation and drying of the body represents one method to measure water balance and flux inside the body compartment of animals but is labor intensive and the loss of water by evaporation is difficult to be avoided during desiccation. In this context, indirect water measurements offer more suitable solutions of estimating individual water intake and water flux even at free ranging conditions.

Isotope dilution technique

One of these indirect methods is the isotope dilution technique (Holleman et al., 1982) including D_2O and Tritium water (TOH). Isotope dilution techniques became the method of choice to measure water flux, water intake, total body water and body composition because the isotope crosses the body barriers at the same rate as body water and mixes completely with body fluid (Holleman et al., 1982; Penman and Wright, 1987; Atti et al., 2000). In addition, stable isotopes are not toxic to the experimental subjects (Pinson, 1952), and can be applied in the field under nomadic husbandry conditions (Lifson and McClintock, 1966; Holleman, et al., 1982, Atti et al., 2000).

Table 2 and 3 give an overview of published data on total body water estimated in sheep and goat breeds by the isotope dilution technique.

Table 2: Total body water (TBW % of body mass) in different sheep breeds at different physiological status

Breed	N	Sex	Age (Year)	Isotope	TBW (%BM)	Experimental Conditions	Reference
English Leicester (castrated)	1	M	Adults	TOH	36.8		Panretto, 1963
Border Leicester X Merino (castrated)	1	M		TOH	51.1		Panretto, 1963
Merino (non – pregnant)	7	F		TOH	51.3 - 74.2		Panretto, 1963
Merino (selected for wool production)	12	F	4.3 - 4.9	TOH	128.7	Wet season	MacFarlane et al., 1966
Merino (selected for wool production)	10	F	4.3 - 4.9	TOH	113.7	Dry season	MacFarlane et al., 1966
Merino	12	F	4.3 - 4.9	TOH	137.5	Wet season	MacFarlane et al., 1966
Merino	10	F	4.3 - 4.9	TOH	100	Dry season	MacFarlane et al., 1966
Merino	9	F		TOH	39.0 - 70.2		Panaretto, 1968
Weathers	4	F		TOH	61.2 - 72.0		Panaretto, 1968
Merino - Weather (cross breed)	2	F		TOH	56.6 - 62.1		Panaretto, 1968
Blackface (pregnant)	13	F	2.5 - 5	D_2O	97.7 - 113.8		Foot and Greenhal, 1970
Uda	4	M	1 - 1.3	TOH	80.5 ± 1.2	Hot-dry season	Aganga et al., 1989
Yankasa	4	M	1 - 1.3	TOH	73.6 ± 0.8	Hot-dry season	Aganga et al., 1989
Barbary (fat tailed)	16	F		D_2O	49 - 69		Atti et al., 2000
German blackhead mutton	8	F	2.3	D_2O	58.3 ± 3.1	Temperate condition	AL-Ramamneh et al., 2010

Table 3: Total body water (TBW % of body mass) in different goat breeds at different physiological status

Breed	N	Sex	Age (Year)	Isotope	TBW(%BM)	Experimental conditions	Reference
Toggenburg (castrated)	3	M	Adults	TOH	64.4 - 75.7		Panretto, 1963
Saanen (castrated)	7	M	Adults	TOH	55.6 - 72.7		Panretto, 1963
Goats	13	F	Adult	TOH	72.5 - 54.8		Panaretto and Till, 1963
Bedouin	10	F	Adult	TOH	68.8 ± 2.2	4 day water deprivation	Shkolnik et al., 1980
Bedouin	10	F	Adult	TOH	76.3 ± 2.1		Shkolnik et al., 1980
Goats (lactating)	4	F	Adult	TOH	83.5		Maltz and Shkolnik, 1980
Goats (lactating)	4	F	Adult	TOH	72.18	4 day water deprivation	Maltz and Shkolnik, 1980
Goats	15	F	2-5	D_2O	58.3 ± 4.9		Brown and Taylor, 1986
Sahel	4	M	1 - 1.3	TOH	77.8 ± 0.4	Hot - dry season	Aganga et al., 1989
Maradi	4	M	1 - 1.3	TOH	66.1 ± 2.8	Hot - dry season	Aganga et al., 1989
Boer	8	F	6	D_2O	60.9 ± 2.3	Temperate condition	AL - Ramamneh et al., 2010

Using stable isotope techniques in living animals is based on injecting the experimental animal with a known amount and concentration of isotope (D_2O, in this case) and allow sufficient time for D_2O molecules to pervade the entire body water (equilibrium period) which is different from one subject to another related to body weight, size, physiological status and animal species and the route by which D_2O is given to the animal (Degen et al., 1981; Holleman et al., 1982; Atti et al., 2000). A base line blood sample is taken before isotope administration to determine the background level of D_2O in the experimental subject. Subsequently, blood samples are collected to follow the decline in the specific activity of the hydrogen isotope in the body water through time indicating the loss of labeled water and therefore the outward water flux rate (Kanto and Clawson, 1980; Degen et al., 1981; Holleman et al., 1982; Atti et al., 2000). In adult animals with a constant flux rate the specific activity declines because of the loss of labeled water from the animal via defecation, urination, evaporation, and panting and due to drinking, preformed, and metabolic water (Holleman et al., 1982; Oftedal et al., 1983). A detailed description of the method can be found in the materials and methods section of chapter 2.

Errors in isotope dilution methods are mainly caused by overestimates and are due to the loss of isotopes during application, and errors in the estimated isotope enrichment of the dose (Holleman et al., 1982; Atti et al., 2000). Lack of equilibrium of the isotopes within body fluids which mainly depends on the route of isotope administration or reduced turnover rate of animals deprived of food and water during the equilibrium period. However, there are several potential disadvantages using the isotope dilution method. First, this method is somewhat time consuming as the animal must be under food and water restriction for 5 - 6 h (depending on the species and size) to allow the isotopes to equilibrate within the total body water. Second, the cost of isotopes and subsequent quantitative analysis can become significant. Collectively they limit the number of animals which can be studied (Holleman et al., 1982).

Drinking behaviour

Another indirect method to estimate water intake is based on the knowledge of animal behaviour by understanding the factors that can affect animal productivity and estimate the amount of water intake (Murphy, 1992). Continuous recording systems using time - lapse video records can be a valuable technique for measuring the drinking behavioural pattern in animals without any disturbance (Das et al., 1999; Keskin et al., 2005). Usually, animals drink more than their actual water needs (Yang et al., 1981). This extra water intake probably acts as a reliable method to maintain homeostasis. Murphy (1992) reported several factors that have been shown to influence drinking behavior of dairy cattle including their feeding pattern, water temperature, water administration methods in a trough or bowl, flow rates into water bowls or animal dominance if water bowls are shared. In addition to several factors altering water consumption of dairy cattle are dry matter intake, nature of the diet, milk production, environmental temperature and humidity.

Keskin et al. (2005) found that Awassi sheep spent about < 1 % of a 24 h period (less than 5 min / day) on drinking. They found that the physiological status of the animal also affects the drinking behaviour, as pregnant sheep spent less time for drinking than growing or lactating sheep. Several studies reported that peak drinking activity among sheep occurred near sunrise and sunset and is associated with feeding time (Shrefer and Hohenboken 1980, Das et al. 1999). Further details are given in each chapter of this study.

Scope of the thesis

The general aim of this study was to evaluate whether D_2O dilution technique would accurately predict water intake and turnover in sheep and goats under different practical management practices (water restriction and shearing) by comparing the results with

measured water intake. Another focus was the species comparison between sheep and goats in relation to their use of water.

Three experiments were conducted using German blackhead mutton sheep and Boer goats to compare the water intake and the drinking behaviour in both species under a free watering regime. Two consecutive trials were performed to characterize the drinking behaviour and the water intake in both species under *ad libitum* and restricted water administration. Finally, shorn and unshorn German blackhead mutton sheep were compared in their drinking behaviour and water intake and thermoregulation using infrared thermography and other physiological parameters.

In each study, we used the D_2O dilution technique to estimate individual water intake and total body water content and compared it with direct individual measurement of water intake by weighing water buckets before and after water administration.

The objectives of this thesis were:

(1) To evaluate the D_2O dilution technique for estimation of water metabolism and water intake in German blackhead mutton sheep and Boer goats under different practical management practices.

(2) To test whether species differences exist under temperate conditions in relation to water metabolism and drinking behaviour.

(3) To verify the postulated superiority of goats in economizing water resources compared to sheep when subjected to restricted water administration.

(4) To evaluate whether the species differences might be due to differences in coat morphology and to examine the impact of fleece in sheep on water requirement for thermoregulation.

The second topic is presented in detail in chapter 2, while the third topic is discussed in chapter 3; the following chapter 4 contains the fourth topic. The first topic is presented in every chapter from this study.

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Chapter 2

Deuterium oxide dilution accurately predicts water intake in sheep and goats

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Abstract

The aim of this study was to test whether the deuterium oxide dilution technique accurately predicts water intake in sheep and goats. Two other issues were also studied: a comparison of water intake in sheep and goats and an assessment of whether observations of drinking behaviour can accurately measure the water intake. In this study, 8 dry Boer goats and 8 dry German black head mutton ewes were kept under controlled stable conditions. Animals had access to hay and water *ad libitum*. Diurnal drinking behaviour was recorded by video. Individual daily water intake was measured and estimated for two weeks by re - weighing water buckets and from water kinetics using the deuterium oxide dilution technique, respectively. In addition, dry matter intakes were directly measured and were significantly higher in sheep than in goats. The average daily water consumption by drinking differed significantly between the two species, with higher intakes in sheep than in goats. Total body water expressed as a percentage of body mass did not differ between species. Measurement methods of total water intake (TWI) using deuterium oxide dilution and re - weighing water buckets did not differ significantly in both species ($P = 0.926$). Results obtained for measured and estimated TWI confirm that the isotope dilution technique gives reliable results for estimates of water intake in sheep and goats. The higher amount of water intake in sheep was also reflected by their drinking behaviour. Sheep spent approximately 0.3 % per 24 h drinking, while Boer goats spent only 0.1 %. However, measured and estimated total water intake were only moderately correlated to the daily time spent drinking. The lower water intake found in Boer goats confirms a superior water management capacity compared with black head mutton sheep even under temperate conditions.

Keywords: sheep; goats; water intake; isotope dilution; total body water; behaviour

Introduction

Various factors have been shown to influence water intake in domestic small ruminants including climatic conditions (King, 1983; Squires, 1993; Sirohi et al., 1997), species or breed (Aganga et al., 1989; Squires, 1993; Silanikove, 2000; Ferreira et al., 2002; Keskin et al., 2005; Salem et al., 2006), physiological state and age (Das et al., 1999). In addition, water intake is linearly related to dry matter intake (MacFarlane and Howard, 1972; Nocek and Braun, 1985) and thus influenced by feeding regime and food composition (King, 1983; Silanikove, 1989; Sirohi et al., 1997; Ferreira et al., 2002; Salem et al., 2006)

An animal's net requirement for water can be calculated as the sum of the minimal losses in faeces and urine, evaporative losses, the water gained by the body in growth and pregnancy, and that lost by secretion in milk (Freer et al., 2007). Measurements of water intakes showed that the intakes are generally greater than calculated minimal requirements which might be due to the isotonic urine excretion by animals (Larvor, 1983). Water intake in sheep ranges between 2 and 4.6 l per kg dry matter intake (DMI) for adult animals when kept under temperate conditions and is reported to increase to 4.2 and 12 l per kg DMI under arid conditions (Lynch et al. 1972; Freer et al., 2007).

Species differences in water requirements are of major importance for a sustainable use of limited water resources e.g., in arid and semi arid areas and represent important criteria for the selection of the most appropriate type of domestic animal kept under these conditions (Devendra, 1990; Silanikove, 2000). Several studies have confirmed differences between sheep and goats in their water consumption and water conservation capacities. Mutton Merino lambs had a 49 % higher water intake per kg mass gain than Boer goats (Ferreira et al., 2002). Higher water turnover rates were also found in sheep compared with goats kept under tropical conditions in Nigeria (Aganga et al., 1989). The lower water turnover rates in goats suggest that goats are better adapted to withstand dehydration than sheep under dry climatic conditions (Silanikove, 2000). Differences between sheep and

goats in drinking frequency were in the same direction as water intake, so measurement of drinking behaviour may be an easy way to measure the water intake (Keskin et al., 2005).

Several methods have been applied to evaluate the water intake. The direct measurement of water consumed is not applicable under most extensive (and nomadic) husbandry conditions in which sheep and goats in arid and semi arid areas are mainly kept. In this context, the isotope dilution technique (Lifson and McClintock, 1966) is a suitable method for estimating individual water flux under free range conditions and has been applied in the field in many species (Aggrey, 1982; Aganga et al., 1989; Atti et al., 2000). Isotopes used include the nontoxic stable ^{2}H in deuterium oxide (D_2O) and ^{3}H in tritiated water (TOH), which is a weak radioactive beta emitter. Aganga et al. (1989) applied tritiated water to determine water spaces in sheep and goats. However, for free range studies D_2O appears to be a better alternative for environmental reasons and welfare considerations. The accuracy of the D_2O dilution technique in estimating total body water (TBW), water turnover rate, body composition and milk intake was confirmed in several studies in sheep (Holleman et al., 1982; Penman and Wright, 1987; Atti et al., 2000). However, to the authors' knowledge no such studies applying D_2O are available for goats.

This study was undertaken to evaluate the D_2O dilution technique in sheep and goats. We also tested the hypothesis of a species difference in water intake and measurements of drinking behaviour were included to evaluate the possibility of their usefulness in measuring water intake.

Materials and Methods

Animals and management

In this study, 16 dry animals (8 German black head Mutton ewes and 8 Boer goats) were involved. Sheep originated from the Experimental Station Relliehausen of Göttingen

University and the goats from a herd owned by the Department of Animal Sciences, University of Göttingen. Animals were transferred to the experimental pens 1 week before to the start of the trial for acclimatization and were kept for two consecutive weeks at the Department of Animal Sciences under controlled stable conditions (room temperature 13.6 ± 0.4 ºC; relative humidity 49.1 ± 9.7 %, means ± SD; light schedule: 10 h dark and 14 h light). Animals were kept in four different rooms in individual straw pens (1.5 x 2.0 m) separated by species. Sheep and goats were on average 2.3 ± 0.5 and 6.0 ± 1.8 years old. Sheep and goats were weighed at the beginning and the end of the experiment and had a body mass of 69.0 ± 8.3 kg and 64.2 ± 3.2 kg, respectively. Hay from ryegrass dominated grassland with an average DM content of 85.7 ± 1.2 % and water was available *ad libitum*. Daily samples of hay were oven dried at 105 º C for 24 h to determine the DM content. The moisture content was calculated as [(wet sample weight – dry sample weight) / wet sample weight] x 100.

Water intake studies

Water was individually provided in open buckets (10 l). Water and hay intakes were individually measured on a daily basis by weighing and re - weighing food and water buckets for each animal with an electronic scale to the nearest 1 g (Sartorius model CP34000, Sartorius AG, Goettingen, Germany). To estimate water loss by evaporation from the bucket surface, a control bucket was placed in the stable and re - weighed daily. The re - weighing of the control water bucket showed that water loss via evaporation from the surface was negligible under the present temperate stable conditions.

Water intake was estimated from water kinetics for two consecutive weeks by using the D_2O dilution technique. Before to the isotope administration a 5 ml blood sample was drawn from the jugular vein into blood tubes containing sodium citrate to determine the background level of D_2O. A dose of 0.3g D_2O / kg body mass of 99.90 % purity (Euriso -

top GmbH, Saarbruecken, Germany) was intramuscularly injected at two body sites. The actual dose given was gravimetrically measured by weighing the syringe before and after the administration of D_2O to the nearest 0.001 g (Sartorius model CW3P1 - 150IG - 1, Sartorius AG). Blood samples (~ 5 ml) were collected at 6 and 24h and 2, 4, 7, 9, 11 and 14 days after the application of D_2O. The samples were then centrifuged within 30 min of collection at 3500 rpm for 10 min. The plasma fraction was pipetted into glass vials and then frozen at - 20 ºC until the determination of the D_2O concentration.

Earlier work showed that tracer concentrations in plasma samples are the same as in vacuum sublimated water samples (Riek et al., 2007). Therefore, plasma samples from sheep and goat were analyzed for D_2O concentrations. Analyses were carried out at the Competence Centre for Stable Isotopes (KOSI, Göttingen University, Germany). Isotope ratios of ^2H were measured using an on - line high temperature reduction technique in a helium carrier gas described previously (Gehre et al., 2004) and expressed relative to the Vienna standard mean ocean water (VSMOW), which is the international reference standard for D_2O. Individual samples were measured in triplicate and the averages calculated.

Calculations

Isotopic equilibrium concentration and fractional water turnover were computed for each animal by extrapolating the regression of the D_2O concentrations (C_t) on time by the regression equation:

$$C_t = C_0 \times e^{-k \times t} \quad (1)$$

where C_0 is the equilibrium concentration (intercept) for D_2O, k is the fractional water turnover (slope) and t is the time elapsed since tracer administration (Holleman et al.,

1982). Isotope equilibration and pre - dose baseline concentration were then used to calculate the D_2O dilution space (V_d) in kilogram by using the equation from Schoeller et al. (1986):

$$V_d = [D \times APE_{dose} \times 18.02 \text{ g / mole}] / [MW_{dose} \times 100 \times (C_0 - C_b) \times R_{std}] \quad (2)$$

where D = dose given in grams, APE_{dose} = atomic enrichment of the dose in percent (= 99.90 %), MW_{dose} = molecular weight of the dose (D_2O = 20.02), C_0 = isotope equilibrium concentration expressed as delta D v. VSMOW, C_b = pre - dose baseline concentration, and R_{std} = ratio of deuterium to hydrogen in VSMOW, i.e. 1.5574×10^{-4}.

The D_2O dilution space was divided by 1.04 to calculate the TBW content as compiled data suggest that the dilution spaces for D_2O are overestimated by 4 % (Schoeller, 1983). As body mass did not differ significantly between the beginning and the end of the trial, no corrections for changing pool size were necessary. The body water fraction, which is TBW content as a percentage of body mass was calculated as TBW in kg divided by body mass in kg.

Finally total water intake of the animals (TWI) was calculated as the product of TBW and k according to Oftedal et al. (1983),that is:

$$TWI = L + G = TBW \times k \quad (3)$$

where L = amount of daily water lost, G = amount of daily water stored and k = daily water turnover rate. TWI includes preformed and metabolic water from food and drinking water. Metabolic water was calculated from the feed composition. It was assumed that 1 g metabolized carbohydrates, fat and protein yielded 0.56, 1.07 and 0.42 gram water, respectively (Maynard et al., 1981). Measured TWI was calculated as

TWI = water drunk + metabolic water + preformed water. (4)

Drinking behaviour

Individual's drinking behaviour was observed during the two experimental weeks every two to three days for 24 h by using a time - lapse (8 fold) video recording system. Infrared lights were used to facilitate data recording during night. For each animal, 6 - 8 observations were available and analyzed with the Interact® 7.0 system (Mangold international GmbH, Amstorf, Bavaria, Germany). Drinking behaviour was defined as follows:

- drinking frequency (number of drinking bouts)
- drinking duration (time in minutes when the animal was actively engaged in the ingestion and swallowing of water).

Statistical analysis

Statistical analyses were performed with the software package Statistical Analysis Systems version 9.01 (Statistical Analysis System Institute (SAS), 2001). The nonlinear regression procedure was used for extrapolating the regression of C_t on time by equation (1). For further statistical analyses averages per animal were used for all traits. Because of the small sample sizes and non - normal distribution of the data, nonparametric procedures were used. The differences between species were tested using the NPAR1WAY procedure in SAS which performs a nonparametric test across a one - way classification. For a better understanding, means ± S.d. are presented in the tables, but significance given is based on the Exact Wilcoxon Two - Sample Test (two sided p - values).

Kendall's Tau b rank correlations (τ) were estimated between water drunk, measured and estimated TWI and drinking behaviour across species, based on averages per animal.

We are aware of the difficulties of a two species approach and therefore followed suggestions to minimize these shortcomings outlined in detail in Garland and Adolph (1994).

Results

Significant species differences were found for nearly all traits measured, with the exception of body mass and TBW (Table 1). Daily water drunk (Table 1) as measured by re - weighing water buckets, differed significantly between the two species with higher amounts for sheep than for goats. These species differences were also maintained when relating water intake to metabolic body mass (water intake per kg body mass$^{0.75}$). DMI differed significantly between the two species with higher intakes in sheep than in goats. The species difference was maintained when expressed as amount of DMI per kg body mass$^{0.75}$, similar to water drunk. When calculating water intake per kg DMI German blackhead mutton sheep had a higher intake than Boer goats.

The body water fraction ranged between 54 to 63 % for sheep and 58 to 64 % for goats, with no significant differences between species. Sheep had significantly higher TWIs than goats, whether measured or estimated. The ratios between estimated TWI (D_2O dilution) and measured TWI (re - weighing water buckets) for both sheep and goats indicate that the D_2O method predicts water intake in both species with high accuracy (Table 1) with only small variations (- 4.2 to 8.9 %). The behavioural observations showed that the drinking frequency was about five times higher in sheep than in goats, with goats ingesting less than half the amount per bout (Table 2). The ratio between water drunk and drinking duration illustrates this species difference in drinking intensity.

Table 1: Body mass (BM), water drunk, dry matter intake, total body water, and total water intake (TWI) in black head mutton sheep and Boer goats kept under stable conditions (numbers represent averages per animal; means ± SD)

Parameter		Sheep *n* = 8	Goat *n* = 8	*P*
Body mass	(kg)	69.0 ± 8.3	64.2 ± 3.2	0.328
	($kg^{0.75}$)	23.9 ± 2.2	22.7 ± 0.9	0.328
Water drunk	($l\ d^{-1}$)[1]	4.7 ± 0.9[a]	2.3 ± 0.4[b]	< 0.001
	(g/kg BM $^{0.75}$)	195.5 ± 30.8[a]	103.6 ± 18.9[b]	< 0.001
Dry matter intake	($kg\ d^{-1}$)	1.6 ± 0.3[a]	1.3 ± 0.3[b]	0.028
	(g/kg BM $^{0.75}$)	68.4 ± 8.6[a]	57.2 ± 12.5[b]	0.021
Water drunk / dry matter intake	($l\ kg^{-1}$)	2.9 ± 0.3[a]	1.9 ± 0.2[b]	< 0.001
Total body water	(% of BM)	58.3 ± 3.1	60.9 ± 2.3	0.195
Measured TWI[2]	($l\ d^{-1}$)	5.5 ± 0.1[a]	3.0 ± 0.5[b]	< 0.001
	(g/kg BM $^{0.75}$)	230.5 ± 34.5[a]	132.9 ± 25.0[b]	< 0.001
Estimated TWI[3]	($l\ d^{-1}$)	5.3 ± 0.9[a]	3.0 ± 0.4[b]	< 0.001
	(g/kg BM $^{0.75}$)	219.3 ± 23.4[a]	143.1 ± 19.3[b]	< 0.001
Estimated TWI / Measured TWI	(%)	95.8 ± 5.6[a]	108.9 ±8.5[b]	< 0.003

[1] Measured by reweighing water buckets.

[2] TWI = metabolic water + water drunk + preformed water from moisture content of the hay.

[3] Estimated by D_2O dilution.

[a, b] values within the same row with different superscripts differ significantly; P - values: Exact Wilcoxon Two - Sample Test, two sided.

Table 2: Daily drinking behaviour (frequency and duration) and water intake in black head mutton sheep and Boer goats kept under stable conditions, based on 24 h video recordings (numbers are averages per animal; means ± SD)

Parameter		Sheep *n* = 8	Goat *n* = 8	*P*
Drinking frequency	(*n* / 24 h)	17.39 ± 11.03[a]	3.60 ± 1.55[b]	< 0.001
	(*n* / h)	0.72 ± 0.46[a]	0.15 ± 0.06[b]	< 0.001
Drinking duration	(min / 24 h)	4.06 ± 1.98[a]	1.48 ± 0.53[b]	0.002
	(min / h)	0.17 ± 0.08[a]	0.06 ± 0.02[b]	0.002
Water drunk / drinking bout	(ml / bout)	14.60 ± 7.05[a]	37.63 ± 29.24[b]	0.002
Water drunk / drinking duration	(ml / min)	57.29 ± 23.97	77.76 ± 27.12	0.083

64 video recordings in sheep (8 per animal), 53 recordings in goats (6.6 per animal).

[a, b] values within the same row with different superscripts differ significantly; P - values: Exact Wilcoxon Two-Sample Test, two sided.

Table 3: Kendall's Tau b correlations (τ) between measured and estimated total water intake (TWI) by using D_2O, water drunk (WD in g / kg $BM^{0.75}$), dry matter intake (DMI in g / kg $BM^{0.75}$) and drinking behaviour across species, based on averages per animal

	Measured TWI	WD[1]	DMI	Drinking frequency	Drinking duration
Estimated TWI	0.983***	0.967***	0.700***	0.717***	0.633***
Measured TWI		0.983***	0.717***	0.700***	0.650***
WD[1]			0.700***	0.683***	0.633***
DMI				0.483**	0.500**
Drinking frequency					0.650***

[1]Measured by reweighing water buckets. *** P < 0.001; ** P < 0.01

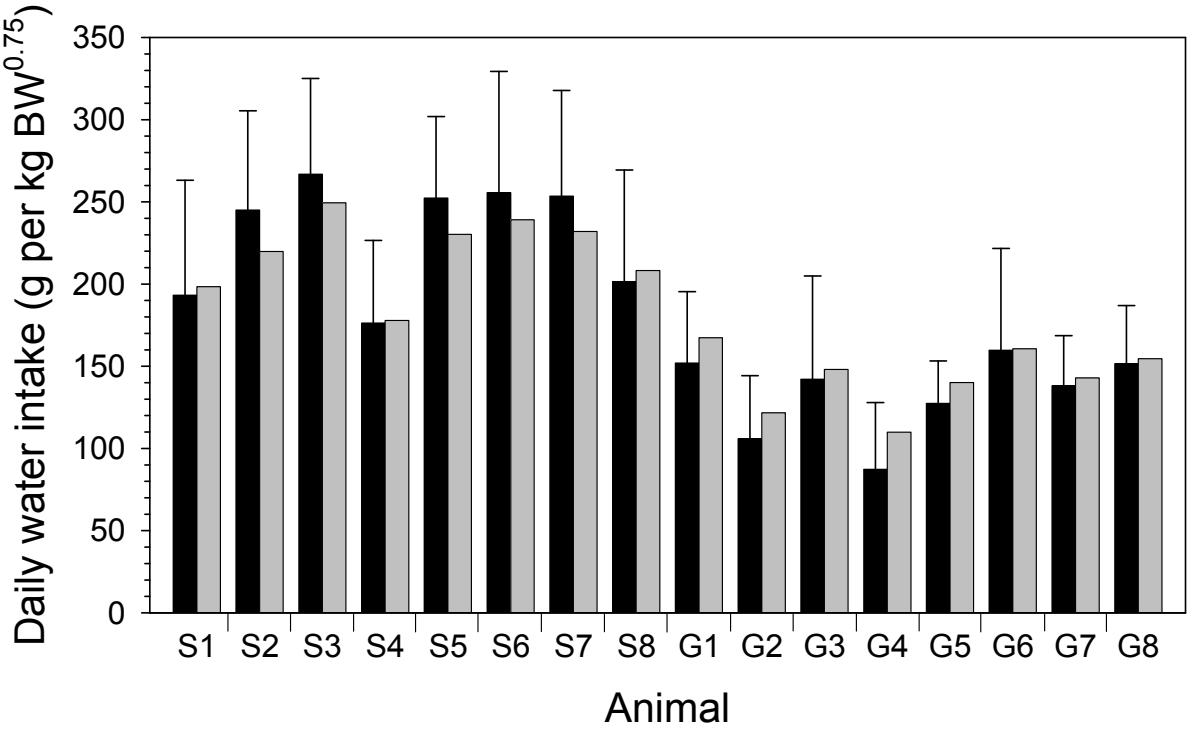

Figure 1: Comparison between measured (by reweighing water buckets, black columns) and estimated daily water intake (by D_2O dilution, gray columns) in individual sheep (S) and goats (G). Values for measured intakes are means ± SD of daily measurements for two weeks and values for estimated intakes are individually calculated over a two week period (see text for details).

However, the correlations between drinking behaviour and measured or estimated water intakes were found to be only of moderate magnitude (0.48 to 0.72; Table 3). In contrast, TWI estimated by D_2O provided a very good predictor for the measured amount of water drunk (τ = 0.967). Pairwise individual ratios of TWI (measured vs. estimated) are shown in Figure 1 and suggest a higher variability in sheep than in goats. The differences between both methods were not significant (p = 0.574 for sheep and p = 0.382 for goats, respectively; Exact Wilcoxon Two - Sample Test, two sided).

Discussion

To our knowledge, this study is the first to show that the deuterium oxide dilution technique is a means of accurately measuring water intake in sheep and goats. The study also confirmed previous findings (Aganga et al., 1989; Ferreira et al., 2002) of higher water intakes in sheep compared with goats and also showed that measurement of drinking time and frequency are only of moderate usefulness in measuring water intake.

The very close relation between measured and estimated TWI (τ = 0.983) confirms that the isotope dilution technique gives reliable results for water intake in sheep and goats. The variations of - 4.2 to 8.9 % found between measured and estimated water intake in both species are in the range of previous results for other species including birds (Nagy and Costa, 1980; Degen et al., 1981). Variations in water intake could be explained by the loss of deuterium through faeces and urine and by evaporation during the equilibration period, which lasts approximately 6 h in sheep and goats (Holleman et al., 1982; Oftedal et al., 1983). However, these losses are normally negligible compared with the amount injected.

In the present study, the body water fraction estimated by D_2O ranged between 54 to 63 % and 58 to 64 % in sheep and goats, respectively. These results are similar to values estimated by the dilution of TOH in indigenous breeds of sheep (Uda, Yankasa) and goats (Sahel, Maradi) ranging between 73.6 to 80.5 % and 66.1 to 77.8 %, respectively (Aganga et al., 1989). Ranjhan et al. (1982) reported TOH spaces of 71.9 to 77.4 % of crossbred sheep in India, while Panaretto (1963) found TOH spaces between 36.8 to 74.2 and 55.6 to 75.7 % for sheep and goats, respectively. Several studies have shown that the estimates of TBW determined by TOH or D_2O dilution in sheep and goats are in close agreement with those derived from post mortem desiccation methods (Panaretto, 1963; Panaretto and Till, 1963; Atti et al., 2000).

The species differences found in this study are in agreement with earlier reports. Higher DMI in sheep compared with goats have also been found by Aregheore (1996) and Van et al. (2007) who explained this observation by higher nutrient requirements and genetic potential for growth in sheep. Ferreira et al. (2002) showed that goats need less water to synthesize 1 kg of weight gain than sheep.

The water turnover rates in this study were higher in sheep than in goats. Similar results were also found for native sheep (Uda, Yankasa) and goats (Sahel, Maradi) in Nigiria under natural climatic conditions with 2.52, 2.63, 0.86 and 1.09 l / day per animal, respectively (Aganga et al., 1989). Our findings also agree closely with the results of Aggrey (1982) in West African dwarf sheep and goats kept in Ghana during the dry season with 2.06 and 1.28 l / day for sheep and goats, respectively.

Silanikove (2000) analyzed the physiological basis of the superior water management in goats emphasizing the role of the rumen as water reservoir that can be utilized during dehydration and rapid rehydration. Adaptation strategies of goats to withstand dehydration include their small body size, low metabolic requirements, efficient utilization of high fibre forage, the ability to minimize water losses via urine and faeces, and to reduce nitrogen requirements via urea recycling and nitrogen conservation (Casey and Van Niekerk, 1988; Silanikove, 2000). Merino sheep have been selected for their productive performance on high quality pastures and therefore probably need a higher water turnover for the excretion of nitrogen in the urine (Ferreira et al., 2002) as has been shown in the Blesbok and Impala (Fairall and Klein, 1984). In this context, it is of interest to note that this suggested superiority of goats for desert conditions was also found in this study where animals were kept under temperate climatic conditions.

The correlation between measured and estimated TWI and drinking behaviour was found to be of only moderate magnitude. This moderate relation might be explained by the fact that observations of drinking behaviour do not account for other sources of water

intake such as water from ingested feed. This observation is similar to previous results on suckling behaviour which suggest that the time spent suckling is not a useful predictor to measure milk transfer in mammals (Higgins et al., 1988; Cameron, 1998). Comparable to suckling, intensity of water ingestion (water swallowed per minute) might be variable between individuals and influenced by for example, drinking motivation that cannot be adequately evaluated by observation. Furthermore, in a previous study the direct observation of drinking behaviour in sheep showed that drinking bouts are very short, lasting approximately ≤ 1 min (Das et al., 1999). The use of time lapse video recording (eightfold) as in this study might not be suitable to identify such short drinking bouts with high accuracy.

In this study, German blackhead mutton sheep spent approximately 0.3 % of time per day for drinking, while Boer goats spent only 0.1 %. Similar low values for diurnal water ingestion were also reported for stall - fed sheep, which invested < 1 % (0.12 ± 0.02) of their activity during 24 h drinking (Das et al., 1999). The species differences in drinking behaviour could result from a higher metabolic rate in sheep, higher DMI, differences in activity level, and thermoregulative capacities. These results indicate that ethological observations are a good predictor to compare drinking behaviour in sheep and goats but appear to be less suitable to predict the amount of water ingested in both species.

The D_2O dilution technique estimated water intake in sheep and goats under stall - feeding conditions with high accuracy. The present results suggest that the isotope dilution method offers a viable technique to measure individual water consumption. This technique has the advantage that water flux can be evaluated individually while animals are kept in herds under extensive free ranging conditions. Thus, normal drinking behaviour will not be disturbed by non representative housing conditions such as stables. Behavioural studies of water intake by direct observations require much less technical equipment than isotope analyses and could be easily applied under difficult field conditions. However, the

moderate correlations between drinking behavior traits and the amount of water drunk show their limited suitability for measurement of water intake.

The lower water intake in Boer goats compared with black head mutton sheep when kept under temperate conditions confirms the superior water management capacity in goats found by other authors in particular for tropical conditions. For a better understanding of the underlying adaptive mechanisms, a parallel study of several breeds (genotypes) per species bred for different purposes and kept under different climatic conditions would be suitable.

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Chapter 3

Effect of water restriction on drinking behaviour and water intake in German blackhead mutton sheep and Boer goats

Abstract

The present study was conducted to evaluate and compare the physiological responses of sheep and goats to water restriction using the deuterium dilution technique (D_2O) to predict total water intake in both species. In two consecutive trails, 10 dry Boer goats and 10 dry German black - head mutton ewes were randomly allocated into two treatment and two control groups. In the control groups (n = 5, for each species) water was offered *ad libitum*, while the treatment groups (n = 5, for each species) received water 3 h / d on experimental days 8 - 14 and 6 h every 48 h on experimental days 15 - 22. Respiratory rate, rectal temperature, body weight, and drinking behaviour were also recorded. Total water intake was estimated by D_2O for each animal. Water restriction for 21 h / d or 42 h / 2 d had no significant ($P > 0.05$) effect on water intake, feed intake, water intake to dry matter intake ratio or body mass in both species. The absence of differences between species in their water intakes were also confirmed by using D_2O. However, sheep had higher respiratory rates and rectal temperatures than goats in both control and treatment groups. Both species showed the capability to tolerate a moderate water shortage by activating several physiological mechanisms and behavioural strategies.

Key words: small ruminants; water deprivation; isotope dilution; total body water; drinking behaviour

Introduction

In arid environments, ranging animals have to walk long distances in search for food and water, and are usually faced with low nutritive value feeds and water scarcity. Thus, theses animals have developed various behavioural, morphological and physiological adaptation mechanisms to enable them to survive, and in particular, to tolerate dehydration (Kay, 1997; Atti et al., 2000; Alamer and Al - hozab, 2004; Hamadeh et al., 2006).

The impact of water deprivation on animals varies depending on various factors, such as species (Al - Qarawi and Mousa, 2004), breed (Alamer, 2006), physiological status (Hamadeh, et al., 2006), age (Mengistu, et al, 2007), diet (Silanikove, 1992), ambient temperature, humidity (Alamer and Al - hozab, 2004) and water restriction interval (Misra and Singh, 2002). Compared to most other mammals, where losses of water over 15 % of body mass can be fatal (Shkolnik et al, 1980), ruminants are able to tolerate water losses of up to 18, 20, 25 and more than 40 % of their body mass as reported for cattle, sheep, camels and the desert black Bedouin goats, respectively (Shkolnik et al., 1980). This greater tolerance to water loss is mainly attributed to the rumen acting as a water reservoir (Silanikove, 2000).

Several studies evaluated the effect of water restriction in ruminants on body mass (Li et al., 2000; Jaber et al., 2004), milk production (Hamadeh et al., 2006), feed intake (Alamer and Al - hozab, 2004), blood parameters (Abdelatif and Ahmed, 1994; Casamassima et al., 2008), total body water (El - Hadi, 1986), and several physiological responses (e.g. body temperature, respiratory rate, urine volume and concentration etc.).

The capability of desert sheep and goats (i.e. Awassi sheep and Bedouin goats) to withstand water shortages by reducing feed intake and therefore endogenous heat production and subsequent water requirement for evaporative cooling has been demonstrated (Silanikove, 1992; Ayoub and Saleh, 1998; Jaber et al., 2004; Alamer, 2006). During water deprivation, increased release of ADH and aldosterone act to

conserve body water by the absorption of salt and water from the gut and kidney. Moreover, the renal function is adjusted by reduced urinary and fecal water excretion through concentrated urine and the excretion of dry faeces (Brosh et al., 1987; Ahmed and Abdelatif, 1994; Silanikove, 2000; Jaber et al., 2004; Alamer, 2006; Mengistu et al., 2007). Several studies have shown, a superior ability of goats to tolerate dehydration (Brosh et al., 1987; Silanikove, 1994, 2000; Misra and Singh, 2002), most likely because of their early domestication in hot and arid environments. The capability of goats to survive under harsh environmental conditions is the result of several physiological characteristics, such as small body size, low metabolic requirements, efficient utilization of high fibre forage, the ability to minimize water losses via urine and faeces, reduced nitrogen requirements etc. (Silanikove, 2000).

The amount of water animals can drink during one visit to a watering point varies according to the degree of dehydration, time allowed drinking and stocking density at the watering point (King, 1983). Therefore, most mammals can be divided into those, which replenish lost water rapidly, and those, which do so gradually (Adolph, 1982). Silanikove (1989) reported that upon rehydration, ruminants, such as cattle, sheep, camels, and goats can drink an equivalent of up to 18 – 40 % of their body mass, within 3 to10 minutes. In general, ruminants can replace 15 - 20 % of their body mass at the first drinking and 20 – 25 % within 1 to 1.5 hours (King, 1983).

Reports on the effect of water restriction on sheep and goats are mostly based on indoor experiments, due to the difficulty to measure individual water intake under free ranging condition. In this context, the isotope dilution technique provides a suitable method for estimating individual water flux under free ranging conditions. To the authors' knowledge, there have been no previous comparative studies on the capabilities of goats and sheep enduring water shortage using the deuterium dilution technique as a method for estimating the amount of water intake. In the present study, we investigated the effects of

a moderate water restriction on water and feed intake in German black - head mutton sheep and Boer goats under temperate environmental conditions. We tested whether the isotope dilution technique (using deuterium oxide) is a suitable method to estimate individual water intake under water restriction compared to direct measurement (re - weighing water buckets). In addition, drinking behaviour was recorded to investigate the behavioural response to water shortage.

Material and methods

Animals and management

In two consecutive trails, twenty dry females (10 German black - head mutton sheep and 10 Boer goats) with an average age of 1.8 ± 0.1 years (sheep) and 4.3 ± 0.5 years (goats) were involved. Animals were kept under temperate conditions in individual pens (1.5 X 2.0 m) on straw at the Department of Animal Sciences, University of Goettingen, Germany. Temperatures and relative humidity averaged 12.7 ± 1.2 °C and 75.3 ± 4.6 % or 8.9 ± 2.8 °C and 71.0 ± 4.2 % in the sheep and goats stables, respectively (mean ± SD). Each pen was equipped with an individual feed through and water bucket and light schedule was kept constant at 16 L : 8 D with lights on at 0500 and lights off at 2100 h.

Animals of both species were randomly allocated into two treatment and two control groups of 5 animals each. In the two control groups (n = 5 for each species), water was offered *ad libitum* (24 hours / day) throughout the experimental period.

In the two treatment groups (n = 5 for each species) water was restricted according to the schedule outlined in Table 1. In brief, the experiment was subdivided into three experimental periods: in period 1 (experimental day 1 - 7) animals were adapted to the water restriction regime by limiting access to water gradually from 15 to 3 h per day. During the second period of the experiment (experimental days 8 - 14) animals of the

treatment groups had excess to water for 3 h / day. In the last period of the experiment (experimental days 15 - 22) animals had excess to water only every second day for 6 h.

Water intake studies

Water drunk by individuals was recorded daily (24 h) by weighing and re - weighing water buckets (10 l) before and after water administration. Water refusals were discarded and the buckets refilled after cleaning. Corrections for water evaporation were made by placing a separate bucket (10 l) containing water in an adjacent area to measure the amount of water lost by evaporation. The actual amount of water consumed by the animals was calculated by subtracting the evaporated amount from the total water intake.

Total water intake includes preformed and metabolic water from food and drinking water. Metabolic water was calculated from the feed composition. It was assumed that 1 gram of metabolized carbohydrates, fat and protein yield 0.56, 1.07 and 0.42 gram of water, respectively (Maynard et al., 1981). Measured TWI was calculated as TWI = water drunk + metabolic water + preformed water.

Water intake was also estimated from water kinetics for two consecutive weeks by using the D_2O dilution technique in both control and treatment groups. On experimental day 8, prior to the isotope administration a 5 ml blood sample was taken from the jugular vein into blood tubes containing sodium citrate to determine the background level of D_2O. Immediately after, a dose of approximately 200 mg D_2O / kg body mass of 99.90 % purity (Euriso - top GmbH, Saarbruecken, Germany) was injected intramuscularly at two sites of the body. The actual dose given was measured gravimetrically by weighing the syringe before and after the administration of D_2O to the nearest 0.001 g (Sartorius model CW3P1 - 150IG - 1, Sartorius AG, Goettingen, Germany). Blood samples (approximately 5 ml) were collected at 12 and 24 h and 2, 4, 7, 9, 11, and 14 days after the application of D_2O.

The samples were then centrifuged within 30 min of collection at 3500 rpm for 10 min. The plasma fraction was pipetted into glass vials and frozen at - 20 ºC until analysis.

Earlier work showed that tracer concentrations in plasma samples are the same as in vacuum sublimated water samples (Riek et al., 2007). Therefore, plasma samples from sheep and goats were analyzed for D_2O concentrations. Analyses were carried out at the Competence Centre of Stable Isotopes (KOSI, Göttingen University, Germany). Isotope ratios of ^2H were measured using an on - line high temperature reduction technique in a helium carrier gas described previously (Gehre et al., 2004) and expressed relative to the Vienna standard mean ocean water (VSMOW), which is the international reference standard for D_2O. Individual samples were measured in triplicate and the averages calculated. Total body water and TWI were estimated from water kinetics according to established formulas described in detail elsewhere (Al - Ramamneh et al., 2010).

Rye grass hay with an average DM content of 85.7 ± 1.2 % was offered *ad libitum* daily at 10.00 h; thereafter feed was supplied when the feed buckets were found nearly empty and the remaining feed was measured to determine individual daily feed consumption.

Physiological reactions

Respiratory rate was measured daily by counting the rate of flank movement for one minute between 15 : 00 and 15 : 30 h. Rectal temperature was measured to the nearest 0.1 °C once per week between 13 : 00 and 14 : 00 h. Individual body mass were recorded to the nearest 10 g at weekly intervals.

Drinking behaviour

Drinking behaviour (duration and frequency) for each animal was observed during experimental periods 2 and 3 (Table 1) every two to three days by using a time - lapse (8 -

fold) video recording system for 24 hours. Infrared lights were used to facilitate data recording during night. Periods from 05 : 01 to 21 : 00 h and from 21 : 01 to 05 : 00 h were considered as daytime and nighttime, respectively. For each animal 2 recordings per period were available (total of 4 observations per animal) and analyzed with the Interact ® 8.0 system (Mangold International GmbH, Germany). During period 3 (Table 1), recordings were only made on days when water was available. Drinking behaviour was defined as follows:

- drinking frequency (number of drinking bouts)
- drinking duration (time in minutes when the animal was actively engaged in the ingestion and swallowing of water).

Table 1: Water restriction regime in black - head mutton sheep and Boer goats treatment groups during the adaptation period (experimental period 1), 3 h / d water restriction (experimental period 2), and 6 h / 2 d (experimental period 3), (grey cells = water restriction; white cells = water available).

Experimental design			Day hours						Night hours
Experimental period	Day	Watering (h)	05-07	07-10	10-13	13-16	16-19	19-21	21-05
Period 1 (Adaptation)	1	15							
	2	12							
	3	9							
	4	6							
	5	3							
	6	3							
	7	3							
Period 2	8	3							
	9	3							
	10	3							
	11	3							
	12	3							
	13	3							
	14	3							
Period 3	15	0							
	16	6							
	17	0							
	18	6							
	19	0							
	20	6							
	21	0							
	22	6							

Statistical analysis

Data on water intake recorded in the treatment groups during period 3 were averaged across the entire period of 7 days. In addition, for the same period average water intake was also computed for the four days with water availability only. Behavioural data were averaged for the two recording days per period.

Analysis of variance was performed based on averages per animal using the PROC MIXED procedure of the software package Statistical Analysis System version 9.01 (SAS, 2001). For all traits with the exception of those related to the deuterium oxide dilution technique, the model included the fixed effect of species, treatment, the experimental period, their interactions, and the random effect of animals. The model (1) was:

$Y_{ijklm} = \mu + S_i + T_j + P_k + S*T_{ij} + S*P_{ik} + T*P_{jk} + S*T*P_{ijk} + A_l + e_{ijklm}$

Where: Y_{ijklm}: observation value; μ: overall mean; S_i: species; T_j: treatment; P_k: experimental period; A_l: random effect of animals and e_{ijklm}: random error.

Data related to deuterium oxide dilution were analyzed with PROC GLM procedure using the following model (2):

$Y_{ijk} = \mu + S_i + T_j + S*T_{ij} + e_{ijk}$

Where: Y_{ijk}: observation value; μ: overall mean; S_i: species; T_j: treatment; e_{ijk}: random error. All values were presented as means ± standard deviation. The significance level was set at $P < 0.05$.

The research conducted in this study was performed in accordance with the guidelines established by the German Animal Welfare Act.

Results

Feed and water intake

No significant species differences were found across the entire experiment for water drunk or dry matter intake (Table 2, 3, 4). However, when expressed as ratio water intake / dry matter intake (WI / DMI) significantly higher values were found for sheep than for goats. When calculated across each experimental day per period, treatment effects were not significant for water and DMI or WI / DMI whether expressed on a whole body mass basis or on a metabolic mass basis (Figure 1, Table 2, 4). However, for period 3, when animals received water only for 6 h every second day, water drunk on watering days were nearly doubled and averaged 235.2 ± 13.9 and 259.8 ± 81.2 g / d / $BM^{0.75}$ ($P = 0.574$) and 4.1 ± 0.3 and 3.4 ± 0.5 g / DMI in sheep and goats ($P = 0.05$), respectively.

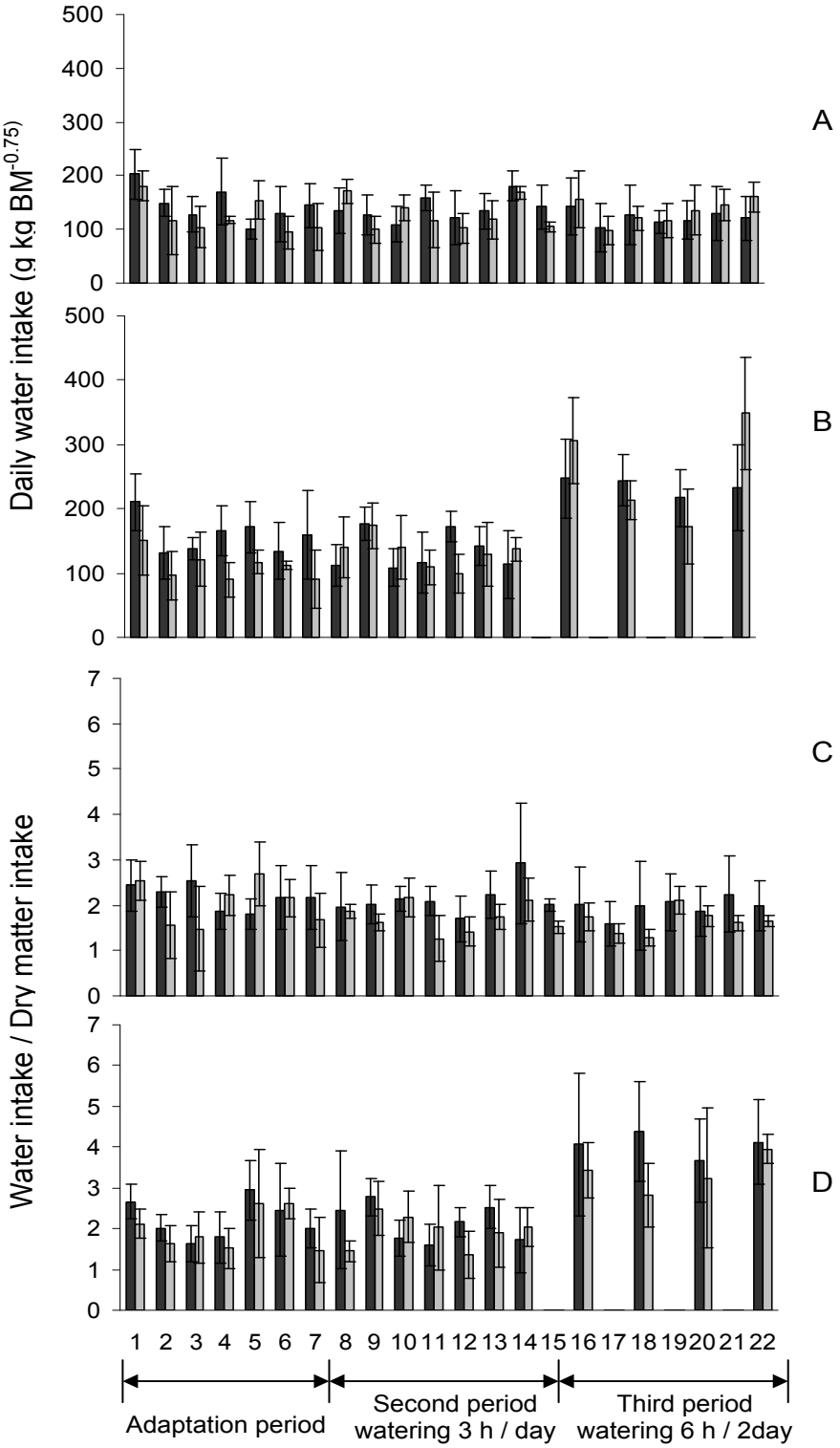

Figure 1: Average daily water intake (g kg $BM^{-0.75}$) and water intake per dry matter intake (WI / DMI) for black - head mutton sheep (black bars) and Boer goats (gray bars) receiving water *ad libitum* (A, C) or water restricted groups (B, D) with access to water for 3 h per day (experimental period 2) or 6 h every two days (experimental period 3), see Table 1 for details (Means ± SD).

Table 2: Analyses of variance for average body mass, water drunk, dry matter intake, water drunk to dry matter intake ratio, respiratory rate, rectal temperature, drinking behaviour (total frequency and duration), water intake per drinking bout and duration in black - head mutton sheep and Boer goats during the experimental periods in control (water *ad libitum*) and treatment groups (water restriction; see table 1 for details)

Traits	Species (S)	Treatment (T)	S*T	Period (P)	S*P	T*P	S*T*P
Body mass (kg)	0.226	0.516	0.787	0.005	0.002	0.751	0.446
Water drunk (L d^{-1})	0.480	0.728	0.829	0.053	< 0.001	0.889	0.046
Water drunk (g kg $BM^{-0.75}$)	0.312	0.868	0.819	0.019	< 0.001	0.896	0.043
Dry matter intake (kg d^{-1})	0.339	0.675	0.561	0.020	< 0.001	0.271	0.675
Dry matter intake (g kg $BM^{-0.75}$)	0.441	0.798	0.561	0.071	< 0.001	0.265	0.650
WI / MBW[*1]	0.042	0.761	0.927	0.001	0.513	0.689	0.509
Respiratory rate (breath min^{-1})	< 0.001	0.063	0.004	< 0.001	< 0.001	0.180	0.074
Rectal temperature (° C)	< 0.001	0.005	0.930	0.005	0.435	0.290	0.752

Table 2 continued.

Traits	Species (S)	Treatment (T)	S*T	Period (P)	S*P	T*P	S*T*P
			Behavioural observations				
Water drunk (L d^{-1})	0.307	0.032	0.826	0.002	0.424	0.001	0.834
Water drunk (g kg $BM^{-0.75}$)	0.456	0.016	0.801	0.001	0.432	0.001	0.741
Total drinking bouts (n)[1]	0.017	0.187	0.987	0.733	0.012	0.031	0.529
Drinking bouts (n h^{-1})[2]	0.060	0.004	0.152	0.012	0.025	0.025	0.065
Total drinking duration (min d^{-1})[*3]	0.497	0.073	0.749	0.277	0.011	0.020	0.627
Drinking duration (min h^{-1})[*4]	0.927	< 0.001	0.985	0.188	0.098	0.280	0.198
Water drunk / drinking bout [*5]	0.047	0.034	0.416	0.107	0.208	0.107	0.382
Water drunk / min [*6]	0.944	0.002	0.668	0.259	0.003	0.322	0.081

[1,2,3,4,5,6] For explanations see Table 4

Table 3: Average body mass, water drunk, dry matter intake, water intake to dry matter intake ratio, respiratory rate, and rectal temperature in black - head mutton sheep and Boer goats during the adaptation period (experimental period 1) in control groups (water *ad libitum*) and in treatment groups (water restriction; see table 1 for details). Values are means ± SD.

Trait	Sheep		Goats	
	Control N=5	Treatment N=5	Control N=5	Treatment N=5
Body mass (kg)	57.8 ± 4.7^{e}	57.1 ± 3.1^{e}	60.0 ± 3.7^{e}	58.2 ± 1.8^{e}
Water drunk (l d^{-1})	3.1 ± 0.7	3.3 ± 0.6	2.7 ± 0.6	2.3 ± 0.3
Water drunk (g kg $BM^{-0.75}$)	146.2 ± 30.1^{c}	158.8 ± 26.1^{c}	124.4 ± 31.0^{c}	111.1 ± 16.9^{c}
Dry matter intake (kg d^{-1})	1.4 ± 0.3^{e}	1.6 ± 0.4^{e}	1.4 ± 0.2^{e}	1.3 ± 0.1^{e}
Dry matter intake (g kg $BM^{-0.75}$)	68.7 ± 12.9	75.5 ± 16.2	63.1 ± 10.1	60.1 ± 5.9
WD / DMI[*1]	2.2 ± 0.3^{Ac}	2.2 ± 0.3^{Ac}	2.0 ± 0.4^{Bc}	2.0 ± 0.3^{Bc}
Respiratory rate (breath min^{-1})	36.7 ± 4.7^{Ac}	35.9 ± 2.9^{Ac}	20.1 ± 2.1^{Bc}	20.5 ± 2.9^{Bc}
Rectal temperature (° C)	39.0 ± 0.4^{Aac}	38.7 ± 0.4^{Abc}	38.1 ± 0.1^{Bac}	37.8 ± 0.3^{Bbc}

*[1]WD / DMI: Water drunk per dry matter intake

[A, B]: Significant differences between species $P < 0.05$

[a, b]: Significant differences between treatment $P < 0.05$

[c,d,e]: Significant differences between periods $P < 0.05$

Table 4: Average body mass, water drunk, dry matter intake, water drunk to dry matter intake ratio, respiratory rate, and rectal temperature in black - head mutton sheep and Boer goats. Values are given as means ± SD for control (water *ad libitum*) and treatment groups with water restrictions of 3 h / d (experimental period 2) and 6 h / 2 d (experimental period 3).

Trait	Experimental period 2				Experimental period 3			
	Sheep		Goat		Sheep		Goat	
	Control N=5	Treatment N=5	Control N=5	Treatment N=5	Control N=5	Treatment N=5	Control N=5	Treatment N=5
Body mass (kg)	58.4 ± 5.0^{d}	58.2 ± 2.9^{d}	60.3 ± 3.7^{d}	58.7 ± 1.9^{d}	58.0 ± 5.3^{c}	57.2 ± 2.7^{c}	61.1 ± 4.0^{c}	60.1 ± 2.0^{c}
Water drunk (l d^{-1})	2.9±0.7	2.8±0.5	2.8±0.3	2.8±0.2	2.7±0.9	2.4±0.5	2.8±0.3	2.8±0.2
Water drunk (g kg $BM^{-0.75}$)	137.9 ± 26.2^{d}	134.3 ± 25.5^{d}	131.3 ± 17.6^{d}	132.6 ± 8.9^{d}	124.76 ± 35.7^{e}	117.6 ± 23.3^{e}	129.7 ± 14.9^{e}	129.9 ± 12.9^{e}
Dry matter intake (kg d^{-1})	1.4 ± 0.2^{d}	1.4 ± 0.3^{d}	1.7 ± 0.3^{d}	1.5 ± 0.1^{d}	1.4 ± 0.5^{c}	1.3 ± 0.5^{c}	1.8 ± 0.2^{c}	1.6 ± 0.1^{c}
Dry matter intake (gkg$BM^{-0.75}$)	65.6±6.4	65.3±13.8	76.6±9.5	72.0±6.1	64.8±17.9	63.3±21.0	80.5±10.0	75.0±7.1
WD / DMI[*1]	2.2 ± 0.5^{Ad}	2.1 ± 0.4^{Ad}	1.7 ± 0.2^{Bd}	1.9 ± 0.1^{Bd}	2.0 ± 0.5^{Ae}	2.0 ± 0.5^{Ae}	1.6 ± 0.1^{Be}	1.7 ± 0.1^{Be}
Respiratory rate (breath min^{-1})	36.1 ± 2.1^{Ad}	34.1 ± 3.5^{Ad}	18.4 ± 0.8^{Bd}	19.1 ± 1.0^{Bd}	32.0 ± 0.9^{Ae}	25.1 ± 1.9^{Ae}	17.6 ± 1.1^{Be}	18.7 ± 1.2^{Be}
Rectal temperature (° C)	38.7 ± 0.3^{Aad}	38.6 ± 0.4^{Abd}	38.1 ± 0.1^{Bad}	37.8 ± 0.3^{Bbd}	38.8 ± 0.3^{Aae}	38.3 ± 0.3^{Abe}	37.7 ± 0.6^{Bae}	37.3 ± 0.4^{Bbe}

*[1] WD / DMI: Water drunk per dry matter intake

[A, B]: Significant differences between species $P < 0.05$

[a, b]: Significant differences between treatment $P < 0.05$

[c,d,e]: Significant differences between periods $P < 0.05$

The absence of clear differences between species in their water intakes were also confirmed by using the D_2O dilution method in estimating TWI in both species (Table 5). The TBW content expressed as percentage of body mass estimated by D_2O dilution ranged between 65.0 to 74.0 % in sheep and 68.5 to 73.5 % in goats with significantly ($P = 0.018$) higher mean values in goats than in sheep in both the control and treatment groups. No significant treatment effects were found for water intakes estimated by the D_2O dilution method (Table 5). The TBW showed a tendency to decrease under water restriction treatment in both species ($P = 0.093$).

The differences between estimated TWI (by D_2O) and measured TWI (by re - weighing water buckets) were moderate, ranging between – 4.9 and + 11.4 % in sheep and - 15.8 and - 7 % in goats, respectively, with significantly lower mean values in goats than in sheep ($P < 0.001$). Measurement methods of total water intake using deuterium oxide dilution or re - weighing water buckets did not differ significantly in both species ($P = 0.364$).

Table 5: Average total body water, total water intake (TWI) measured by weighing water buckets or estimated by the deuterium dilution technique in black - head mutton sheep and Boer goats. Values are means ± SD.

Trait	Sheep		Goat	
	Control N = 5	Treatment N = 5	Control N = 5	Treatment N = 5
Total body water (% body mass)	69.0 ± 3.3^{B}	68.0 ± 2.6^{B}	72.5 ± 1.0^{A}	69.8 ± 1.3^{A}
Measured TWI (g kg $BM^{-0.75}$)	164.6 ± 34.5	158.8 ± 30.6	170.6 ± 19.5	168.8 ± 12.2
Estimated TWI (g kg $BM^{-0.75}$)	171.9 ± 31.8	163.8 ± 23.0	152.9 ± 18.4	147.0 ± 9.6
Estimated TWI / measured TWI (%)	105.6 ± 4.7^{A}	104.6 ± 7.2^{A}	89.7 ± 3.4^{B}	87.3 ± 3.2^{B}

$^{A, B}$: Significant differences between species $P < 0.05$

Physiological reactions

Body mass was not significantly influenced by species or water regime (Table 2, 4). However body mass increased during the second and third experimental period ($P = 0.005$) compared to the adaptation period.

Sheep had significantly ($P < 0.001$) higher respiratory rates and rectal temperatures than goats in both the control and treatment groups (Table 3, 4). When exposed to water restriction (periods 2 and 3), sheep decreased their respiratory rates while goats tended to increase theirs, resulting in a significant species x treatment interaction (Table 2, 4). Under the water restriction treatment, rectal temperatures were significantly reduced in both species (Table 4).

Drinking behaviour

Control animals in both species spent approximately 0.2 % of the 24 h day drinking (2.6 ± 0.7 and 2.7 ± 0.7 min / day for control sheep and goats, respectively). Sheep visited water buckets more often than goats ($P = 0.017$) during the experimental periods 2 and 3 (Table 6). When time to water access was limited, animals increased the frequency of drinking bouts and the time spent drinking during the hours of water availability; in addition, more water was consumed per minute drinking. Goats consumed significantly more water per drinking bout than sheep ($P = 0.047$).

Table 6: Daily water drunk, drinking behaviour (total frequency and duration), water intake per drinking bout and duration in black-head mutton sheep and Boer goats during the experimental periods for control (water *ad libitum*) and treatment groups with water restrictions of 3 h / d (experimental period 2) and 6 h / 2 d (experimental period 3) based on 24 h video recordings (numbers are averages per animal; means ± SD)[*1]

Traits	Experimental period 2				Experimental period 3			
	Sheep		Goat		Sheep		Goat	
	Control N=4	Treatment N=4	Control N=4	Treatment N=4	Control N=4	Treatment N=4	Control N=4	Treatment N=4
Water drunk (l d^{-1})	3.1±0.6^{bd}	2.6±0.8^{ad}	3.2±0.4^{bd}	2.9±0.5^{ad}	2.6±0.9^{bc}	4.7±1.2^{ac}	3.1±1.1^{bc}	5.3±1.0^{ac}
Water drunk (g kg $BM^{-0.75}$)	147.7±22.2^{bd}	121.9±38.4^{ad}	142.9±17.0^{bd}	132.5±19.5^{ad}	119.1±34.0^{bc}	224.0.±53.9^{ac}	139.8±52.4^{bc}	245.1±45.9^{ac}
Total drinking bouts (n d^{-1})[*2]	15.3±3.9^{A}	10.6±6.6^{A}	7.6±2.5^{B}	3.8±1.7^{B}	11.3±4.3^{A}	10.5±5.8^{A}	8.1±2.3^{B}	6.5±2.7^{B}
Drinking bouts / h (n h^{-1})[*3]	0.6±0.2^{bc}	3.5±2.2^{ac}	0.3±0.1^{bc}	1.3±0.6^{ac}	0.5±0.2^{bd}	1.8±1.0^{ad}	0.3±0.1^{bd}	1.1±0.5^{ad}
Total drinking duration (mind^{-1})[*4]	3.0±0.1	1.9±1.2	2.5±0.8	1.4±0.4	2.1±0.8	2.0±0.5	2.8±0.6	3.0±0.7
Drinking duration (minh^{-1})[*5]	0.12±0.01^{b}	0.6±0.4^{a}	0.11±0.03^{b}	0.5±0.1^{a}	0.09±0.03^{b}	0.3±0.1^{a}	0.11±0.02^{b}	0.5±0.1^{a}
Water drunk / drinking bout[*6]	9.9±0.9^{Ab}	13.0±3.4^{Aa}	20.9±8.8^{Bb}	41.4±20.9^{Ba}	11.1±2.3^{Ab}	30.0±21.6^{Aa}	19.6±11.8^{Bb}	45.1±27.6^{Ba}
Water drunk / Min [*7]	49.4±7.8^{b}	76.1±25.2^{a}	61.9±24.2^{b}	99.7±30.7^{a}	59.3±15.8^{b}	115.5±17.6^{a}	53.5±24.1^{b}	82.6±9.9^{a}

$^{A, B}$: Significant differences between species $P < 0.05$

[a, b]: Significant differences between treatment $P < 0.05$

[c,d]: Significant differences between periods $P < 0.05$

[*1]: Only concurrent water intake values on video recording days were included.

[*2]: Based on 24h video recordings (average of 2 records / animal). Water availability hours for control group 24 hour per day, restricted groups during the second period 3 hours / day and 6 hours / 2 days in third period.

[*3]: Drinking bouts per hour with water availability.

[*4]: Total duration during the water availability hours.

[*5]:Drinking duration per hour with water availability.

[*6]: Water intake based on metabolic body mass ($BW^{0.75}$) divided by total drinking bouts

[*7]: Water intake based on metabolic body mass ($BW^{0.75}$) divided by total drinking duration $(min)^{*3}$. Only concurrent water intake values on video recording days were included.

Most of the daily water intake was observed during the daytime in both species (Figure 2). Consequently, nocturnal drinking behaviour (i.e. between 21 : 00 and 05 : 00 h) accounted for only a small percentage of daily drinking frequency (4.0 and 3.8 %) and duration (2.2 and 3.0 %) in control goats and sheep, respectively.

Higher drinking frequency was associated with feeding times at 10 : 00 and 16 : 00 h for both control groups (sheep and goats). Control sheep tended to ingest water earlier than goats after light onset (Figure 2). After 42 hrs of water withdrawal (period 3), the drinking patterns were similar for both species (Figure 2) and most of the drinking activity occurred within the first 2 hours after water availability (63 and 67 % in goats and sheep, respectively).

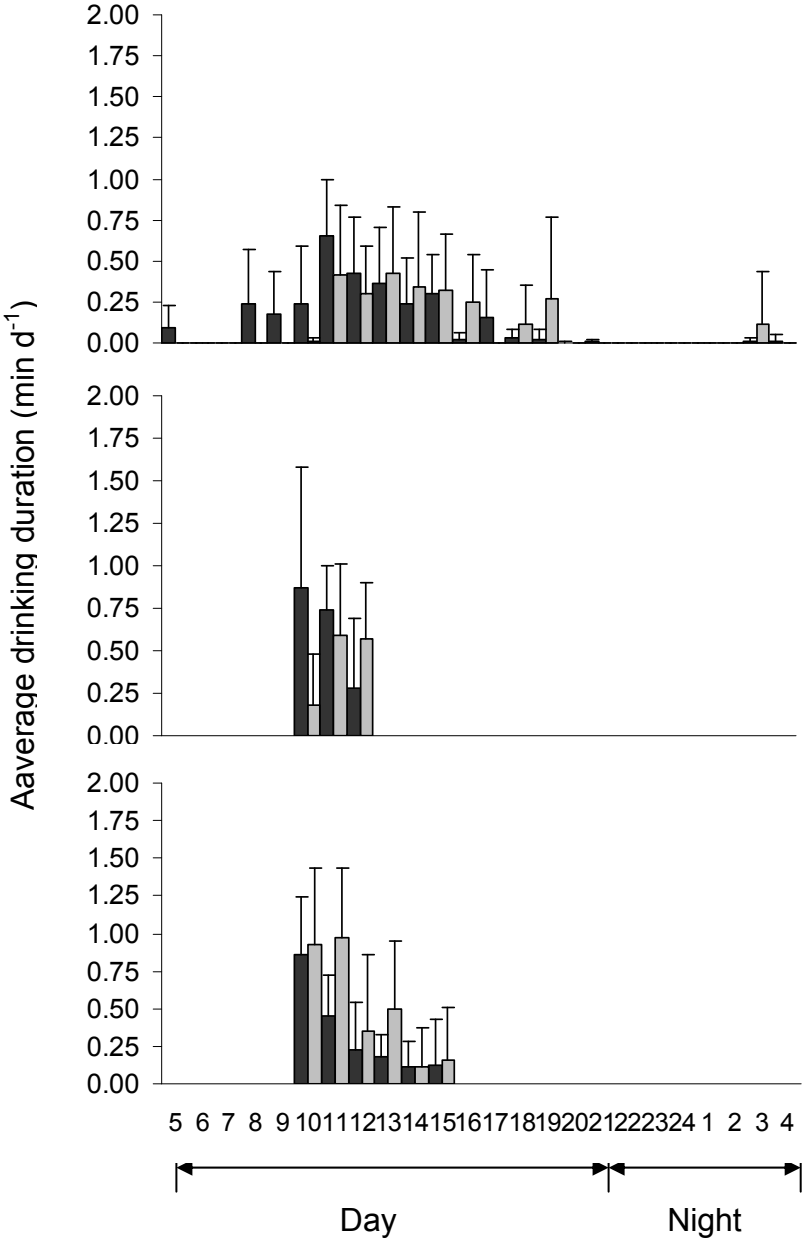

Figure 2: Average diurnal drinking duration (Mean ± SD) for black - head mutton sheep (black bars) and Boer goats (gray bars). A: control groups receiving water *ad libitum*, across experimental periods 2 and 3. B: water restricted groups receiving water 3 h per day (10 : 00 – 13 : 00 h), experimental period 2. C: water restricted groups receiving water 6 h every two days (10 : 00 – 16 : 00 h), experimental period 3.

Discussion

Water deprivation in our study did not induce major changes in water intake, dry matter intake, and body mass in both species. However, the significant effects of period and the respective S x P and S x T x P interactions are complex and partly erratic and thus difficult to interpret (Table 2). These effects can be attributed to differences in climatic conditions between stables and periods. In this context, it is of interest to note, that animal variance for water drunk accounted for 80.2 % of the total variance, indicating great individual variation.

This small impact of water restriction found in the present study may be mainly attributed to the temperate environmental conditions prevailing during the experiment. The effect of water deprivation is very variable, depending on various factors, such as species, age, diet, ambient temperature, and humidity (Misra and Singh, 2002). It has been shown that feed intake in sheep with access to water only every 24, 48, and 72 hours, is not affected when temperatures range between 4 to 25 ° C (Singh et al., 1976), which is in accordance with the results found in our study. It is not surprising, that desert adapted breeds such as Awassi ewes can withstand a once per two day watering regime under semi - arid conditions, with little physiological disturbances (Jaber at al., 2004). However, in our study we used a temperate adapted sheep breed, showing similar capabilities to withstand water shortages, indicating a higher degree of tolerance towards water restriction than so far assumed for this breed.

Several water restriction studies pointed out that body mass losses in ruminants associated with a reduction in water and feed intake are greatly influenced by environmental temperatures and body water loss (Silanikove, 1992; Alamer, 2006, 2009). Shkolnik et al. (1980) found that during 4 days of water deprivation, black Bedouin goats lost 25 to 30 % of their body mass. The TBW was found to be reduced from 76 to 69 % while the animals continued to eat normally under arid desert conditions. Under the

present water restriction regime and temperate climatic conditions, the TBW of sheep and goats was not significantly reduced, indicating that animals were able to adjust to the water deficit. Similarly, Freudenberger and Hume (1993) found no effect of water deprivation (50 % of the main daily intake) on TBW in feral goats kept under semi - arid conditions. Water restriction had also no effect on feed intake and body mass in our study, which is in agreement with results obtained under different environmental conditions (Brosh et al., 1986; Hadjigeorgiou, et al., 2000; Misra and Singh, 2002) in adult goats and sheep.

When animals are faced with water shortage, they activate several water saving mechanisms to minimize water losses and keep essential physiological systems unimpaired (Silanikove, 2000). Increasing the watering interval (from 24 to 72 h) has been reported to significantly reduce rectal temperatures in Sudanese desert sheep (Abdalatif and Ahmed, 1994). In the present study, rectal temperatures were also significantly reduced in water restricted sheep and goats. This observation may indicate a decrease in endogenous metabolic heat production to reduce water requirements for evaporative cooling (Degen, 1977; Ismail et al., 1996), as it is known that water restriction induces a decrease in metabolic rate, indicating a water conservation mechanism (Choshniak et al., 1995).

However, the capacity and speed of fluid replacement appears to be higher in the more arid - adapted mammals (King, 1983). Silanikove (1992) reported that ruminants are able to withstand dehydration and replenish the entire water loss in one drinking bout by storing the ingested water in the rumen, which controls water consumption and distribution inside the body. In general, ruminants can replace 15 – 20 % of their body mass at the first drinking bout and 20 – 25 % within 1 to 1.5 hours (King, 1983). In Northern Nigeria for example Yankasa ewes have been observed to drink an equivalent of up to 30 % of their body mass within 2 to 3 minutes during the dry season with ambient temperatures ranging from 19 - 30 °C (Aganga et al., 1990).

Our observation of drinking behaviour in sheep and goats revealed interesting differences in behavioural strategies between both species. Under water restriction, goats were able to increase their water intake per drinking bout to about 0.9 ± 0.5 l / bout compared to 0.5 ± 0.4 l / bout in sheep, while the amount of water intake per minute drinking was identical in goats and sheep (2.0 ± 0.5 *vs* 2.0 ± 0.6 l / minute). The higher frequency of drinking events in sheep may be related to their evaporative cooling mechanisms, because sheep panted more frequently than goats. It is suggested, that sheep require more water to regulate their body temperature due to their longer fleece, which increases their endogenous heat production. In this context, it is of interest to note that the TBW and the ratio between estimated and measured TWI was significantly different between goats and in sheep, which is in accordance with results found earlier (Al - Ramamneh et al., 2010).

The loss of stable isotopes in faeces particularly in ruminants and the possible influences of high roughage content in the rumen on the isotope equilibration due to high fecal production has been discussed (Midwood et al., 1993). However, our results on water intake both measured (by re - weighing water buckets) and estimated (by the isotope dilution method) suggest, that the isotope dilution method provides a viable method to estimate TWI in black - head mutton sheep and Boer goats under temperate controlled conditions.

The higher TBW found in Boar goats compared to sheep suggests that they may have developed different physiological responses to adapt to water shortages and heat stress, which could be a crucial factor for survival in these harsh environmental conditions.

Conclusion

The results of the present investigation confirm and extend previous reports, on the capability of sheep and goats to withstand water shortages. This tolerance to water restriction allows them to maximize the use of pastures as animals could graze and browse at long distances from the watering points (Nicholson, 1987). These findings may be used for planning the distribution of watering points in dry areas.

The comparison between sheep and goats under temperate conditions revealed some remarkable differences. Sheep had higher rectal temperatures, WI / DMI, and respiratory rates, and a lower TBW content than goats. It is open to question whether these characteristics indicate underlying species differences in the endogenous metabolism. In particular, the wooly hair coat of sheep in contrast to the skin morphology of goats may play an important role for the better adaptability of the latter to water scarcity. Furthermore, our ethological studies underline that behaviour offers a most effective tool for short term adaptations of the animals to water restriction, indicated by the ability of restricted goats to drink more water per bout than sheep.

For a better understanding of the differences in adaptive mechanisms in sheep and goats under water restriction, further studies on several breeds (genotypes) per species bred for different purposes and kept under different climatic conditions are necessary. In this context, the isotope dilution method offers a viable method to measure individual water consumption, which can be applied successfully under extensive free ranging conditions in arid and semi - arid areas with water scarcity.

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Chapter 4

Effect of shearing on water intake in German blackhead mutton sheep

Abstract

The present study was conducted to investigate the effect of wool coverage on water metabolism in relation to thermoregulation in sheep using the deuterium dilution technique (D_2O) to predict total water intake before and after shearing and to evaluate the physiological, thermoregulative reactions and drinking behaviour responses of sheep. Fourteen dry German blackhead mutton ewes were randomly allocated into two groups: a control group (n = 7) that was already shorn (wool length: 2.3 ± 0.8 cm, mean ± SD) and a treatment group (n = 7) that was left unshorn (wool length: 10.6 ± 1.2 cm). After two weeks, the treatment ewes were shorn. Individual feed and water intake were recorded daily during the experimental period of 71 day. Water intake was also estimated from water kinetics using D_2O dilution twice for two consecutive weeks. Air temperature, relative humidity and respiratory rate were measured daily, while body mass, rectal and animal surface temperatures (using infrared thermography) and wool length were measured weekly. Before shearing, treatment and control ewes differed significantly ($P < 0.05$) in DMI (52 ± 4 vs. 59 ± 4 g / kg body mass$^{0.75}$), water intake (165 ± 17 vs. 134 ± 18 g / kg body mass$^{0.75}$), respiratory rate (66 ± 5 vs. 31 ± 4 breath / min), rectal temperature (39.3 ± 0.2 vs. 38.8 ± 0.1 °C) and surface temperatures (rump: 19.3 ± 0.3 vs. 24.5 ± 0.6 °C; leg: 25.8 ± 2.4 vs. 27.4 ± 1.6 °C). However, after shearing these differences disappeared. The same trend in water intake between groups was confirmed using the isotope dilution technique. Our study demonstrated that shorn German blackhead mutton ewes were more efficient in their thermoregulative responses compared to unshorn ewes under temperate climatic conditions.

Key words: Deuterium oxide; sheep; thermoregulation; wool; water turnover

Introduction

Sheep are the most widely distributed domestic ruminants and exist in a broad range of climatic conditions. Their thermoregulative strategies include behavioural, morphological, and physiological mechanisms to maintain their body temperature and body water (King, 1983; Silanikove, 2000a; Marai et al., 2007). Thermoregulation is part of a homeostatic mechanism to keep the organism at optimum operating temperature within certain boundaries, even when the air temperature (T_a) is very different (Ruben, 1995; Grigg et al., 2004). These mechanisms can be divided into acting on the modulation of the rate of heat production and those modulating the rate of heat flow into or out of the organism (Bligh, 1998). Water serves as a main medium for the transport of heat from the body core to the surface via sensible heat transfer (flow of energy into or out of the organism by convection, conduction or radiation). Water is also used for the insensible heat transfer via evaporation (energy transfer from the organism to the environment). During evaporation a thermal gradient through the peripheral body tissues from the core to the skin is created or enhanced. Evaporative cooling involves the transfer of water to water vapour, which requires 2443 J per gram of water converted (Schmidt - Nielsen, 1997). Thus, there is a close link between thermoregulative mechanisms, water turnover and the use of water for metabolic processes (MacFarlane et al., 1966).

The evolution of subcutaneous (fatty tissue) or supercutaneous (hair) thermal insulation influences the flow of heat down the thermal gradient from the organism to the environment (de Lamo, 1990; Schmidt - Nielsen, 1997; Bligh, 1998). In this light, sheep fleece morphology has a considerable impact on heat dissipation from their skin surface and the possible loss of body water through thermoregulative processes. During domestication, the coat morphology of sheep was considerably changed. The wild type has a double coat with a coarse outer (produced by primary hair follicles) and a fine inner coat (derived from secondary hair follicles), still present in some primitive sheep breeds

(e.g., Soay). The modern wooled sheep (e.g., Merino) has instead a single - coat where all fibres are produced by both primary and secondary follicles which are similar in their physical characteristics (Ryder, 1981; Sumner and Bigham, 1993, Galbraith, 2010). However, skin and coat morphology is not homogenous and varies among body regions. Skin thickness decreases dorsally to ventrally on the trunk and proximally to distally on the limb (Scott, 1988) with a positive correlation between skin thickness, mean fibre diameter and staple length (Gregory, 1982). In sheep, the thinnest skin is found on the pinnae and the axillary, inguinal and perianal areas with an average thickness of general body skin in adults of 2.6 mm (Lyne and Hollis, 1968). Such areas with shorter hair and thinner skin are considered to act as “thermal windows” for heat dissipation and have received particular attention in thermal physiology (Fowler, 1994; Mauck et al., 2003). The thermal energy that flows between the outer surface of the animal’s body and the environment can be measured via the radiated electromagnetic waves. Infrared thermography offers an excellent non - invasive tool for measuring this infrared radiation on the boundary layer of an animal (de Lamo, 1990; Gerken, 1996, 2010; Gerken et al., 2006; McCafferty, 2007; Schwalm et al., 2008).

The conditions of the outer coat layer are modified by fleece removal, resulting in a change of thermal conductance. Shorn sheep are known to better withstand exposure to high T_a’s (Klemm, 1962; Arnold et al., 1984; Marai et al., 2007; Piccione et al., 2010). On the other hand, the importance of residual fibre length after shearing to prevent heat loss under cold or wet climatic conditions was outlined by Burnham et al. (1996) and Gerken (2010). However, there are only few reports available on the effect of shearing on water intake and water turnover in sheep. In Welsh Mountain sheep exposed to T_a’s of 20, 30 and 40 °C for periods of 8 hr, more sweat was discharged when the animals were shorn (Johnson, 1973). MacFarlane et al. (1958) found that water intake is related closely to respiratory rate in Merino sheep. Peppin Merino sheep selected for high wool production

drank 3.8 l of water / day under dry weather conditions compared to 3.1 l in the unselected control ewes. The application of tritiated water revealed an increase in water turnover of 13.7 % in wooled sheep compared to unselected ewes for high wool production (MacFarlane et al., 1966). MacFarlane and Howard (1970) reported that water turnover and storage in Merino sheep increased after shearing.

Therefore, the aim of this investigation was to study the effect of wool coverage on water metabolism in relation to thermoregulation in German blackhead mutton sheep. We applied the D_2O dilution technique (Lifson and McClintock, 1966) to estimate water intakes and water turnover and compare these results with direct measurements of water intake before and after shearing. Thermoregulative reactions were recorded including respiratory rate, rectal temperature, drinking behaviour, and changes in body surface temperatures determined by infrared thermography and were considered in the broader context of water use for thermoregulation.

Materials and Methods

The research conducted in this study was performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the University of Göttingen, Germany.

Animals and Management

The experiment was conducted between June and September 2008 at the Department of Animal Sciences, University of Göttingen, Germany. Fourteen dry German black head mutton ewes 1.1 ± 0.4 years old (mean ± SD) were allowed to adjust to the environment for one week and were then randomly distributed into two groups: the control sheep (n = 7) were already shorn in May 2008 while the treatment sheep (n = 7) were left unshorn. After

two weeks (day 15), the treatment ewes were shorn with a commercial sheep shearing machine.

Sheep were kept on straw in individual pens (1.5 x 2.0 m) in order to monitor individual feed and water intake offered *ad libitum* during the whole experimental period of 71 days. Individual intakes of rye grass hay (85.7 ± 1.2 % dry matter content) and water were measured directly on a daily basis by re - weighing the residual feed and water with an electronic scale (Sartorius model CP 34000, Sartorius AG, Göttingen, Germany). Corrections for water evaporation were made by placing a bucket (10 l) containing water in an adjacent area to measure the amount of water lost by evaporation. The actual amount of water consumed by the animals was calculated by subtracting the evaporated amount from the total water intake. The T_a and relative humidity in each room were monitored throughout the trial with thermo - hygrographs (Tiny view - Plus, Gemini data loggers, Germany) placed at a height of 1.0 m from the floor. Average T_a and relative humidity were 19.9 ± 0.4 ° C and 61.5 ± 1.1 % before shearing (day 1 to 15) and 21.0 ± 0.4 ° C and 62.2 ± 1.1 % two weeks after shearing (day 16 - 30) and 19.4 ± 0.4 ° C, 72.4 ± 0.7 % during the last two weeks of the experiment (day 57 - 71). In the stable, light schedule was kept constant (16 h light to 8 h dark, light: 0500 to 2100 h).

Water Kinetic Studies

Water intake was also estimated for two experimental periods at day 1 - 15 and at day 57 - 71, from water kinetics using D_2O. Prior to the isotope administration, a 5 ml blood sample was taken from the jugular vein into blood tubes containing sodium citrate to determine the background level of D_2O. A dose of 0.2 g D_2O / kg live body weight of 99.90 % purity (Eurisotop GmbH, Saarbrücken, Germany) was then injected intramuscularly at two body sites. The individual amount administrated was determined by the weight of the animals, which was measured with a scale prior to the isotope application (Sartorius CW3P1 -

150IG - 1, Sartorius AG, Göttingen, Germany). The actual dose given was gravimetrically measured by weighing the syringe before and after the administration to the nearest 0.001 g. Blood samples (approximately 5 ml) were collected at 12, 24 h and 2, 4, 7, 9, and 11 days after the application of D_2O for water turnover measurements. Earlier work showed that tracer concentrations in plasma samples are the same as in vacuum sublimated water samples (Riek et al., 2007). Therefore, plasma samples from sheep were analyzed for D_2O concentrations. Analyses were carried out at the Competence Centre of Stable Isotopes (KOSI, Göttingen University, Germany). Isotope ratios of ^2H were measured using an on - line high temperature reduction technique in a helium carrier gas described previously (Gehre et al., 2004) and expressed relative to the Vienna standard mean ocean water (VSMOW), which is the international reference standard for D_2O. Individual samples were measured in triplicate and the averages calculated. Total body water and total water intake (TWI) were estimated from water kinetics according to established formulas described in detail elsewhere (Al - Ramamneh et al., 2010).

Total water intake includes preformed and metabolic water from food and drinking water. Metabolic water was calculated from the feed composition. It was assumed that 1 gram of metabolized carbohydrates, fat and protein yielded 0.56, 1.07 and 0.42 gram water, respectively (Maynard et al., 1981). Measured TWI was calculated water drunk + metabolic water + preformed water.

Physiological Measurements

Respiratory rate was measured daily by counting the rate of flank movement for one minute between 15 : 00 and 15 : 30 h. Rectal temperature and body mass were recorded on a weekly basis.

The animals' surface temperature was determined by measuring the infrared radiation using infrared thermography equipment (Thermovision® 900 system, AGEMA

Infrared System AB, Danderyd, Sweden; resolution: 0.1 ° C, accuracy: ± 0.5 ° C, temperature range: – 30 ° C to 1.500 ° C). A scanner operating in the 8 to 12 µm band of the infrared spectrum was used as infrared detector. The signals are used by a processor to generate single infrared frames built up like a TV picture with an image resolution of 230 elements per line, 136 lines, and 272 pixels per line. The measurement formula is (AGEMA, 1992):

$I_m = I(T_{obj}) * \tau * \varepsilon + \tau * (1 - \varepsilon) * I(T_a) + (1 - \tau) * I(T_{atm})$

Where

$I(T)$ = Thermal value

I_m = Thermal value for the measured total radiation

τ = Efficient atmospheric transmission

ε = Emissivity of the object

T_{atm} = Atmospheric temperature

T_a = Air temperature

For each measurement the following object parameters have to be entered by the operator: emissivity of the object, object distance, relative humidity, atmospheric temperature and reflected Ta. The average emissivity (ε) of sheep wool was determined to be 0.980.

Infrared images were recorded on a weekly basis for both control and treatment animals. Additionally, images were also recorded on the day of shearing (day 15) and on the first 3 days after shearing (i.e., experimental days 16, 17, 18) for the treatment animals, resulting in 119 pictures for treatment ewes and 108 for the control group. The information from the images was analyzed by the integrated software ERIKA 3.00 which allows the calculation of means, standard deviation, minimum, maximum, and median across the measurement points of specific areas or geometric figures where temperatures are converted to a calibrated scale (Figure 1).

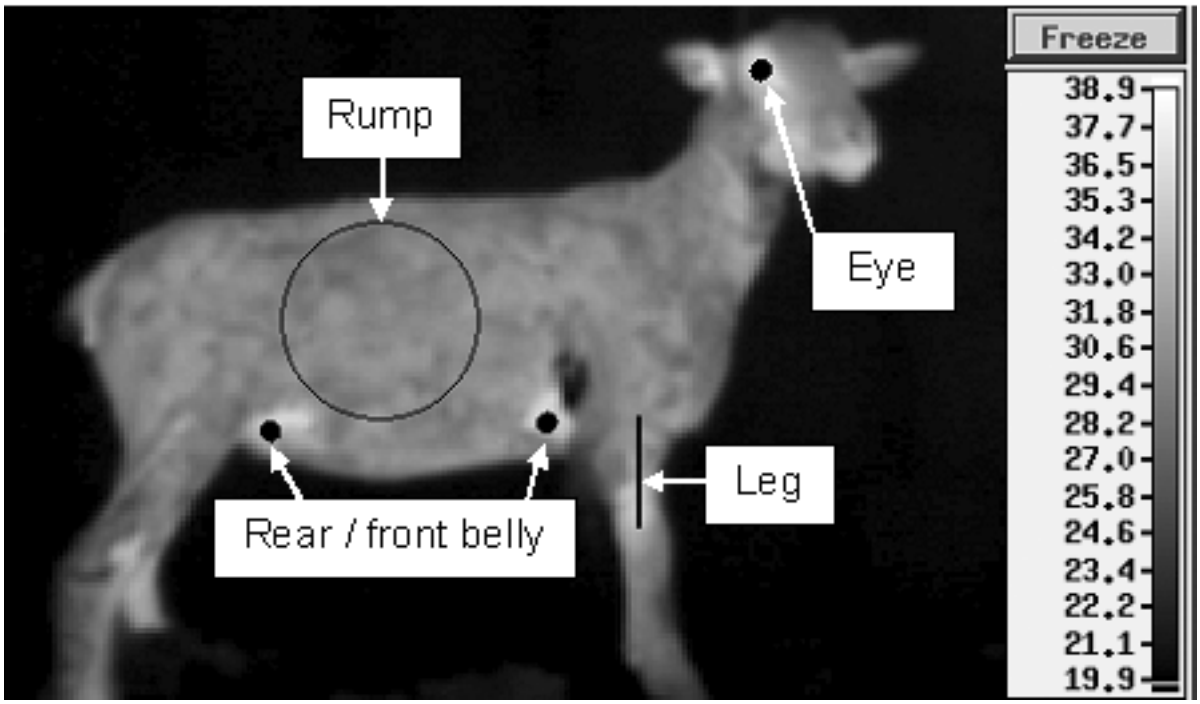

Figure 1: Thermographic image of a control sheep (see text for details) indicating the geometric areas used to determine the radiative heat loss (circle for rump, dot for eye, rear and front belly, and a straight line for the fore leg, distance from the *ginglymus* to *patella).* Control ewe with average wool length of 2.3 ± 0.8 cm at room temperature 19 °C and 76 % relative humidity. The scale on the right side indicates the surface temperatures ranging from low (dark shades) to high (light shades). Temperatures are: rump, 23.2 ± 0.5 °C (range 22.1 – 25.8 °C); eye, 34.0 °C; rear belly, 32.8 °C, front belly, 31.8 °C; leg, 24.1 ± 1.4 °C (range 22.5 – 27.3 °C).

Several representative body regions were chosen to evaluate the surface temperature distribution across the body: eye, rump, front, and rear belly and fore leg (upper part of the leg, from the *ginglymus* to the *patella*). For simplicity, predefined geometric figures were used during analyses as shown in Figure 1: a point for eye, rear belly and front belly, a line for the fore leg and a circle for the rump. In treatment ewes, front and rear belly temperatures were not measurable due to the wool coverage before shearing.

In addition, coat fibre depth was measured using a ruler and recorded as the distance between the skin surface and the coat surface in a vertical position at three different positions (front, rear shoulder and rump) on both sides of the body.

Drinking Behavior

Drinking behavior was observed every two to three days by using a time - lapse (8 - fold) video recording system during the first two weeks (day 1 - 15) and last two weeks (day 57 - 71) of the experiment for 24 hours. Infrared lights were used to facilitate data recording during night. For further video analysis, the Interact® 8.0 system (Mangold International GmbH, Germany) was used. Individual drinking behavior (frequency and duration) and diurnal drinking duration (across 24 h) were analyzed. For the present study, 87 video observations were included (45 from 7 control ewes and 42 from 6 treatment ewes) before shearing (day 1 - 15) and 98 recordings (49 from 7 control and 7 treatment ewes) after shearing (day 57 - 71). Drinking behavior was defined as follows:

- drinking frequency (number of drinking bouts)
- drinking duration (time in minutes when the animal was actively engaged in the ingestion and swallowing of water).

Statistical Analysis

For statistical analyses of differences between treatment groups, averages were calculated across 3 periods covering days 1 - 15 (before shearing, period 1), 16 - 30 (after shearing, period 2) and 57 - 71 (after shearing, period 3). Data were then subjected to analyses of variance using the PROC MIXED procedure of the software package Statistical Analysis System version 9.01 (SAS, 2001). The model included the fixed effects of treatment and period and the random effect of the animal. The model was:

$Y_{ijk} = \mu + TP_i + A_j + e_{ijk}$

Where:

Y_{ijk}: observation value; μ: overall mean; TP_i: combined treatment and period effect; A_j: random effect of the animals and e_{ijk}: random error. An integrated Tukey test was used to detect differences between means with a 5 % significance level. All values are presented as means ± SD.

For further analysis of the relations between daily or weekly recorded traits, a multivariate analysis was conducted using the hierarchical cluster procedure of SPSS (version 18.0). Data were standardized by Z - transformation and Euclidian distances were obtained by average linkage.

Results

Water intake, physiological and behavioral reactions

At the beginning of the trial (day 1), wool length averaged 2.3 ± 0.8 cm (mean ± SD) and 10.6 ± 1.2 cm in control and treatment ewes, respectively. Shearing (day 15) resulted in a very evenly distributed hair length of about 0.6 ± 0.2 cm with an average fleece weight of 3.5 ± 0.5 kg. The linear regressions of wool length on experimental days revealed no growth in control animals (Wool length, cm = 2.3027 + 0.001 day, P = 0.78), while hair length significantly increased by about 1 mm per day in treatment ewes after shearing (Wool length, cm = 0.4912 + 0.0115 day, $P < 0.05$).

Prior to shearing, treatment sheep had a lower dry matter intake (g / $BM^{0.75}$), drank more water (g / kg $BM^{0.75}$), had higher WI / DMI ratios and their respiratory rate was 2 - times that of the control animals (Table 1, Figure 2). Immediately after shearing, newly shorn animals significantly reduced their water intake and respiratory rate (Figure 2). Across the two weeks after shearing (period 2) the two treatment groups only differed in uncorrected dry matter intake and respiratory rate, which were both significantly lower in newly shorn sheep compared to the control animals. Six to 8 weeks after shearing, there

were no significant differences between treatment groups (period 3). The simultaneous daily variations of water intake and respiratory rates shown in Figure 2 indicate that respiratory rate followed T_a more closely than water intake.

Table 1: Average body mass, water drunk, dry matter intake, water intake to dry matter intake ratio, and respiratory rate in blackhead mutton sheep before (d 1 - 15) and after (d 16 - 30 and d 57 - 71) shearing (see text for details, values are means ± SD)

Item	Period 1 (d 1 – 15)		Period 2 (d 16 – 30)		Period 3 (d 57 – 71)	
	Control	Treatment Before shearing	Control	Treatment After shearing	Control	Treatment After shearing
Body mass (kg)	58.4 ± 9.4^{ab}	58.8 ± 9.1^{ab}	59.5 ± 8.7^{a}	55.3 ± 8.0^{c}	57.4 ± 8.4^{b}	53.4 ± 8.0^{d}
Metabolic body mass (kg)	21.1 ± 2.5^{ab}	21.2 ± 2.5^{ab}	21.4 ± 2.3^{a}	20.2 ± 2.2^{c}	20.8 ± 2.3^{b}	19.7 ± 2.3^{d}
Water drunk (l d^{-1})	2.9 ± 0.7^{bc}	3.5 ± 0.6^{a}	2.9 ± 0.6^{b}	2.5 ± 0.5^{cd}	2.6 ± 0.8^{cbd}	2.3 ± 0.5^{d}
Water drunk (g / kg $BM^{0.75}$)	134.3 ± 18.0^{b}	164.9 ± 16.7^{a}	134.8 ± 21.8^{b}	123.2 ± 19.7^{bc}	122.4 ± 29.7^{bc}	115.0 ± 19.9^{c}
Dry matter intake (kg d^{-1})	1.2 ± 0.2^{a}	1.1 ± 0.1^{bc}	1.1 ± 0.1^{ab}	1.0 ± 0.1^{cd}	1.0 ± 0.2^{bc}	0.9 ± 0.1^{d}
Dry matter intake (g / kg $BM^{0.75}$)	59.0 ± 5.1^{a}	52.0 ± 4.1^{b}	53.2 ± 7.0^{ab}	47.5 ± 2.5^{bc}	49.4 ± 6.2^{bc}	45.4 ± 6.8^{c}
WI / DMI	2.3 ± 0.3^{c}	3.3 ± 0.4^{a}	2.6 ± 0.5^{bc}	2.8 ± 0.4^{b}	2.5 ± 0.5^{bc}	2.6 ± 0.7^{bc}
Respiratory rate (breath / min)	31.0 ± 3.9^{de}	66.1 ± 4.7^{a}	39.5 ± 5.3^{b}	30.8 ± 2.0^{e}	37.3 ± 3.5^{bc}	34.4 ± 3.0^{cd}

^{a,b,c,d,e}: Mean values within the same row with no common superscript differ significantly ($P < 0.05$)

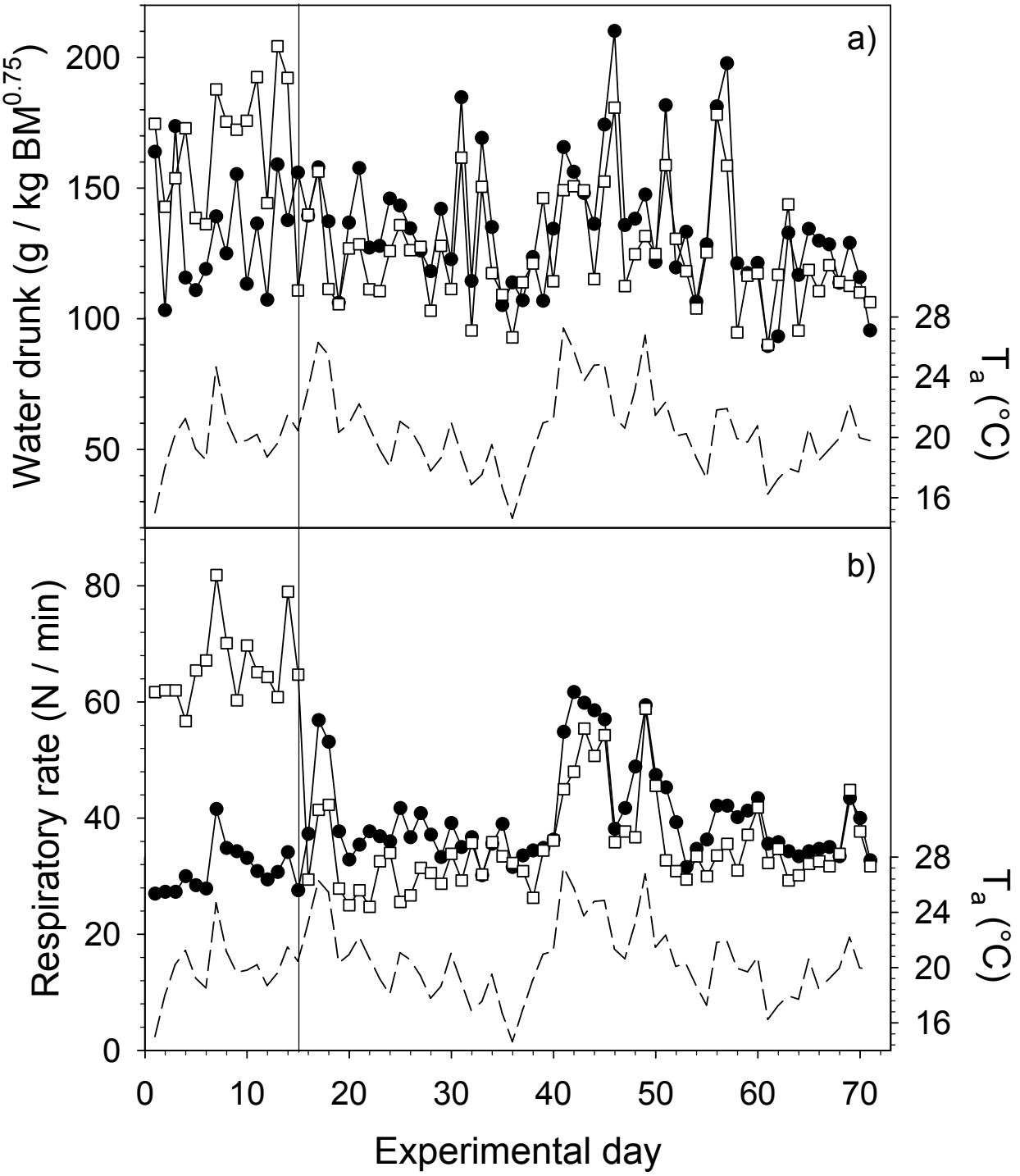

Figure 2: Average daily water intake a) and respiratory rate b) for control (●; n = 7, shorn animals) and treatment (□; n = 7) groups of German blackhead mutton ewes before (day 1 – 15) and after shearing (day 16 - 71), indicated by the vertical line. The corresponding ambient temperature (T_a) is depicted as a dashed line.

Rectal temperatures paralleled the development of respiratory rate with significantly higher values before shearing in the treatment group and an immediate decline after shearing to values even below those of the control animals (Table 2, Figure 3). After shearing, both experimental groups did not differ in average rectal temperatures (Table 2, periods 2 and 3).

Table 2: Rectal and body surface temperatures in °C (rump, eye, leg and front and rear belly) measured by infrared thermography for control and treatment groups of blackhead mutton ewes before and after shearing (see text for details, values are means ± SD)

Item	Period 1 (d 1 - 15)		Period 2 (d 16 – 30)		Period 3 (d 57 – 71)	
	Control	Treatment Before shearing	Control	Treatment After shearing	Control	Treatment After shearing
	N = 7	N = 7	N = 7	N = 7	N = 7	N = 7
Rectal temperature	38.8 ± 0.1^{b}	39.3 ± 0.2^{a}	38.8 ± 0.1^{b}	38.8 ± 0.2^{b}	38.8 ± 0.1^{b}	38.9 ± 0.2^{b}
Eye temperature	33.6 ± 1.1	33.8 ± 1.2	34.3 ± 0.6	34.0 ± 0.8	34.1 ± 0.8	33.0 ± 1.9
Rump temperature	24.5 ± 0.6^{c}	19.3 ± 0.3^{f}	25.4 ± 0.4^{b}	29.3 ± 0.5^{a}	22.2 ± 0.1^{e}	23.8 ± 0.5^{d}
Leg temperature	27.4 ± 1.6^{abc}	25.8 ± 2.4^{c}	28.2 ± 1.6^{ab}	29.0 ± 0.9^{a}	25.5 ± 2.4^{c}	26.6 ± 1.5^{bc}
Front belly temperature	33.6 ± 0.8^{ab}	NA	34.2 ± 0.6^{a}	33.5 ± 0.6^{ab}	32.9 ± 1.2^{b}	33.1 ± 1.1^{b}
Rear belly temperature	34.1 ± 1.4^{a}	NA	34.1 ± 1.2^{a}	33.6 ± 1.0^{ab}	31.1 ± 2.5^{c}	32.2 ± 1.9^{bc}

NA: Not available due to wool cover

^{a,b,c,d,e,f}: Mean values within the same row with no common superscript differ significantly ($P < 0.05$)

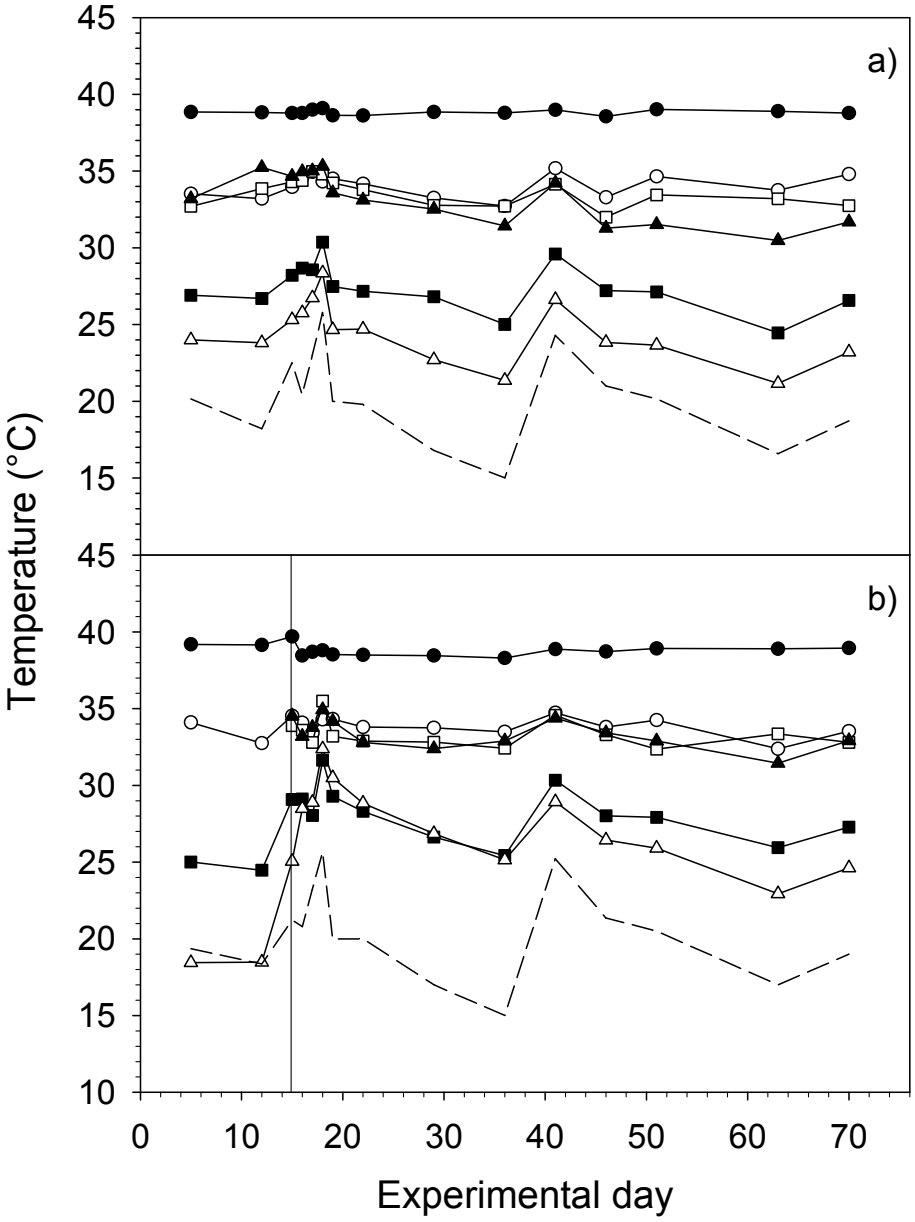

Figure 3: Average rectal temperature (●), body surface temperatures (eye: ○, leg: ■, front belly: □, rear belly: ▲, rump: Δ; measured by infrared thermography, see text for details) and air temperature (---) in relation to experimental days for control a) (shorn animals) and treatment b) German blackhead mutton sheep before and after shearing, indicated by the vertical line. Values are means of seven animals.

Recorded drinking behavior did not reflect the treatment differences found for the water intakes as drinking duration and frequencies were not different between experimental groups. On average, animals spent between 0.1 and 0.3 % of the 24 h day by drinking and had 11.5 to 19.1 drinking bouts / day.

Infrared thermography

Figure 3 depicts the observed changes in measured rectal and surface temperatures for control and treatment animals. Surface temperatures of leg and rump varied very closely with T_a, while temperatures of eye, front and rear belly remained quite stable irrespective of large environmental changes in temperatures. The lowest fluctuation was observed for rectal temperatures.

The determination of surface temperatures by infrared thermography revealed also considerable differences between body regions, with the lowest values (range: 19.3 - 29.3 °C) found for rump and leg temperatures, while eye, front and rear belly temperatures ranged between 31.1 and 34.3 °C (Table 2). Across all measurement periods, there was no discernable treatment difference for eye temperature. The longer fleece in treatment sheep led to significantly lower surface temperatures of the rump (period 1). In newly shorn sheep, rump temperature increased significantly (Table 2) and remained significantly higher than in control sheep. Apart from rump temperature no further significant differences were detected between control and treatment ewes after shearing (periods 2 and 3).

Deuterium dilution technique

The differences in water intake between control and treatment ewes were confirmed by using D_2O dilution technique. Total body water (TBW) content expressed as percentage of body mass was significantly lower in wooled sheep before shearing (62.2 to 76.1 %)

compared to the control group (66.6 to 85.1 %, P = 0 .042). After shearing the TBW content significantly increased (P < 0.001) in newly shorn animals and reached 78.5 % of body mass in period 3 (Table 3), but the values were not significantly different from the control animals. Treatment ewes had significantly (P < 0.05) higher measured (re - weighing water buckets) and estimated (D_2O dilution) TWI compared to the control group before shearing (Table 3). After shearing, these differences between treatment and control group disappeared. The ratio between estimated and measured TWI was not significantly different before (P = 0.727) and after (P = 0.786) shearing, ranging between + 0.6 to + 16.1 % in control and + 2.4 to 17.5 % in treatment animals (Table 3).

Table 3: Average total body water, total water intake (TWI) measured by weighing water buckets or estimated by the deuterium dilution technique and the ratio between estimated and measured TWI in blackhead mutton ewes before and after shearing (see text for details, values are means ± SD)

Item	Period 1 (d 1-15)		Period 3 (d 57-71)	
	Control N = 7	Treatment before shearing N = 7	Control N = 7	Treatment after shearing N = 7
Total body water (% of BM)	72.3 ± 6.0^{bc}	65.5 ± 4.9^{c}	84.1 ± 5.2^{a}	78.5 ± 6.1^{ab}
Measured TWI[1] (g / kg $BM^{0.75}$)	164.5 ± 18.8^{b}	191.5 ± 16.8^{a}	147.7 ± 31.9^{bc}	138.3 ± 19.9^{c}
Estimated TWI[2] (g / kg $BM^{0.75}$)	165.4 ± 21.2^{b}	195.8 ± 17.1^{a}	171.1 ± 35.0^{ab}	162.2 ± 29.2^{b}
Estimated TWI / Measured TWI (%)	100.6 ± 7.1^{b}	102.4 ± 5.3^{b}	116.1 ± 6.7^{a}	117.5 ± 14.9^{a}

^{a,b,c}: Mean values within the same row with no common superscript differ significantly ($P < 0.05$)

[1] TWI = metabolic water + preformed water from moisture content of the hay + water drunk

[2] TWI estimated by D_2O dilution

Cluster analysis

The present cluster analysis shows that the traits recorded differ largely in their relations with water intake. From Figure 4, two main clusters appear: traits related to body surface temperatures are in close relation to ambient temperatures, while the main second cluster is formed by respiratory rate, wool length, dry matter intake and rectal temperature.

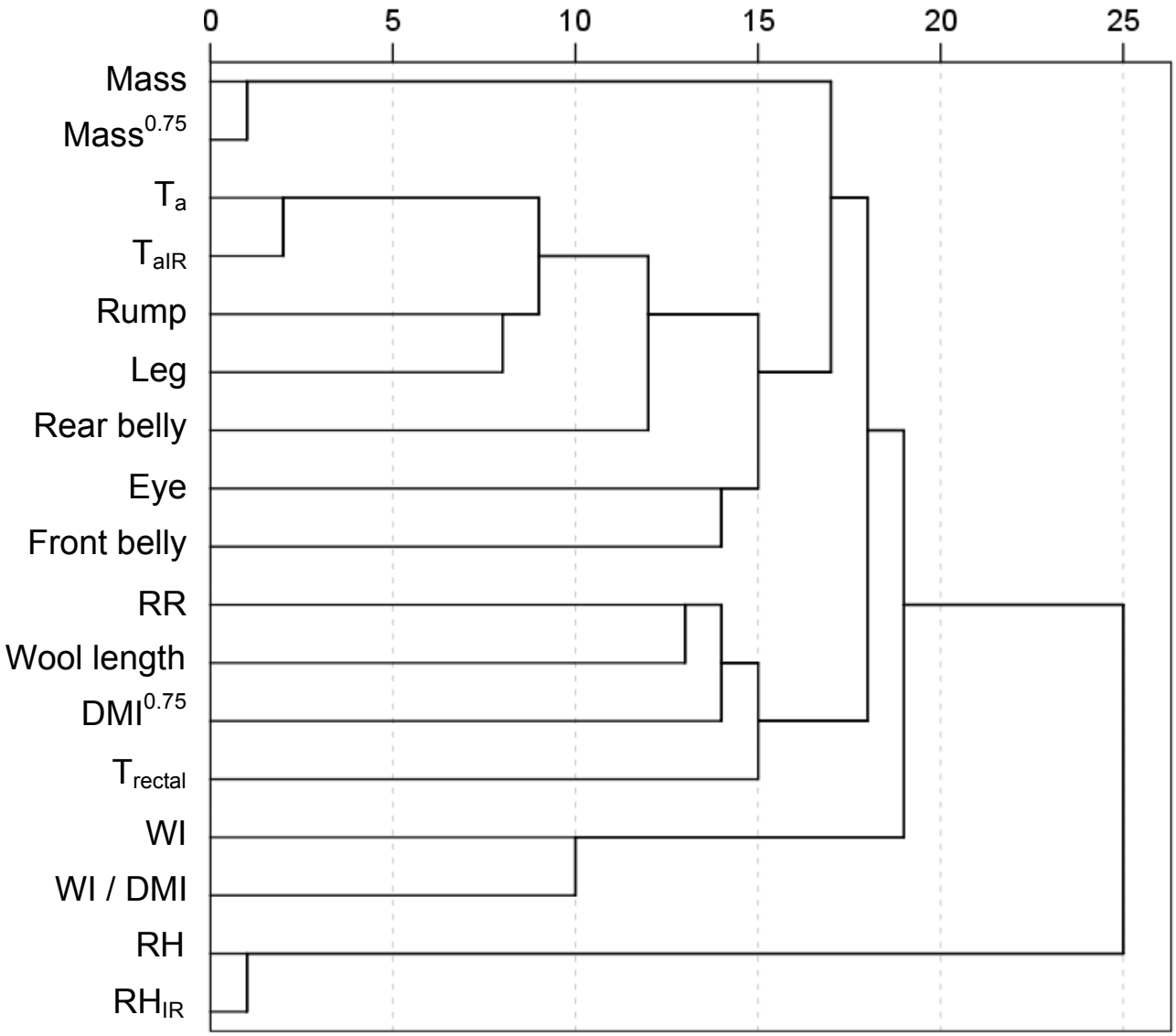

Figure 4. Dendrogram of a cluster analysis using average linkage across various traits measured. Data, from both groups (control and treatment) after shearing, were transformed to Z - values and distances rescaled (Mass (kg), $Mass^{0.75}$ ($kg^{0.75}$), T_a (°C): air temperature, T_{alR} (°C): air temperature during infrared thermography, rump, rear belly, eye, front belly (°C), RR (N / min): respiratory rate, $DMI^{0.75}$ (kg / $mass^{0.75}$): dry matter intake per metabolic body mass, T_{rectal} (°C): rectal temperature, WI (l / d): water intake, WI / DMI (l / kg / d): water intake per dry matter intake, RH (%): relative humidity, RH_{IR} (%): relative humidity during infrared thermography).

Discussion

Water intake, body water and physiological responses

In the present study wooled sheep showed reactions similar to the physiological and behavioral responses described for ruminants when exposed to thermal stress (Christopherson, 1985; Silanikove, 1992; Marai et al., 2007; McKinley et al., 2009; Bernabucci et al., 2010) including increased water intake, respiratory rate and rectal temperature, and lower DMI intake. According to the quantification proposed by Silanikove (2000b), treatment sheep underwent medium to high heat stress according to their panting rate. After shearing, respiratory rate immediately declined to significantly lower levels compared to the control animals. The same similar pattern was found for rectal temperature. Similarly, MacFarlane et al. (1958) found that water intake was closely related to the respiratory rate in Merino sheep. Woolly sheep had a lower TBW content before shearing due to higher water losses by the respiratory tract through evaporative cooling via panting. Our results also agree with MacFarlane and Howard (1970), who reported that water turnover and storage in sheep increased after shearing. The authors attributed this to a shift of water from evaporative cooling via respiration to the extracellular fluid (Ternouth and Beattie, 1970). Pennisi et al. (2004) described increased panting rates in unshorn 9 - month old Comisana ewes, but found no differences in rectal temperatures between shorn and unshorn lambs under traditional range conditions. This disagreement might be due to differences in breed, age and housing conditions.

Infrared thermography

The low surface temperature of wooled animals (period 1) indicate that less body heat was radiated to the outer surface layer of the animals. In the present study, shearing led to an immediate shift in thermoregulative mechanisms from evaporative cooling (via panting) to heat radiation via the skin even under the prevailing moderate temperate conditions.

Among the body regions analysed, only rump surface temperature significantly changed after shearing.

The dissipation of body temperature from a sheep skin covered by wool has been shown to be quite complex. Sheep are considered in an intermediate position between horses and cattle (species, in which sweating prevails) and pigs (in which panting is the main pathway for heat dissipation) with regard to the importance of sweating in thermoregulation (Hörnicke, 1987). However, in a fully wooled sheep evaporation of sweat or body water from the skin is hindered, because a fleece contains air spaces with water vapor, which is in equilibrium with the water either absorbed or adsorbed on the wool fibers (Gatenby et al., 1983). When the skin is covered by a heavy pelage, passive transfer of water vapor across the skin is negligible. In that case, the high humidity of the air immediately above the skin prevents significant water vapor transfer unless the hair is strongly ruffled. Also, transient heat production, due to absorption of sweat or water in the fleece, may result in additional heat loads for the animal (Gatenby et al., 1983). In the thermal windows the hair is so short that both sensible and insensible water loss is possible (de Lamo et al., 1990). However, in unshorn sheep, the thermal windows at the front and rear belly were partly covered by the wool, thus reducing the possible heat transfer.

Infrared thermography offers an excellent tool to detect thermal windows within the outer layer of the body surface (Gerken, 2010; Gerken et al. 2006) and also allows visualizing changes in the superficial blood flow as shown by the comparison of sheep before and after shearing. However, infrared thermography is of limited suitability to evaluate changes in the metabolic rate due to shearing, because it measures radiative heat loss, not total non - evaporative heat loss (Autio, 2008). Infrared thermography has been used to determine rates of heat loss, but the estimation of the metabolic costs

caused by panting for increased thermoregulative mechanisms due to the impact of wool cover requires the cross - calibration with existing metabolic methods (McCafferty, 2007).

Relationships between traits

The traits recorded differ in their relations with water intake and from the cluster analysis two main clusters are suggested (Figure 4). We suggest that the traits recorded via infrared thermography are more closely related to environmental conditions, in particular T_a. Similarly, Gatenby et al. (1983) measured the temperature and relative humidity in the fleece of Clun Forest ewes of 7 cm thickness at different distances from the skin using a thermocouple and found that on the surface of the fleece the temperature closely followed T_a, but deeper in the fleece there was much less change.

In the present study, respiratory rate, rectal temperature and dry matter intake on the other hand are possibly better indicators for metabolic processes and appear to be strongly influenced by wool length. Similarly, studies on horses (Autio, 2008) and our own studies on llamas (unpublished) only showed a low to medium relationship between infrared thermography measurements and core body temperature. These results are in contrast to Stewart et al. (2005) who recorded the eye temperature of cattle in animal welfare studies.

However, the present suggestion of two main clusters in relation to water intake need to be confirmed with a larger number of animals, as the present study only involved 7 sheep per experimental group. In this context, it should be noted, that animal variance for water and dry matter intake per metabolic body weight accounted for 82.50 and 60.23 %, respectively of the total variance, indicating huge individual variation.

Fleece morphology

Selection for wool during domestication had a considerable impact on thermoregulative metabolism. Interestingly, when sheep were released into the wild, Bigham and Cokrem (1984) observed a gradual reduction in the secondary / primary follicle ratio and total fiber production in subsequent generations, indicating a successive regression to a more wild type double coat. We hypothesize, that natural selection acts against a single coat. One of the underlying natural selection mechanisms could be the higher demand for water and energy, both natural resources which are frequently of limited availability in the wild, during panting as a main thermoregulative mechanism in fully wooled animals. We suggest that the double coat allows a more flexible heat radiation, because primary follicles are equipped with muscles (Galbraith, 2010) thus allowing to change the opening of the fleece to some extend by ruffling.

Goats are described as superior with regard to water management in comparison to sheep (Silanikove, 1992; Jaber et al., 2004; Alamer, 2006). We suggest that this advantage is mainly due to species differences in skin thickness and hair morphology, with goats having thinner skin and a wild type double hair coat (Devendra and McLeroy, 1982; Sumner and Bigham, 1993). Interesting is the comparison with Angora goats, who also underwent changes in their hair coat morphology during domestication. Hetem et al. (2009) showed similar effects of shearing in Angora goats as in our study with sheep, underlining the impact of modification of fleece morphology on thermoregulation irrespective of species.

Conclusion

This study illustrates that shorn German blackhead mutton ewes are more efficient in their thermoregulation compared to woolly unshorn ewes especially with regard to physiological reactions and their water requirements. In the tropics, hair sheep have been shown to adapt very well to hot climatic conditions (Devendra and McLeroy, 1982). However, to our knowledge, there are no comparative studies for hair and wooled sheep with regard to water requirements. When wool becomes less important as natural resource for human textiles, hair sheep might be a better alternative also due to their presumably lower amount of water required for evaporative cooling, even under European landscape management systems.

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Chapter 5

General discussion

General discussion

In the following chapter, the data obtained for sheep and goats under different practical management practices (watering regime and shearing) discussed in the previous chapters are evaluated to give basic recommendation on water intake. In addition, the suitability of different methods to measure water intake and water turnover will be discussed.

Comparison between sheep and goats

The most important nutrient in animal nutrition is water (NRC, 2001). The results of this thesis demonstrates that the drinking water of sheep and goats should not be discounted and in fact, be part of the nutritional program in animal husbandry. There is a close relationship between food and water intake in animals (MacFarlane and Howard, 1972; King, 1983, Freer et al., 2007), therefore, enough drinking water is a prerequisite for animals to utilize food efficiently. Goats that are native to dry regions have developed several strategies to cope with scarcity of food and water (Silanikove, 2000; Ferreira et al., 2002). Their small body size, low metabolic requirements, the ability to reduce metabolism, the digestive efficiency in relation to feeding strategies, the efficient usage of water, as well as their ability to economize the nitrogen requirements via urea recycling and nitrogen conservation (Silanikove, 2000) influence their superiority for living under harsh environmental conditions.

The results of the first experiment (Chapter 2) confirmed that German blackhead mutton sheep differ from Boer goats in their water and feed intakes and drinking behaviour and are in agreement with earlier reports (Aregheore, 1996; Ferreira et al., 2002; Van et al., 2007) with higher intake in sheep compared with goats. The lower water intake in Boer goats compared to blackhead mutton sheep when kept under temperate conditions confirms the superior water management capacity in goats as an adaptation to the desert environment , the unusual ability to withstand dehydration, minimize water losses via urine

and faeces and a high urine concentration ability (Silanikove, 2000; Daramola and Adeloye, 2009). Goats living in harsh environments represent a climax in the capacity of domestic ruminants to adjust to such areas. Boer goats originated from and were selected in dry arid areas (South Africa) compared with merino sheep that have been selected and farmed on higher quality pastures (higher in protein and lower fiber forage). Therefore, sheep probably need a higher water turnover for excretion of nitrogen in the urine (Fairall and Klein, 1984; Ferreira; 2002). Goats are essentially browsing animals that can distinguish between bitter, sweet, salty and sour taste and show a higher tolerance for bitter taste than do sheep and cattle (Devendra, 1990).

Goats are considered to have a higher concentration of rumen ammonia, and longer retention time that could lead to a better fibre digestion (Devendra, 1990). On the other hand, goats are thought to have a smaller proportion of the gut in relation to body weight, which results in a lower intake of water and feed intake compared with sheep. Ferreira et al. (2002) showed that goats need less water to synthesize 1 kg of weight gain than sheep.

It has been suggested that lower water consumption in goats compared to sheep and other animals is due to the fact that goats have adapted to limited water intake and short - term shortages due to a low water turnover rate (Silanikove, 2000; Daramola and Adeloye, 2009). The adaptation is similar to the camel, an animal that is known for its ability to survive without water for long periods (Silanikove, 2000) and their greater ability to reduce evaporative loss of water, faecal water content and to concentrate urine (Silanikove, 2000; Daramola and Adeloye, 2009).

It has been suggested by several researchers that goats differ in level of feed intake from sheep both on pasture and in confined conditions. Aregheore (1996) and Van et al. (2007) reported that sheep required a higher amount of nutrients for growth and their higher genetic potential for growth is reflected by the higher dry matter intake in sheep

compared to goats. This may reflect the differences in genetic make - up between sheep and goats and in their feed habitats (MacFarlane and Howard, 1972). Animut et al. (2005) showed that goats on pasture spent less time eating and more time idling than sheep. This was suggested to be due to the botanical composition of the diets and to the lower rate of growth of goats than sheep leading to shorter feeding times and lower dry matter intake per body mass (DMI / $BM^{0.75}$) in goats than in sheep.

Water deprivation in the present study (Chapter 3) did not induce major changes in water and dry matter intake, or body mass in both species (more details are mentioned in chapter 3). This small impact of water restriction may be mainly attributed to the temperate environmental conditions prevailing during the experiment. Lynch et al. (1972) and Brown and Lynch (1972) found that merino ewes in the temperate climate of Australia, deprived of drinking water for 12 months or more, could survive and breed and have similar productivity to ewes with water *ad libitum*. In other climates, deprivation will generally have serious consequences, especially in the arid and semi - arid regions.

The relatively large volume of fluid in the alimentary tract of ruminants may maintain water balance for blood and body tissues for the first few days of water deprivation (Louw and Seely, 1982). After dehydration, some desert - adapted species are able to drink water in amounts equivalent to a large proportion of their body mass. For example, Bedouin goats (Choshniak and Shkolnik, 1977), camels (Schmidt – Nielsen, 1979), and desert bighorn sheep (Turner, 1973) are able to drink water in amounts equivalent to 40, 30, and 20 % of their body mass, respectively. However, other species, such as cattle, are not able to rapidly drink water because of problems associated with hemolysis (Bianca, 1970; Louw, 1984). The function of the rumen during rehydration differs among species that are able to ingest large volumes of water over short time periods. For example, in camels, water ingested rapidly passes from the alimentary tract to the blood and body tissues (Schmidt – Nielsen, 1979; Etzion et al., 1984). In desert - adapted goats, large volumes of

rapidly ingested water are released more slowly to the blood and other body tissues, thus minimizing hemolysis and osmotic shock to tissues (Choshniak and Shkolnik, 1977; Louw, 1984).

During the first few days of dehydration, fluid contained in the rumen provides a large portion of the water lost (Turner, 1979; Silanikove, 1992, 2000). Water turnover rates vary among species and are lower in animals from arid areas than those adapted to temperate conditions. This trend is also observed in domestic ruminants with breeds adapted to arid areas having lower water turnover rates than European breeds (MacFarlane and Howard, 1972; Singh et al., 1976; Degen, 1977, King, 1983).

Thus, the unchanged water intake between control and restricted groups in our study confirmed that a new equilibrium was reached by both species. Several reports have documented the capability of desert sheep and goats to withstand water shortage by reducing their feed intake and therefore endogenous heat production and subsequent water requirement for evaporative cooling (Ayoub and Saleh, 1998; Silanikove, 2000). It is possible that in response to this restriction of water intake the restricted sheep and goats had made physiological adjustments in order to reduce their water expenditure such as reduction of rectal temperature and respiratory rate in goats. The lower feed intakes of the restricted groups imply a proportional reduction in heat production of these animals compared with the free access to water. With a lower heat production, the rate of respiratory water loss of the restricted groups would tend to be lower than that of the control groups, which also agree with a previous report by Brown and Lynch (1972) on sheep.

In the third experiment (Chapter 4), an additional impact on the water metabolism of the sheep was caused by the presence of the fleece, increasing the heat load on the animal even under temperate conditions. An increase in water intake in unshorn ewes illustrated a higher water demand for evaporative cooling due to impaired

thermoregulation. Accordingly, animals drunk more water to maintain body fluid homeostasis by refilling fluids lost via urine excretion, sweating and respiration (Silanikove, 1992, Pennisi et al., 2004). Klemm (1962) described that the main dissipation of heat from wooly sheep is respiratory evaporation and is noticeable as panting. The observed rise in the respiratory rate may be attributed to the greater need for respiratory enhancement to dissipate the extra heat imposed on the animals during fleece existence.

Deuterium dilution technique and water turnover

Determination of daily water exchange between animals and their environment is important for the estimation of their water requirements and the evaluation of their adaptability and productivity (El - Nouty et al., 1988). In ruminants, daily water turnover rate is influenced by breed, environmental temperature, animal age, sex, physiological state and the water availability in the environment (MacFarlane and Howard, 1972; King, 1983; El - Nouty et al., 1988).

The species differences in their water intake were also confirmed by using the D_2O dilution technique with higher total water intake estimated for sheep than goats in the first experiment (Chapter 2).

Several studies have shown that the estimates of TBW determined by TOH or D_2O dilution in sheep and goats are in close agreement with those derived from post - mortem desiccation methods (Panaretto, 1963; Panaretto and Till, 1963; Atti et al., 2000). The body water fraction estimated by D_2O ranged between 54 % to 63 % and 58 % to 64 % in sheep and goats, respectively, in the present experiment and is in close agreement with other results (Panaretto, 1963; Panaretto and Till, 1963; El - Nouty et al., 1988; Atti et al., 2000).

El - Nouty et al. (1988) found that TBW in Baladi goats was higher than in two sheep breeds (Bakri and Rahmani) during spring and winter season; in addition, they

found that Baladi goats seem to be more adapted to unfavorable environmental conditions than sheep, which is in close agreement to the present results obtained in the first experiment (Chapter 2).

Under the present water restriction regime and temperate climatic conditions (Chapter 3), the TBW of sheep and goats was not significantly reduced, indicating that animals were able to adjust to the water deficit. Similarly, Freudenberger and Hume (1993) found no effect of water deprivation on TBW in feral goats kept under semi - arid conditions.

Different species of livestock have different rates of water turnover, and in general, animals adapted to dry environments have lower rates of turnover than those in zones that are more temperate. El - Nouty et al. (1988) found that Baladi goats appear to be more adapted to unfavorable environmental conditions than sheep. This is based upon the findings that they had lower water turnover rates, and higher TBW than Barki and Rahmani sheep breed.

The higher TBW in Boer goats compared with German blackhead mutton sheep in the present study may be also explained by the reverse relationship between fat content and TBW. Makinde (1993) found that the amount of total body water was significantly higher in small ruminants compared with pigs and the TBW in sheep and goats ranged between 60 to 85 % of the body weight.

MacFarlane et al. (1966) suggested that water turnover is linked with the use of water for metabolic processes. Furthermore, he found that sheep selected for high wool production drunk 3.8 l / day water. When applying TOH water intake was estimated as 113.7 % compared to the control group that consumed 3.1 l / day water and 100 % under dry weather conditions. MacFarlane and Howard (1970) reported that water turnover and storage in sheep increased after shearing. Ternouth and Beattie (1970) reported an increase in fluid volume after shearing due to lowered respiratory rate and shift of water

from evaporative cooling via respiration to the extracelluar fluid (Ternouth and Beatti, 1970). El - Nouty et al. (1988) reported that sheep and goats exposed to heat during summer and spring season increased their TBW content and their extracelluar fluid volume compared to the winter season due to the increased capacity for water retention upon exposure to heat in order to provide water for the intense evaporative heat loss. MacFarlane (1964) reported that extracelluar fluid volume of Pappin merino sheep was larger in the tropics at all times than in sheep of similar age in the temperate zone.

The higher TBW after shearing in the present investigation (Chapter 4) indicates lower water and D_2O loss through the respiratory tract. We suggest that the presence of the fleece impairs the cutaneous water loss thus contributing to the relatively high body temperature (rectal temperature) of sheep before shearing. However, stomach water will be a small fraction of TBW and thus variation in stomach water is unlikely to account for the poor correlation between TWI measured (by weighing water buckets) or TWI estimated (by D_2O dilution method) in the present studies.

Drinking behaviour

The higher amount of water intake in sheep (Chapter 2) was also reflected by their drinking behaviour. Sheep spent approximately 0.3 % per 24 h drinking, while goats spent only 0.1 %. The higher drinking frequency and duration by sheep could result from a higher metabolic rate in sheep, differences in activity level and thermoregulation capacity between both species.

Our observation of drinking behaviour in sheep and goats revealed interesting differences in behavioural strategies between both species under water restriction (Chapter 3). Water restricted goats were able to increase their water intake per drinking bout to about 0.9 ± 0.5 l / bout compared to 0.5 ± 0.4 l / bout in sheep, while the amount of water intake per minute drinking was identical in goats and sheep (2.0 ± 0.5 vs. 2.0 ± 0.6 l

/ minute). The higher frequency of drinking events in sheep may be related to their evaporative cooling mechanisms, because sheep panted more frequently than goats. It is suggested, that sheep require more water to regulate their body temperature due to their longer fleece, which increases their endogenous heat production.

After shearing (Chapter 4), sheep reduced their time spent in drinking by 40.5 % and reduced their drinking frequency to 0.1 % per day after the removal of the heat load caused by the presence of the compact fleece. Shkolnik et al. (1980) reported that goats drank an equivalent of 30 to 45 % of their dehydrated body weight within 2 min, which reflects a vital process of rapid rehydration.

The high individual variability in drinking behaviour obtained by using time - lapse video recording could be due to the used technique, which is less accurate for detection of short duration behaviour. Das et al. (1999) showed that drinking bouts are very short, lasting approximately one minute or less. Accordingly, the use of time - lapse video (8 - fold) recording in the present studies might not allow identifying such short drinking bouts with high accuracy. Arnold - Meeks and McGlone (1986) observed that behaviour patterns that occurred for very short durations could not be accurately recognized at faster speeds compared with long - time activities such as lying, sleeping, and eating. In addition, observations of drinking behaviour do not account for other sources of water such as water from ingested feed.

Infrared thermography

In the shearing investigation (Chapter 4), sheep were shorn by a shearing machine that left a residual fleece with the length of 1.1 ± 0.2 cm compared to 10.6 ± 1.2 cm before shearing. This reduction of fleece coverage allowed the ewes to dissipate a higher rate of the internal metabolic body heat to the environment through the body surface and the thermal windows, in particular at the rump and leg by conduction, convection and

radiation. Mccafferty (2007) reported that the surface temperature of a mammal will not only be influenced by its skin temperature but also by the thickness, density and quality of hair covering different parts of the body and this may differ between individuals.

Animal coats, which have low heat capacities, can give false surface temperature readings if these are measured using conventional probes that are attached to the animal body. Because body surface temperature is influenced by both body and ambient temperature it would be generally possible to measure core body temperature of an animal using this methodology to understand thermal responses of animals to a range of environmental conditions. In addition, infrared thermography allows the identification of the main sites of heat loss from the body.

Conclusions

From our and previous studies, it can be concluded that water intake and turnover is variable and is influenced by species, breed, physiological state of the animal, prevailing environmental condition (temperature, humidity wind…etc.), length of water deprivation, dry matter intake, moisture content in feed, presence of the coat and shearing time. The differences in water turnover between sheep and goats were reported by many workers and can be attributed to the physiological characteristics of goats as desert animals exhibiting mechanisms of water economy indicating the superiority of goats in tolerating harsh environmental conditions. The results of our studies underline that the D_2O dilution method is not limited to the laboratory or captive animals and could be used on free - living animals in their natural conditions.

However, the present results underline the role of coat morphology on water requirement for thermoregulation. Thus, we found that shorn sheep are more efficient in thermoregulation compared with wooly unshorn sheep.

The D_2O dilution technique, infrared thermography, and video recoding are useful techniques to understand animal reactions to their environment. In general, it can be concluded that animals use behavioral adaptations in combination with physiological and morphological mechanisms for the maintenance of temperature and water balance.

Most studies tend to focus on one species, rather than involving two species simultaneously. In this context, comparative experimentation on water intake provides a better understanding of the productivity and sustainability in sheep and goats under different environmental condition and physiological status. This comparative approach is justified by the fact that goats and sheep are often herded and managed together in the developing countries, and that their feeding and nutrition are quite complementary. Thus, it is important to consider the response of each species to different challenges in order to select the most suitable one for production in a certain environment. Based upon the results of these experiments several research topics warrant future investigation. One topic would be to clarify the genotype differences in their water intake. Other topics would include the use of different animals with different physiological condition (dry, lactating, or milking animals).

The non - invasive nature of infrared thermography technique will continue to provide the basis of future applications and previous studies show that IR can be used to answer many interesting research questions. In addition, it allows to examine thermoregulation of mammals under their natural conditions.

The D_2O dilution technique estimated individual water intake in sheep and goats under stall - feeding conditions with high accuracy that makes it a reliable technique under free ranging conditions.

Drinking behaviour is considered as an important monitoring technique for animal welfare and reaction of animals to different watering regimes to describe the relationship between their behaviour and amount of water ingested.

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Appendix

Curriculum vitae

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2006 – 2007	Researcher, Livestock and Rangeland Directorate.
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