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EFFECTS OF BACTERIAL LIPOPOLYSACCHARIDE AND DEHYDRATION ON FEEDING AND METABOLISM IN BOVINES

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1. SUMMARY

The present thesis studied two situations which negatively affect the productivity of bovines, i.e. Gram-negative bacterial infection simulated by intravenous infusion of bacterial lipopolysaccharides (LPS) and dehydration.

Gram-negative bacteria are responsible for many diseases in cattle (e.g. diarrhoea, respiratory diseases, mastitis). These infections can be simulated in healthy animals by infusion of LPS, which are the major cell wall components of Gram-negative bacteria. LPS trigger the local and systemic reactions observed in the infected organism. The systemic reactions are commonly referred to as acute phase response (APR) and are caused by mediators (especially proinflammatory cytokines) produced primarily by the host's macrophages. The major behavioral symptom of the APR is hypophagia.

Water scarcity is common in several regions of the world. It causes important losses in bovine production because bovines are less well adapted to drought than other ruminants. But dehydration can also occur under normal husbandry conditions, for instance in relation to diarrhoea or rumen acidosis. Continuing water losses via the digestive tract trigger extracellular dehydration followed by cellular dehydration. Dehydration is also accompanied by hypophagia.

The first study examined the effects of LPS on food intake and metabolism in heifers. One aim was to distinguish and better characterize the feeding suppressive and metabolic effects of LPS. Six heifers were infused intravenously for 100 min with either (1) LPS (2 μg/kg body weight) with food ad lib, (2) saline with food ad lib, or (3) saline with pair-feeding to the LPS treatment. Food intake, body temperature, plasma levels of various metabolites and hormones, as well as the respiratory quotient were measured.

LPS reduced food intake and induced pronounced changes in energy turnover, and fat and carbohydrate metabolism that were largely independent of the concomitant food intake reduction. Some of the metabolic changes were biphasic: the first phase resembled a stress response and the second phase reflected a beginning energy deficit. Many of the initial metabolic responses occurred prior to and therefore independent of an increase in circulating tumor necrosis factor.

The second study investigated how lactating cows are able to cope with a sustained water restriction. In a first experiment body weight and meal patterns were recorded with ad libitum access to water and during 8 days of 25 and 50% water restriction relative to ad libitum intake. In a second experiment, indirect calorimetry was combined with nitrogen and energy balance and plasma hormone and metabolite measurements to assess the effects of 50% water restriction on digestion and metabolism.

Food intake and body weight declined during the first 3 days of water restriction depending on the restriction level and stabilized thereafter at a lower level. Nitrogen balance became negative because, relative to intake, nitrogen excretion via urine and milk was higher. In contrast, energy balance was not affected by 50% water restriction. The lower energy intake during 50% water restriction was compensated by a lower milk production, a higher digestibility of organic matter and energy, and, apparently, a more efficient energy utilization. Through these changes and a preserved water balance the cows reached a new equilibrium at a lower water turnover level, which enabled them to cope with a sustained water restriction of 50%. The loss of nitrogen could become limiting for long-term adaptation to water scarcity.

The third study comprised three experiments with non-lactating, rumen-fistulated cows. The aim of this study was to assess the role of rumen fluid hypertonicity in dehydration-induced hypophagia. First, the course of rumen fluid and plasma osmolality before and after an individual test meal was recorded when water was offered ad libitum and on the fifth day of a 65% water restriction period. Then, the effects of intraruminal water infusions on food intake were examined in dehydrated cows. Finally, an attempt was made to pharmacologically inactivate the ruminal osmosensors potentially involved in dehydration-induced hypophagia.

Water restriction reduced test meal size and increased rumen fluid and plasma osmolality. Despite the smaller meal, the prandial increase in rumen fluid osmolality was more pronounced during water restriction than with water ad libitum. Independent of treatment, the test meal had no effect on plasma osmolality. Intraruminal water infusions during water deprivation normalized food intake. Injection of a local anesthetic into the rumen did not attenuate dehydration-induced hypophagia. These

results suggest that rumen fluid hypertonicity, perhaps in interaction with plasma osmolality, contributes to the early satiation induced by dehydration.

Both situations studied here, LPS administration as a model of bacterial infection and dehydration, are two examples of complex homeostatic mechanisms in ruminant animals. The hypophagia observed in both cases is beneficial for the organism. Hypophagia induced by LPS administration is an active host defense mechanism against pathogens that improves the short term chances for survival. On the other hand, dehydration-induced hypophagia must probably be considered as a compromise between nutrient intake and maintenance of the osmotic balance to save the maximum of water.

2. RESUME

Le sujet de cette thèse s'est concentré sur l'étude de deux situations qui affectent négativement la productivité des bovins: les infections par bactéries Gram-négatives, simulées ici par une intraveineuse de lipopolysaccharides (LPS) bactériens, et la déshydratation.

Les infections dues aux bactéries Gram-négatives provoquent des maladies importantes chez les bovins (diarrhées, maladies respiratoires, mammites, ...). Ces infections peuvent être simulées par l'injection de LPS, les constituants pariétaux principaux des bactéries Gram-négatives. Les LPS provoquent chez l'organisme infecté des réactions locales et systémiques. Les réactions systémiques (‚acute phase response'), sont le résultat de médiateurs (entre autres, des cytokines) produits par les macrophages de l'hôte infecté. L'une de ces réactions est une baisse de l'ingestion (hypophagie).

Le manque d'eau est courant dans de nombreuses régions du globe. Les bovins, moins bien adaptés que les autres ruminants, y sont particulièrement sensibles et essuient de grosses pertes. Sous nos latitudes, des raisons pathologiques comme la diarrhée ou l'acidose du rumen peuvent aussi provoquer des déshydratations chez les ruminants. Les pertes d'eau continuelles par le tractus gastro-intestinal causent d'abord une déshydratation extracellulaire, puis une déshydratation cellulaire. L'un des symptômes de la déshydratation est également l'hypophagie.

Dans la première étude, il s'agissait de voir par quels mécanismes les LPS agissent sur l'ingestion et le métabolisme de génisses, et de distinguer et de mieux caractériser l'effet hypophagique et les effets métaboliques. Trois traitements (perfusions) ont été appliqués en chambre de respiration: 1) LPS (2 µg/kg PV) avec fourrage à volonté 2) solution saline avec fourrage à volonté, ou 3) solution saline avec la quantité de fourrage limitée à ce qui avait été ingéré en 1).

La perfusion de LPS a engendré une baisse de l'ingestion, ainsi que des réactions marquées dans le métabolisme de l'énergie, des lipides et des hydrates de carbone. Plusieurs des paramètres sanguins mesurés ont évolué de manière biphasique, la première phase ressemblant à une réaction de stress, la deuxième plutôt à un déficit énergétique. Ces réactions métaboliques étaient largement indépendantes de

l'hypophagie. La plupart d'entre elles devenaient visibles avant que le taux de 'tumor necrosis factor α' n'augmente dans le sang. Ce dernier ne semble donc pas en être responsable.

Le but de la seconde étude était de voir en détails comment des vaches laitières réagissent à une restriction d'eau. Dans un premier essai, l'évolution de leur poids corporel et de leur comportement d'ingestion a été observé avec de l'eau à volonté, puis pendant 8 jours alors que l'eau disponible avait été réduite de 25% et de 50%. Dans un deuxième essai, des bilans azotés et énergétiques, accompagnés d'analyses sanguines, ont été réalisés en chambre de respiration, avec de l'eau à volonté, puis réduite de moitié.

Lors des 3-4 premiers jours de restriction, l'ingestion et le poids corporel des vaches ont diminué, puis ils se sont stabilisés. Le bilan azoté est devenu négatif en raison d'une augmentation des excrétions azotées via l'urine et le lait, proportionnellement à la quantité d'azote ingérée. Par contre, le bilan énergétique n'a pas été affecté par la restriction d'eau. La baisse de l'apport énergétique a été compensée par une diminution de la production laitière, une meilleure digestibilité de la matière organique et de l'énergie et apparemment par une utilisation plus efficace de l'énergie. Le bilan hydrique est resté inchangé, signe que les vaches avaient retrouvé un état métabolique plus économique et équilibré. Seules les pertes d'azote auraient pu nuire à long terme à l'adaptation de ces vaches laitières à une restriction d'eau de 50%.

La troisième étude a examiné le rôle de l'osmolalité ruminale dans l'hypophagie due à la déshydratation chez des vaches taries et fistulées au niveau de la panse. Le premier essai a décrit l'évolution des osmolalités ruminale et sanguine avant et après l'ingestion d'un repas test individuel avec de l'eau à volonté et au cinquième jour d'une période de restriction d'eau de 65%. Un deuxième essai a étudié les effets sur l'ingestion d'injections d'eau directement dans la panse via la fistule. Dans un troisième essai, une tentative a été faite d'inactiver par anesthésie locale des récepteurs osmotiques ruminaux éventuellement responsables de l'hypophagie.

La restriction d'eau a réduit la taille du repas test et augmenté les osmolalités ruminale et sanguine. Malgré le repas moins important, la hausse de l'osmolalité ruminale était plus prononcée dans le traitement avec moins d'eau que dans celui avec de l'eau à volonté. Le repas test n'a eu aucun effet sur l'osmolalité sanguine,

indépendamment du traitement. Les injections d'eau intraruminales, alors que les abreuvoirs étaient fermés, ont normalisé l'ingestion. Les injections intraruminales d'anesthésique n'ont pas atténué l'hypophagie. Ces résultats indiquent que l'hypertonicité du rumen joue un rôle dans l'hypophagie due à la déshydratation, peut-être en interaction avec l'osmolalité sanguine.

L'étude de ces deux situations, infection de LPS et déshydratation, sont deux exemples de la complexité de l'homéostase chez les ruminants. L'hypophagie constatée dans les deux cas est bénéfique pour l'organisme. L'hypophagie induite par la perfusion de LPS est un moyen actif de défense de l'hôte contre le pathogène, conduisant à court terme à de meilleures chances de survie. L'hypophagie induite par la déshydratation représente probablement un compromis entre l'ingestion de nutriments et le maintien de l'équilibre osmotique des fluides pour économiser le plus d'eau possible.

3. INTRODUCTION

Worldwide bovines can be exposed to various suboptimal husbandry conditions and/or stressful situations that negatively affect their productivity. Two of these conditions were examined here, i.e. Gram-negative bacterial infection, simulated in healthy animals by infusion of bacterial lipopolysaccharide (LPS), and dehydration, modeled by either long-term water restriction or short-term water deprivation.

3.1. Infections

Practical importance

Gram-negative bacteria are responsible for many diseases in cattle (Cullor, 1992). In calves Gram-negative bacteria are potential pathogens of the diarrhoea complex (e.g. neonatal coliform septicemia, salmonellosis, campylobacteriosis) which is the most frequent disease in calves younger than 8 weeks in Switzerland (Stärk et al., 1997). In feedlot cattle, respiratory diseases are often problematic. They can also be caused by Gram-negative bacteria (e.g. pneumonia caused by *Pasteurella* and *Actinobacillus*). In dairy cows, Gram-negative bacteria cause mastitis (*E. coli*, *Enterobacter*, and/or *Pseudomonas*) as well as metritis. The study mentioned above (Stärk et al., 1997) also shows that diseases of the mammary gland and low fertility (of course not only caused by Gram-negative bacteria) are the most important problems in Swiss dairy herds. The clinical features associated with Gram-negative bacterial infections appear to be consequences of the host reaction to bacterial cell wall components such as LPS (Cullor, 1992).

LPS are also present in the environment, stemming from the growth of Gram-negative bacteria on plants and other sources of animal feeds. Therefore, animals can be exposed to LPS not only by Gram-negative bacterial infection, but also – at least in pathological cases when the barrier function of the intestinal epithelium is compromised (Bahrami, 1996) – by the translocation of LPS from LPS-contaminated fodder and from Gram-negative bacteria residing in the gastrointestinal tract. In ruminants the rumen fluid contains large amounts of LPS, which increase when animals are fed on

concentrates instead of hay (Janosi et al., 1998). Increased LPS translocation from the forestomachs has been demonstrated in complex disease syndromes such as ruminal acidosis, ruminal stasis and parturient paresis in the cow (Janosi et al., 1998).

Pathophysiological background

Acute microbial infections (bacteria, viruses, fungi) and/or contact with LPS trigger a generalized host defense reaction that is commonly referred to as acute phase response (APR). The APR comprises metabolic, immune, endocrine, neuronal and behavioral reactions (Langhans, 1996; Exton, 1997). The systemic physiological changes of the APR include fever, increased protein synthesis in the liver, enhanced muscle proteolysis, negative nitrogen balance, stimulation of cytokine production, leucocytosis, lowered serum levels of iron and zinc, and often a stimulation of the pituitary-adrenal axis. The behavioral changes include lethargy, inactivity, somnolence, and a reduction of food intake, which is one major focus of this thesis. All these changes are not caused directly by the infective agents or by LPS, but are indirect consequences of a cascade of biological reactions of the host's immune system initiated by infective agents or bacterial products (Janosi et al., 1998). For instance, *in vitro* or *in vivo* exposure of neutrophils, phagocytic cells and platelets to LPS results in an increased synthesis and release of many biologically active substances, in particular various proinflammatory cytokines (e.g. tumor necrosis factor-α [TNFα], interferons, interleukins), certain arachidonic acid-derived lipid-like molecules (eicosanoids), such as prostanoids (prostaglandins and thromboxanes) and leukotrienes, biogenic amines (serotonin, histamine), and oxygen radicals (nitric oxide, hydrogen peroxide, superoxide) (Janosi et al., 1998). All these substances are endogenous intercellular communication molecules (mediators) primarily involved in the process of local inflammation. They are synthesized and released from one cell group and act on other cells through specific receptors (Henson and Murphy, 1989). The exact role of the particular substances is difficult to define because all are part of a network of mediators that control inflammation. Generally, it can be said that these substances have vasoactive, chemotactic, and/or cytotoxic functions; they can also affect cell adherence and synthesis of other mediators (Henson and Murphy, 1989).

In addition to the local effects and depending on the intensity and duration of the immune challenge as well as on the state of the host's immune system, the mediators also trigger the systemic APR mentioned above (Dargel, 1995). Again, it is hard to identify the exact role of a single mediator in triggering the symptoms of the APR, in particular because the interactions between the various mediators are not sufficiently characterized. This holds especially for the feeding suppressive effect. One aim of this thesis is to enhance our knowledge in this area.

3.2. Dehydration

Practical importance

In many areas of the world ruminants routinely have to cope with periods of dehydration. The regions mostly threatened by droughts are Africa (with 44% of land more or less regularly subjected to droughts), South Asia (43%), Central America (32%), Australia (28%) and South America (17%) (Barrow, 1987). In some areas there is theoretically enough rainfall, but the precipitations show a markedly seasonal distribution. Water availability is therefore greatly limited at certain times of the year (Jackson, 1989).

Table 1. *Animal specific characteristics related to drought adaptation*

	camel	cattle	sheep	goat
Walking potential (km)	> 50	< 40	< 40	15
Drinking interval (d)	5 – 9	1 – 2	2 – 3	1 – 2
Consumption of wooden vegetation	+++	-	+	+++
Consumption of grass vegetation	++	+++	+++	++

(Source: von Schutzbar, 1995)

The farm animals mostly used in the Tropics are camels, cattle, sheep and goats. Among the ruminants, cattle have the greatest economical and social value, but are not the best adapted to water scarcity (Table 1). Cattle do not eat wooden vegetation and must be watered at least every second day. Therefore, losses due to water- and food

shortages induced by droughts are most important in cattle (von Schutzbar, 1995). The vulnerability of cattle in Third-world countries was enhanced since several years with the import of European type animals. These animals are used to increase productivity, but are less adapted to the climate (Syrstad, 1991).

Also under common farming conditions in Europe, water intake may at times be insufficient: for example in young animals shortly after weaning, when it can take days before the level of water intake reaches that in milk; or on alpine pasture, where water availability or accessibility can also limit food intake and contribute to the negative energy balance and the milk yield depression observed (Christen, 1992) in dairy cows. Water intake might even be critical during early lactation in high yielding dairy cows, when fluid balance is pushed to its limits. In addition to these "natural" situations, there are pathological situations which can also trigger a more or less severe dehydration. Diarrhoea especially in young calves is accompanied by a dramatic increase in the water content of the feces and can rapidly lead to death by dehydration if no rehydration therapy is applied (Institut de l'élevage, 1994). Moreover, metabolic diseases such as rumen acidosis can also lead to a dehydration-like state. An imbalance in the ruminal microflora that favors the lactate-producing bacteria (for example when too much concentrates are fed) increases the rumen osmolality and causes an inflow of water into the rumen (Institut de l'élevage, 1994). In these two last situations systemic dehydration is not derived from a reduced water intake. Rather, the enhanced water losses are the reason for dehydration.

These few examples show that a lack of water due to external or internal unavailability is a practical problem that warrants further attention.

Physiological background

Water is an essential component of the organism that has several functions in the body, as a solvent for biochemical reactions, as a transport medium, for the maintenance of cell structures, and for thermoregulation. Body water accounts for approximately 65% of body mass and is stored in 3 different compartments (Grossman, 1990):

-the intracellular compartment, which contains about 2/3 of the total body water;

-the interstitial compartment (about 80% of extracellular water), with the fluid that forms a thin film on the outside of each cell and fills the space between adjacent cells;
-the vascular compartment (20% of extracellular water), which contains the blood plasma.

The intracellular and the extracellular compartments are separated by a semipermeable membrane. The exchanges of body fluids across the cell membrane happen by diffusion. There is a net movement of water when the concentration of solutes that cannot cross the cell membrane differs between the intra- and extracellular environment. In this case water will move into the region of higher concentration until the differences are eliminated (=osmosis).

Water enters the organism via the gastrointestinal tract, where it is absorbed into blood plasma. From here, water and solutes diffuse into the interstitial spaces so that the entire extracellular fluid becomes diluted. This creates an osmotic pressure gradient, which results in the movement of water into the more concentrated cellular fluid compartment.

During water deprivation or in the pathological cases mentioned above, this process can be reversed. In case of water deprivation water continues to be lost from plasma because of milk production, salivation, urination, digestive needs and evaporation from skin and lungs. In case of diarrhoea or rumen acidosis, the water losses via the digestive tract are abnormally high. This produces an increase in the concentration of solutes in plasma. As a result, water is drawn into venous capillaries from the interstitial fluid, which in turn becomes increasingly concentrated and thus promotes the efflux of cellular water into the extracellular space. In the short term, the movement of water from the cells into the extracellular compartment is sufficient to postpone a severe drop in plasma volume (Grossman, 1990). With a long lasting lack of water, the inevitable drop in plasma volume and blood pressure activate additional compensatory mechanisms (e.g. the renin – angiotensin system) and can ultimately be fatal.

In comparison to monogastric animals, in which the kidneys play the dominant role in fluid and electrolyte homeostasis, ruminants have a more complex regulation (Silanikove, 1994). The rumen, with a mean fluid capacity of about 182 L in bovines (Ewing and Cole, 1994), must be considered as a water reservoir that can be partly used

when drinking water is in short supply (Silanikove, 1994). This characteristic feature explains the capacity of the ruminants to withstand a greater degree of dehydration than most monogastric animals. Another feature of ruminants is the huge secretion of saliva. Mainly depending on the diet consumed, dairy cows can produce up to 250 L saliva per day (Silanikove, 1994). The amount of water and sodium secreted with saliva per day is therefore equivalent to about 5 times that of plasma content. Yet, this huge saliva secretion does not require a proportionally higher water intake because the secreted saliva is reabsorbed and recycled. Despite of these particularities ruminants react like non ruminants when water is scarce, i.e. they both reduce their food intake.

3.3. Hypophagia

A reduction in food intake (hypophagia) is common to both situations studied in this thesis, bacterial infection and dehydration. Hypophagia can be characterized by describing the animals' feeding behavior (meal pattern analysis). Animals eat during distinct periods of time (meals) which may include short breaks, but which are separated from other meals by longer intervals (intermeal intervals) (Forbes, 1995). A reduction in food intake can therefore occur through either a decrease in meal frequency, and/or through a decrease in meal size. Meal pattern analysis during hypophagia can help to understand the mechanisms involved because meal frequency and meal size appear to be in part controlled by different mechanisms (Langhans and Scharrer, 1992)

Hypophagia is a nonspecific phenomenon often observed during disorders of numerous physiological systems (Porter, 1998). The mechanisms which trigger the hypophagia can be different from situation to situation and are often not well known. This holds particularly for ruminants. The major goal of this thesis is therefore to learn more about the mechanisms of hypophagia during bacterial infection and dehydration in ruminants.

3.4. Questions examined in this thesis

The first study deals with the effects of Gram-negative bacterial infection on food intake and metabolism. The infection was simulated by a prolonged low-dose LPS infusion in heifers. This first study was designed to distinguish the direct metabolic effects of LPS from secondary effects due to the LPS-induced reduction in food intake.

The second study analyzed the effects of a long-term water restriction of 8 and 9 days on meal patterns and metabolism in lactating cows. The specific aims were to identify and quantify the adaptation and compensation mechanisms to the dehydration-induced reduction in food intake.

The third study addressed the possible role of rumen fluid and plasma hypertonicity in dehydration-induced hypophagia.

4. EFFECT OF A PROLONGED LOW-DOSE LIPOPOLYSACCHARIDE INFUSION ON FOOD INTAKE AND METABOLISM IN HEIFERS

based on:

Steiger, M., M. Senn, G. Altreuther, D. Werling, F. Sutter, M. Kreuzer and W. Langhans. 1999. *J. Anim. Sci.* 77: 2523-2532.

4.1. Introduction

Administration of lipopolysaccharides (LPS) at various doses and by various routes produces the generalized pathophysiological responses that usually accompany bacterial infections, including a decrease in food intake and disturbances of carbohydrate, protein, and fat metabolism (Lohuis et al., 1988; Cullor, 1992). The known metabolic effects of LPS are similar in ruminants and non ruminant laboratory animals, which are more often used in such experiments, but the mechanisms underlying the LPS-induced changes in food intake and metabolism are still largely unknown. Moreover, the effects of LPS on food intake and metabolism are usually examined separately, i.e. the metabolic effects of LPS are often tested in food deprived animals (Takeuchi et al., 1997), and they are largely ignored in experiments which focus on the feeding suppressive effects of LPS (Van Miert et al., 1988). Because of the impact of food intake on metabolism, the feeding and metabolic effects of LPS could in fact scarcely be differentiated in most experiments. The aim of the present study was to characterize and distinguish the feeding suppressive and metabolic effects of LPS, in order to better understand the pathophysiology of systemic Gram-negative bacterial infections in ruminants. Therefore, an additional control treatment consisted of saline infusion with feeding restricted to the amount of food consumed after the LPS infusion. The effects of LPS on carbohydrate and fat metabolism were characterized by indirect calorimetry traits and determination of plasma metabolites and hormones. The plasma level of tumor necrosis factor-α (TNFα) was also measured because TNFα is considered to be a major mediator of LPS effects (Beutler and Cerami, 1986). A prolonged low dose infusion of LPS was used, which better simulates the effects of a

severe Gram-negative bacterial infection than single bolus injections (Werling et al., 1996).

4.2. Materials and Methods

Animals

Six healthy heifers (Holstein x Jersey, 311 kg ± 5 kg body weight [mean ± SEM]) received each of the following 3 treatments: 1) LPS infusion with free access to food, 2) saline infusion with free access to food, and 3) saline infusion with feeding restricted to the amount of food consumed in response to the LPS infusion. Each animal began the experiment with the saline or LPS infusion, which were randomized and separated by at least 2 days. Two catheters (Cavafix Certo, B. Braun AG, Melsungen, Germany) were inserted into the left and right jugular veins (one for infusion, one for blood sampling). Ninety min later, the animals were randomly placed for 24 h in one of two respiration chambers. Infusions (1 animal saline, 1 animal LPS) began 90 min after the animals had entered the chambers. Sterile 0.9% saline (NaCl 0.9%, B. Braun Medical AG, Emmenbrücke, Switzerland) or LPS solution (E.Coli 0111:B4, Sigma Chemicals Co, St Louis, MO) were infused (2 μg/kg body weight, 1 mL/min) for 100 min. LPS was freshly dissolved (2 μg/100 mL) in sterile saline just prior to the experiment. The saline infusion plus restricted feeding was done after all animals had received the saline and LPS infusions. Prior to the first saline or LPS infusion, the animals were familiarized with the conditions in the chambers by putting them for 48 h into stalls about the same size that resembled the chambers. All experimental procedures were approved by the Kanton of Zug Veterinary Office.

Feeding

The animals were fed a mixed ration consisting of corn silage (150 g/kg), grass silage (450 g/kg), hay (250 g/kg), and straw (150 g/kg). The dry matter (DM) was 646 g/kg, crude fiber 272 g/kg DM, protein 126 g/kg DM, and net energy for lactation 5.5 MJ/kg DM). To allow the exact recording of food intake in the chambers, food was offered as a complete mixture in separate portions of 3 kg each at time 0 (start of infusion), at 100 min (end of infusion), 4 h, and 6 h. An additional 6 kg of food was

offered at 10 h. For saline infusion with restricted feeding, the pre-determined amounts of food were placed into the troughs at the same time points. The animals were adapted to the ration and to the feeding protocol for at least 10 days prior to the experiment. Tap water was continuously available ad libitum.

Blood samples and rectal temperature

Blood samples were taken every 30 min from 1 h prior to infusion until 6 h after infusion onset. Additional blood samples were obtained at 10 and 22 h. The first 5 mL of each blood sample were discarded. The subsequent 10 mL were drawn into sterile EDTA-tubes (K_2-EDTA vacuum tubes) mixed and immediately placed on ice. After each blood sampling, the catheters were filled with heparin solution (Liquemin, Roche Pharma AG, Reinach, Switzerland) and rectal temperature was measured.

Blood samples were centrifuged immediately at 4°C. The plasma was frozen and stored for 1 wk at -20°C, and then at -70°C until analysis. Plasma glucose, lactate, free fatty acids (FFA), β-hydroxybutyrate (BHB), triglycerides, and glycerol were measured enzymatically with an automatic analyzer (Cobas-Mira, Hoffmann-LaRoche, Basel, Switzerland). Commercially available test kits were used for the determination of plasma cortisol (Diagnostic Products Corp., Los Angeles, CA) and insulin (Pharmacia, Uppsala, Sweden). Plasma tumor necrosis factor-α (TNFα) was determined using the murine fibroblast cell line L929 as described previously (Werling et al., 1996). All these variables were selected for the sake of compatibility with a previous study under similar conditions (Werling et al., 1996), and because of their potential to reflect the LPS effects according to our previous findings (Werling et al., 1996). All analytical procedures are well established in our laboratory and have been validated for their use in ruminants.

Respiratory measurements

Gaseous exchange was measured in the respiration chambers for 24 h. Open circuit calorimetry was used to measure oxygen consumption and the output of carbon dioxide and methane. The two chambers were air conditioned (60.6% relative humidity, 16°C air temperature, and 16.9 m^3/h air flow). Gaseous composition of the

air flowing into and out of the chambers (internal volume: 20 m^3, each) was measured using infrared analyzers (Binos, Leybold-Heraeus, Zurich) for carbon dioxide and methane, and a paramagnetic analyzer (Oxymat 3, Siemens AG, Dietikon) for oxygen. The total volume of air leaving each chamber was recorded with in-line electronic flow meters (Swingwhirl DV 630, Flowtec AG, Reinach). For a detailed description of the chambers see Sutter (1993). The whole system was manually calibrated prior to the onset of the respiratory measurements, and then automatically every 3 h, including one final calibration at the end of the 24-h period. Calibrations lasted about 9 min. Heat production of each animal was estimated from the daily rates of oxygen consumption and carbon dioxide and methane production according to the following equation (Brouwer, 1965): Heat production [kJ] = 16.179 x O_2 [L] + 5.022 x CO_2 [L] - 2.168 x CH_4 [L]. Urinary nitrogen losses were not considered. The respiratory quotient (RQ) and heat production measurements were cumulated over 30 min periods for statistical evaluation and presentation.

Statistics

The Friedman Two-Way analysis of variance by ranks test was used for the paired, non-parametric evaluation of overall treatment and time effects on all variables measured, because we used a pseudo Latin square design, and data were often not normally distributed. This test allows for the analysis of repeated observations on the same experimental unit with a small n-value. When the overall effects were significant, the Wilcoxon signed rank test was used for pairwise comparisons between treatments and for judging changes over time within treatments. The latter was done for each variable by selectively comparing baseline with 1.5 h values (close to the end of infusions) and/or with maximum or minimum values recorded at any time thereafter. Although the saline infusion plus restricted feeding was always applied last, with this treatment the values for most variables were remarkably similar to the values with saline infusion, and the statistical analysis used accounts for potential effects of treatment order and handling in the comparison between the saline and LPS infusion treatments. Data in the text and in graphs are presented as means ± SEM (n = 6). The analyses were done with SYSTAT 5.0 (Systat Inc., Evanston, IL). P-values < .05 were considered significant.

4.3. Results

Food intake

LPS (2 μg/kg BW) infused over 100 min decreased ($P < .05$) food intake (Fig. 1). Food intake suppression reached significance at 4 h, and was not compensated within 24 h, when it still accounted for a 33% difference.

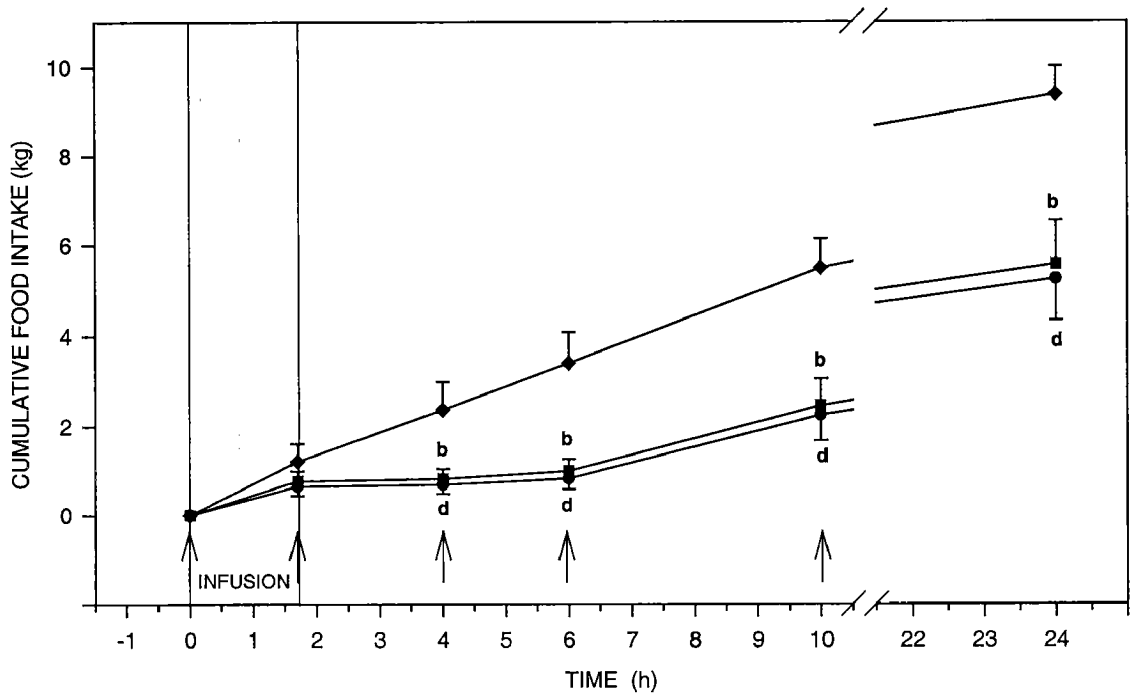

Fig. 1: Cumulative food intake of heifers in response to LPS infusion (2 μg/kg BW, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. b: LPS infusion values are lower ($P < .05$) than saline infusion values. d: Values after saline infusion and after saline infusion plus restricted feeding are different ($P < .05$).

Rectal temperature

Rectal temperature (Fig. 2) increased during the LPS infusion (0 h vs 1.5 h, $P < .05$) and reached a maximum of 39.4°C at 5 h. It was higher ($P < .05$) with LPS than with saline infusion at 1.5, 2, and 6 h. The individual variation of the rectal temperature response to LPS was considerable. With saline infusion plus restricted feeding, rectal temperature slowly decreased (-1 h vs 5 h, $P < .05$) and was lower ($P < .05$) than with saline infusion between 4.5 and 6 h. The treatment differences in rectal temperature had disappeared at 10 h.

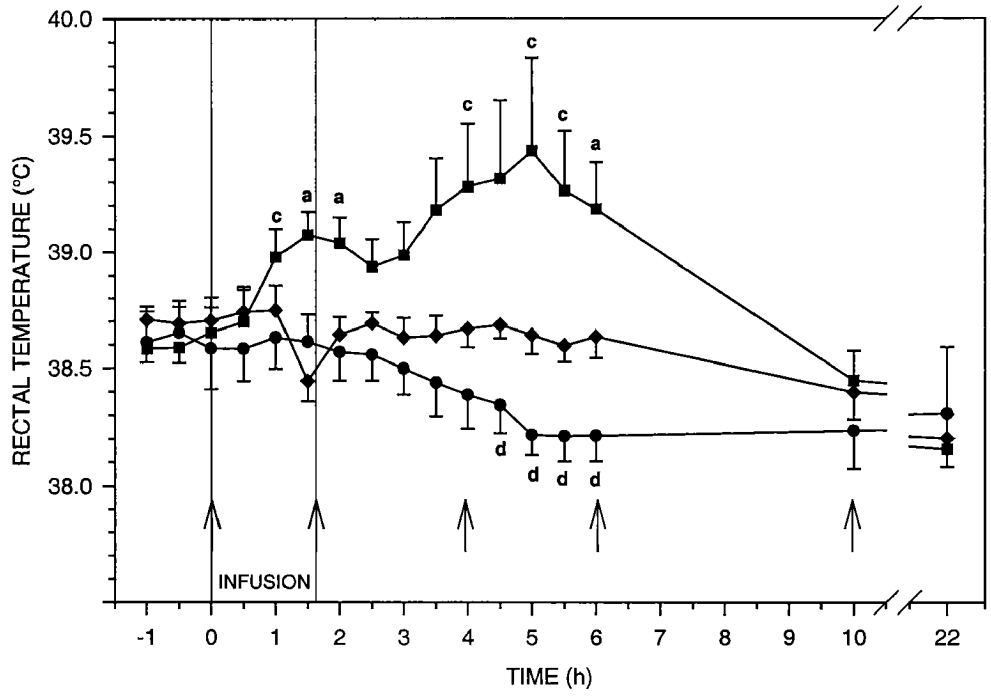

Fig. 2: Rectal temperature of heifers in response to LPS infusion (2 µg/kg BW, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($P < .05$) from the values of the two other treatments. c: LPS infusion values are different ($P < .05$) from values after saline infusion plus restricted feeding. d: Values after saline infusion and after saline infusion plus restricted feeding are different ($P < .05$).

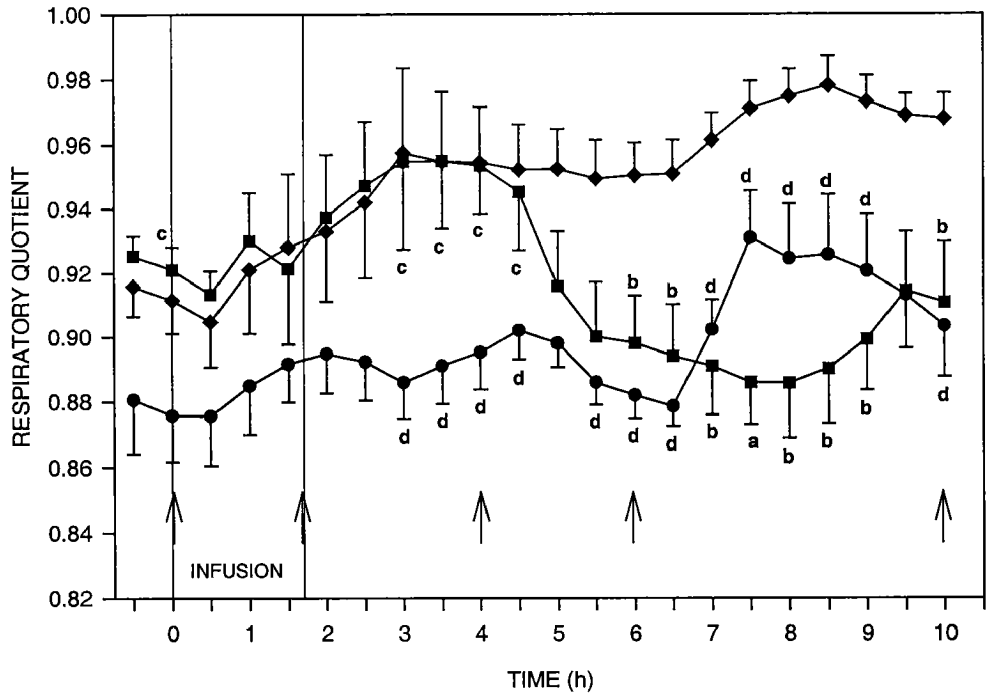

Fig. 3: Respiratory quotient of heifers in response to LPS infusion (2 μg/kg BW, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different (P < .05) from the values of the two other treatments. b: LPS infusion values are different (P < .05) from values after saline infusion. c: LPS infusion values are different (P < .05) from values after saline infusion plus restricted feeding. d: Values after saline infusion and after saline infusion plus restricted feeding are different (P < .05).

Respiratory traits

The RQ (Fig. 3) was calculated until 10 h. It increased with saline infusion (- .5 h vs 8.5. h, P < .05). With LPS infusion the RQ appeared to increase until about 3 h (not significant), and decreased thereafter (4 h vs 8 h, P < .05). With saline infusion and restricted feeding, the RQ remained comparatively low from the beginning until 6.5 h, when it transiently increased (6.5 h vs 7.5 h, P < .05). The low initial RQ with saline infusion and restricted feeding was associated with one heifer that became very excited during insertion of the catheters and had an extremely high plasma FFA concentration

on the day with saline infusion and restricted feeding. Because this animal's RQ was within 3 standard deviations of the mean, it was not excluded for presentation. Without this heifer, the mean RQ at - .5 h was .9.

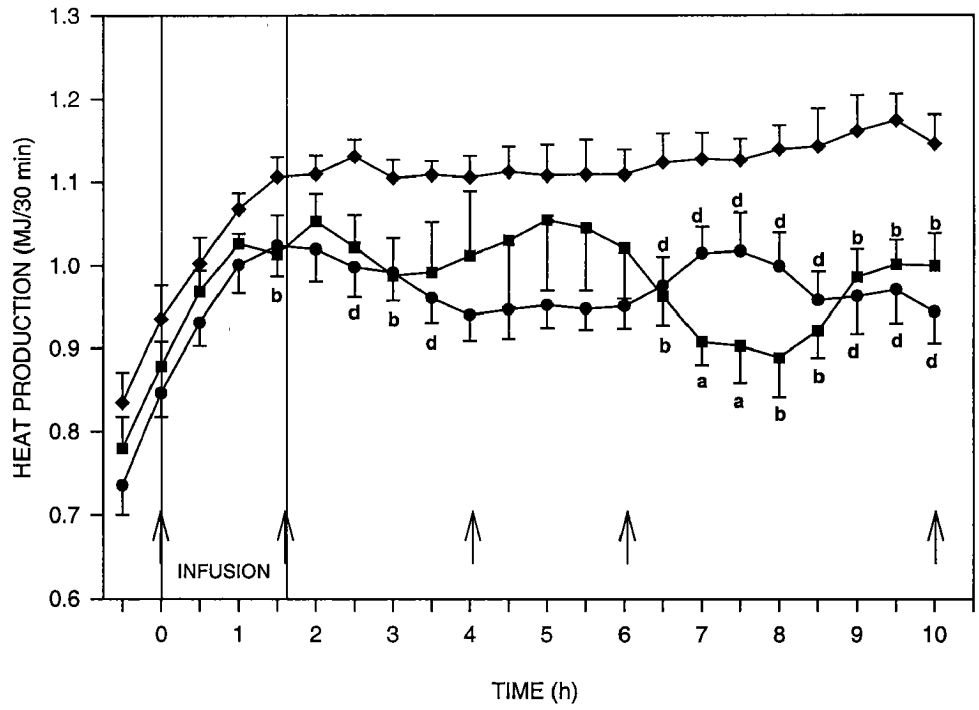

Fig. 4: Heat production of heifers in response to LPS infusion (2 μg/kg BW, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different (P < .05) from the values of the two other treatments. b: LPS infusion values are different (P < .05) from values after saline infusion. d: Values after saline infusion and after saline infusion plus restricted feeding are different (P < .05).

Heat production (Fig. 4) increased initially (- .5 h vs 1.5 h, P < .05) and reached a plateau after approximately 1 to 1.5 h with all 3 treatments. Thereafter, heat production was lower (P < .05) with LPS than with saline infusion at 1.5 h and 3 h, and from 6.5 to 10 h. In addition, heat production appeared to be higher with LPS infusion

than with saline infusion and restricted feeding from 4 to 6 h, but was lower ($P < .05$) with LPS infusion than with saline infusion plus restricted feeding at 7 and 7.5 h. With LPS infusion, the temporal evolution of heat production was similar to the rectal temperature pattern and also was highly individually variable, in particular until approximately 6 h.

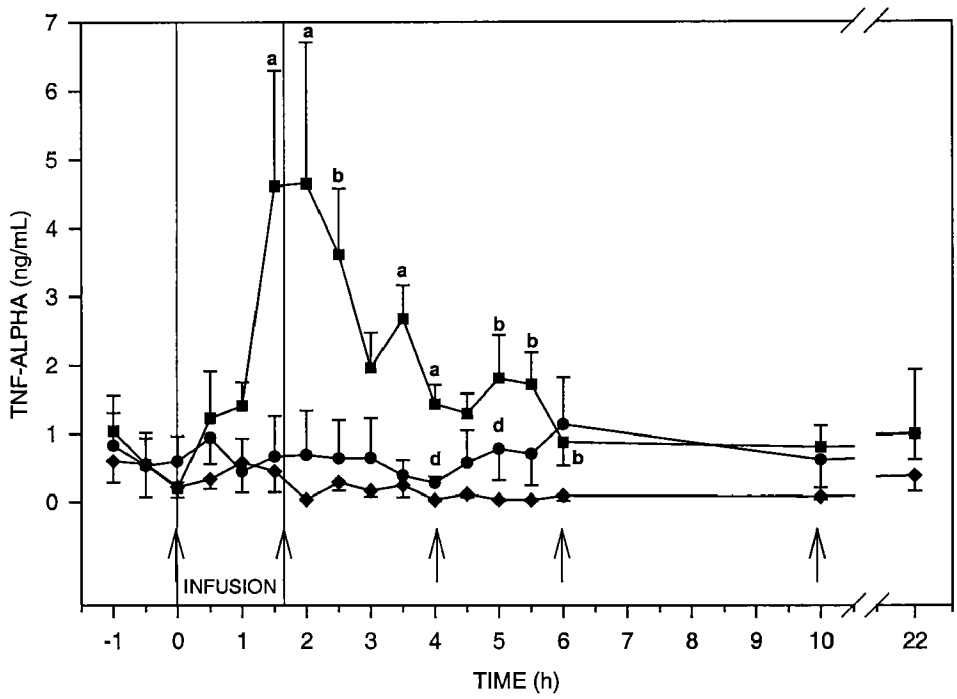

Fig. 5: Plasma tumor necrosis factor-α concentration of heifers in response to LPS infusion (2μg/kg, 1mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($P < .05$) from the values of the two other treatments. b: LPS infusion values are different ($P < .05$) from values after saline infusion. d: Values after saline infusion and after saline infusion plus restricted feeding are different ($P < .05$).

TNFα, cortisol, and insulin

The plasma concentration of TNFα (Fig. 5) sharply increased during the LPS infusion (0 h vs 1.5 h, $P < .05$) and then gradually decreased. Plasma TNFα was higher ($P < .05$) with LPS than with saline infusion or saline infusion and restricted feeding from 1.5 to 2.5, and from 3.5 to 4 h. The difference had disappeared at 10 h. Again, the individual TNFα responses to LPS varied considerably. The plasma concentration of TNFα did not systematically change over time with saline infusion and saline infusion plus restricted feeding.

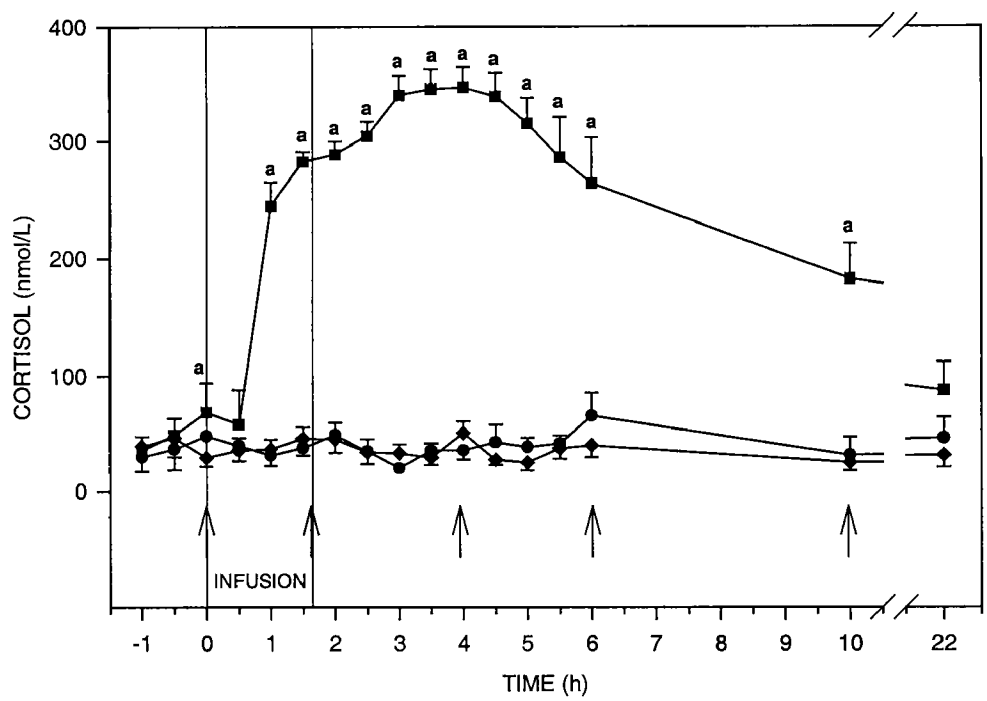

Fig. 6: Plasma cortisol concentration of heifers in response to LPS infusion (2μg/kg, 1mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($P < .05$) from the values of the two other treatments.

With LPS infusion, plasma cortisol (Fig. 6) sharply increased ($P < .05$) between .5 and 1.5 h, and was significantly higher than with saline infusion or saline infusion and restricted feeding from 1 to 10 h. Plasma cortisol did not change over time, and it did not differ between saline infusion and saline infusion plus restricted feeding.

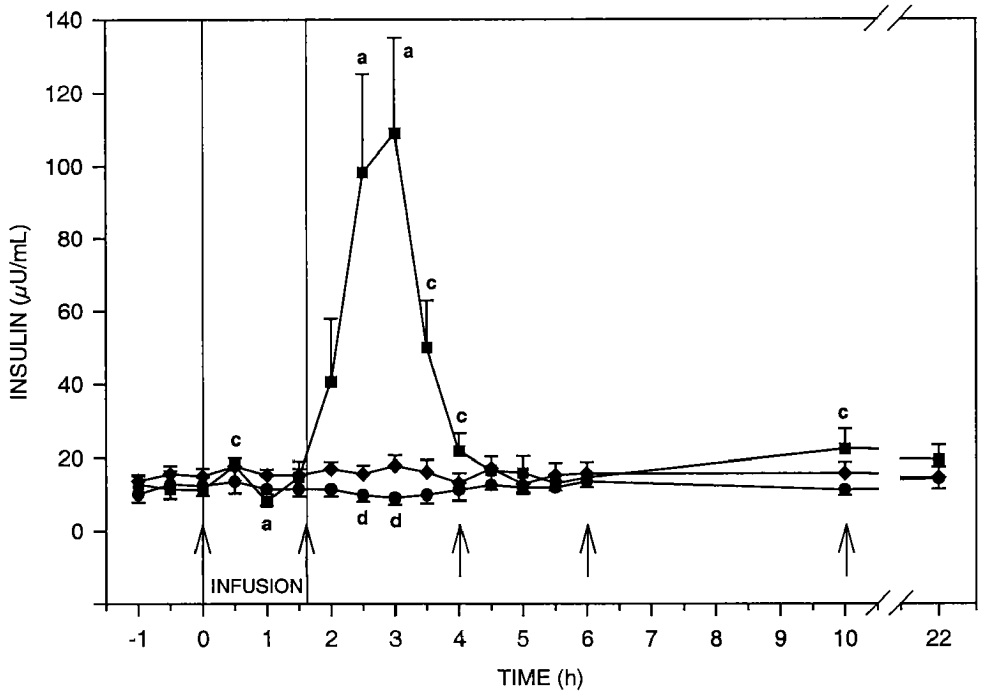

Fig. 7: Plasma insulin concentration of heifers in response to LPS infusion (2 μg/kg, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($P < .05$) from the values of the two other treatments. c: LPS infusion values are different ($P < .05$) from values after saline infusion plus restricted feeding. d: Values after saline infusion and after saline infusion plus restricted feeding are different ($P < .05$).

There was a marked, transient increase in plasma insulin (Fig. 7) with LPS infusion between 2 and 4 h (0 h vs 3 h, $P < .05$), and there was no change over time with saline infusion and saline infusion plus restricted feeding. The plasma insulin concentration was higher ($P < .05$) with LPS infusion than with saline infusion and restricted feeding from 2.5 to 4 h . Due to the low variations, plasma insulin values with saline infusion and saline infusion plus restricted feeding were significantly different at 1, 2.5, and 3 h.

Metabolites

With LPS infusion, the plasma concentration of BHB (Fig. 8) started to decrease between .5 and 1 h, reached a minimum at 3.5 h (0 h vs 3.5 h, $P < .05$), and increased again thereafter (3.5 h vs 10 h, $P < .05$). As a result, plasma BHB was lower ($P < .05$) with LPS infusion than with saline infusion and/or saline infusion plus restricted feeding from 1 to 5.5 h. Plasma BHB did not change over time with saline infusion and saline infusion plus restricted feeding, but was lower ($P < .05$) with saline infusion and restricted feeding than with saline infusion from 1 to 1.5 and from 5 to 5.5 h.

LPS caused a well pronounced biphasic increase in the plasma concentration of FFA (Fig. 9). Plasma FFA increased during the LPS infusion (0 h vs 1 h, $P < .05$), decreased thereafter (1h v. 3 h, $P < .05$) and increased again (3h vs 6 h, $P < .05$) to reach higher values than with saline infusion from 4.5 to 6 h ($P < .05$). With saline infusion and restricted feeding, the plasma concentration of FFA gradually increased (-1 h vs 10 h, $P < .05$) and was higher ($P < .05$) than with saline infusion at 4.5 h and from 5.5 to 10 h. The individual variation of plasma FFA in response to LPS was considerable. With LPS infusion and saline infusion plus restricted feeding, the plasma concentration of FFA was still higher than with saline infusion at 10 and 22 h ($P < .05$).

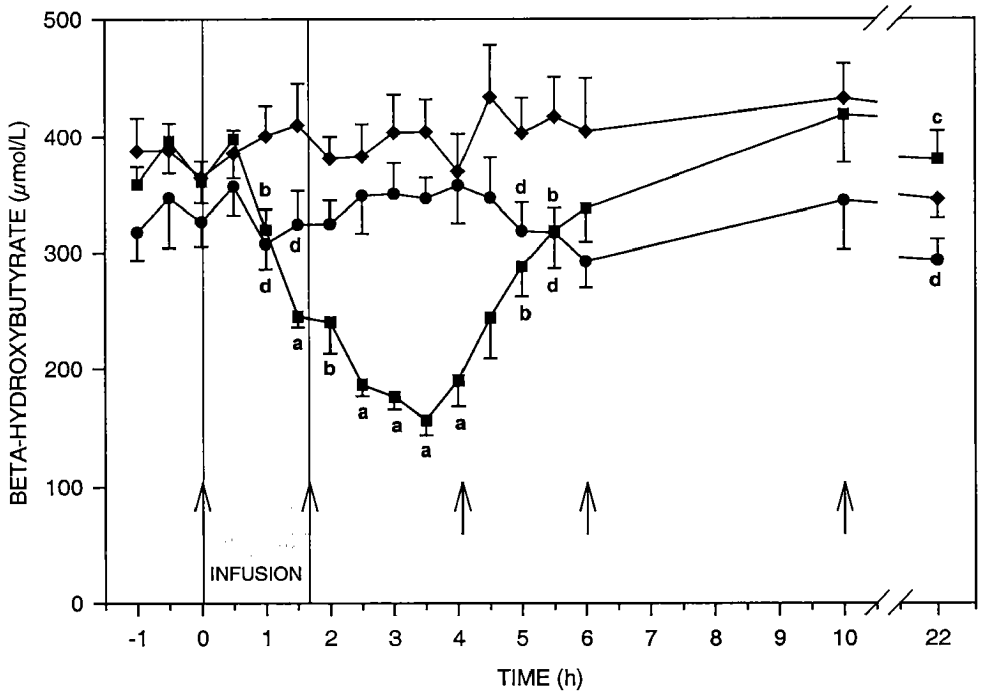

Fig. 8: Plasma β-hydroxybutyrate concentration of heifers in response to LPS infusion (2 µg/kg, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($p < .05$) from the values of the two other treatments. b: LPS infusion values are different ($P < .05$) from values after saline infusion. c: LPS infusion values are different ($P < .05$) from values after saline infusion plus restricted feeding. d: Values after saline infusion and after saline infusion plus restricted feeding are different ($P < .05$).

The changes in plasma concentration of glycerol (Fig. 10) resembled the treatment-induced changes described for plasma FFA except that there was no gradual increase of plasma glycerol with saline infusion and restricted feeding.

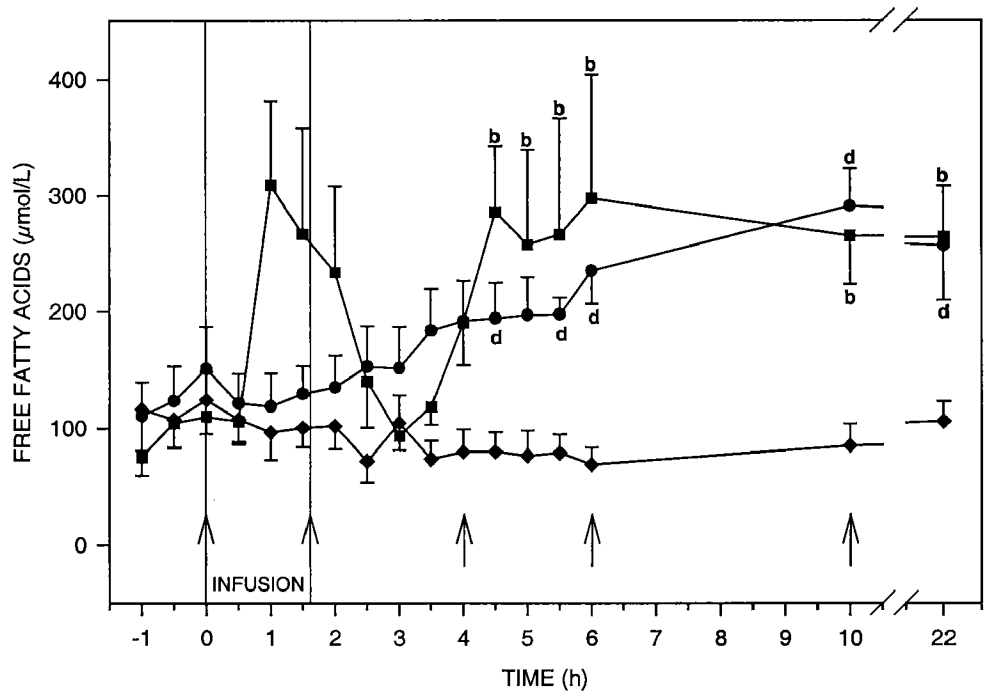

Fig. 9: Plasma free fatty acid concentration of heifers in response to LPS infusion (2 µg/kg, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. b: LPS infusion values are different (P < .05) from values after saline infusion. d: Values after saline infusion and after saline infusion plus restricted feeding are different (P < .05).

There were no consistent treatment effects on the plasma concentration of triglycerides (Fig. 11) until 10 h. With LPS infusion, however, plasma triglycerides decreased between 1 and 2.5 h (P < .05), and were higher (P < .05) than with saline infusion or saline infusion and restricted feeding at 22 h.

With LPS infusion, the plasma concentration of glucose (Fig. 12) first increased (0 h vs 1.5 h, P < .05) and later decreased (1 h vs 4 h, P < .05). As a result, plasma glucose was higher (P < .05) with LPS infusion than with saline infusion or saline

infusion and restricted feeding from 1 to 2.5 h, and lower ($P < .05$) than with saline infusion and saline infusion plus restricted feeding from 3.5 to 10 h. Plasma glucose did not change over time with, and was not different between saline infusion and saline infusion plus restricted feeding, except at infusion onset.

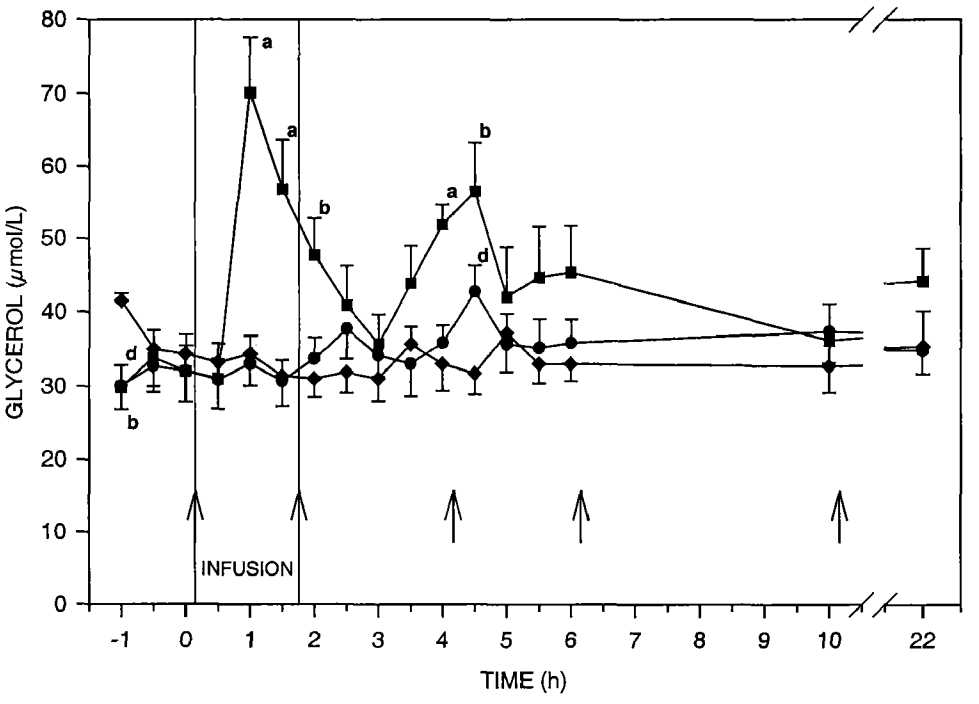

Fig. 10: Plasma glycerol concentration of heifers in response to LPS infusion (2 µg/kg, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($P < .05$) from the values of the two other treatments. b: LPS infusion values are different ($P < .05$) from values after saline infusion. d: Values after saline infusion and after saline infusion plus restricted feeding are different ($P < .05$).

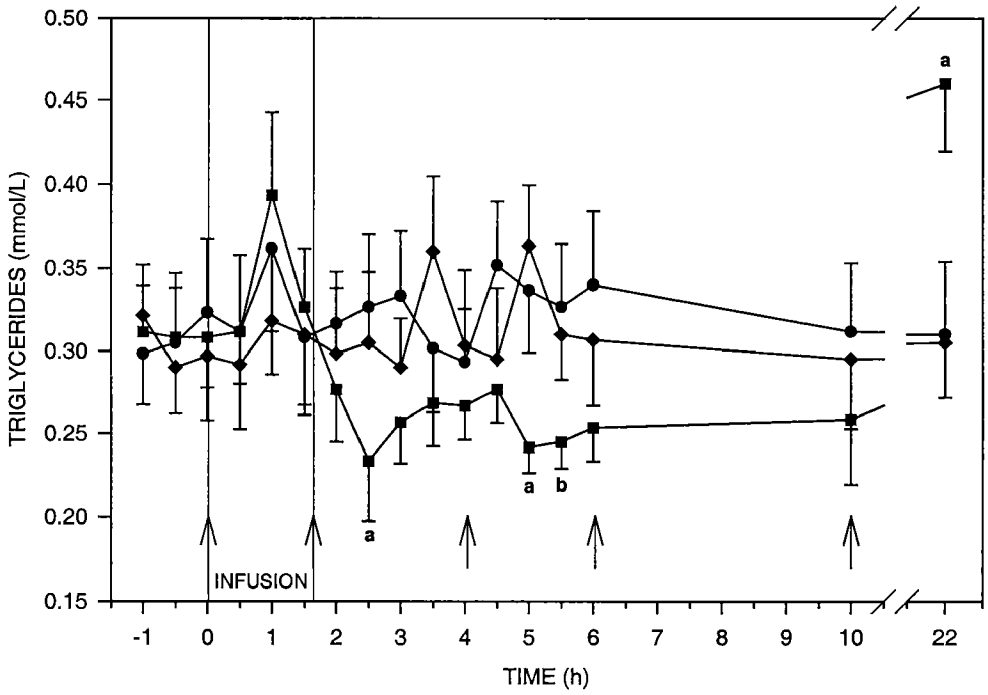

Fig. 11: Plasma triglyceride concentration of heifers in response to LPS infusion (2 µg/kg, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($P < .05$) from the values of the two other treatments. b: LPS infusion values are different ($P < .05$) from values after saline infusion.

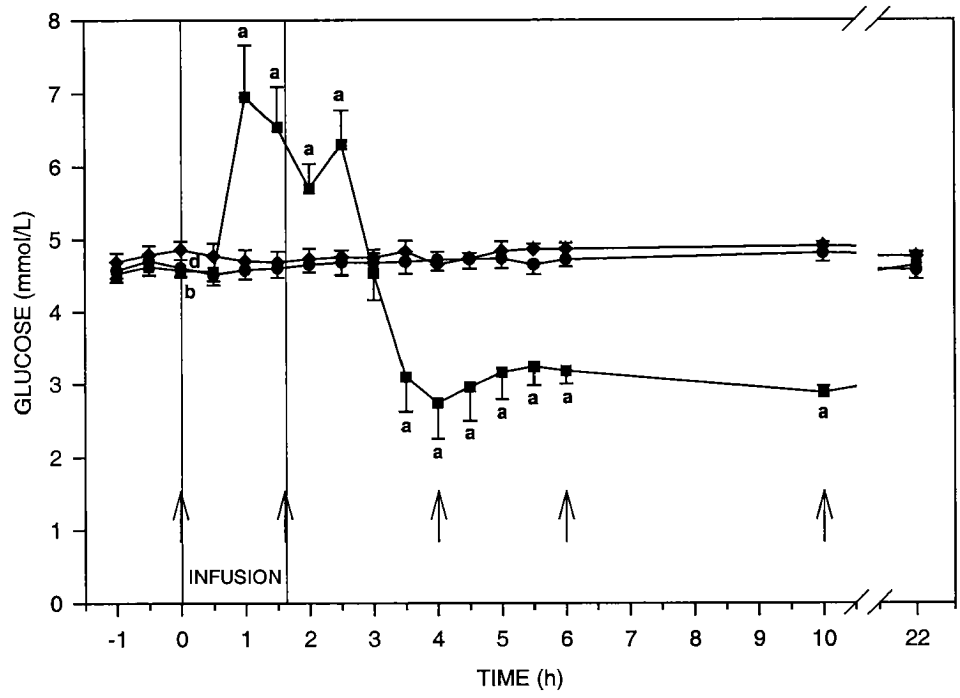

Fig. 12: Plasma glucose concentration of heifers in response to LPS infusion (2 µg/kg, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different (P < .05) from the values of the two other treatments. b: LPS infusion values are different (P < .05) from values after saline infusion. d: Values after saline infusion and after saline infusion plus restricted feeding are different (P < .05).

With LPS infusion, the plasma concentration of lactate (Fig. 13) increased markedly (.5 h vs 1 h, P < .05) and was higher (P < .05) than with saline infusion or saline infusion and restricted feeding from 1 h to 22 h. Plasma lactate slightly decreased over time with, but did not differ between saline infusion and saline infusion plus restricted feeding.

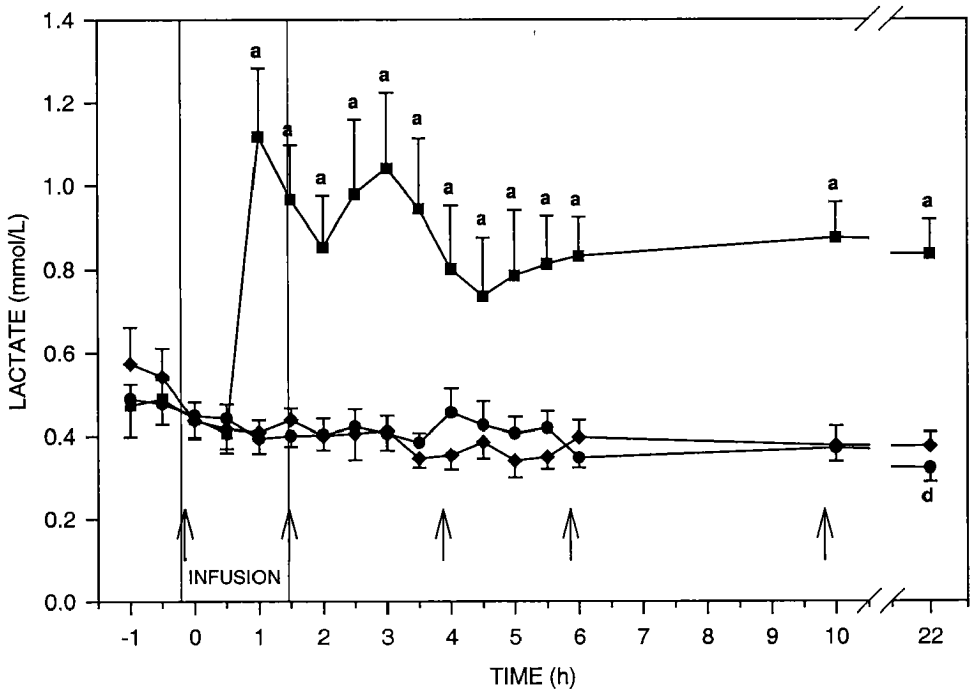

Fig. 13: Plasma lactate concentration of heifers in response to LPS infusion (2 μg/kg, 1 mL/min, 100 min) (-■-), saline infusion (-◆-), and saline infusion plus restricted feeding (-●-). Data points are means ± SEM. N = 6 for each treatment. Arrows indicate time of food presentation. a: LPS infusion values are different ($P < .05$) from the values of the two other treatments. d: Values after saline infusion and after saline infusion plus restricted feeding are different ($P < .05$).

4.4. Discussion

In demonstrating that a prolonged low-dose LPS infusion reduces food intake, increases body temperature, and causes marked changes in energy, fat, and carbohydrate metabolism of heifers, the present results confirm and extend previous findings in ruminants (Takeuchi et al., 1995; 1997; Van Miert et al., 1988; Werling et al., 1996). The saline infusion plus restricted feeding, which allowed to distinguish the direct metabolic effects of LPS from secondary effects due to the LPS-induced reduction in food intake, revealed that the metabolic effects of LPS are largely independent of the

food intake reduction. There seemed to be two distinct phases of metabolic responses to LPS, with the first phase (until about 3 h) resembling a stress reaction (Mizock, 1995) and the second phase reflecting a beginning energy deficit.

The significant hypophagic effect of LPS in non-ruminants and ruminants is well known and presumably mediated by cytokines, although its exact mechanisms have not been fully elucidated. The lack of a compensation for the observed food intake suppression within 24 h after the LPS infusion is in line with previous findings in rats (Langhans, 1996) and suggests that the suppression was not big enough to trigger a compensatory response.

A biphasic fever is the usual reaction of the body to a moderate dose of LPS (Lang et al., 1985; Lohuis et al., 1988); its presence indicates that the infused dose of LPS triggered reactions which are relevant for a severe Gram-negative bacterial infection, but do not approach septic shock (Lang et al., 1985). A fever in response to LPS is presumably due to the prostaglandin-mediated action of cytokines, in particular interleukin-1 (IL-1), on the hypothalamus (Dinarello, 1991; Lohuis et al., 1988).

The evolution of rectal temperature was only in part reflected by heat production: there was an increase in heat production during the initial rise of body temperature, and the increase also tended to be biphasic with LPS. However, after the short initial rise that was similar with all treatments and reflected the equilibration time of the chamber, heat production did not further increase and remained lower with LPS than with saline infusion, whereas rectal temperature continued to rise. This was unexpected because a cytokine-mediated increase in resting energy expenditure is considered to be a major contributor to LPS-induced fever, at least during the initial, rising phase (Klasing, 1988; Kluger, 1989). Given the findings with saline infusion and restricted feeding, the lower heat production with LPS than with saline infusion obviously reflects the lower thermic effect of food due to the reduction in food intake, and not a compensatory metabolic response to insufficient energy supply. Even fasted mammals have only a slightly reduced energy expenditure because basal metabolic rate is scarcely reduced (Müller and Kirchgeßner, 1984). The slightly higher cumulative heat production with LPS infusion than with saline infusion and restricted feeding between 2.5 and 6 h (7.1 ± .5 vs 6.7 ± .2 MJ, mean ± SEM) can not fully account for the maximum difference in rectal temperature (1.2°C at 5 h) between both treatments in

animals with a body weight of more than 300 kg. Therefore, non-oxidative, anaerobic mechanisms of heat production or reduced heat loss may have contributed to the LPS-induced fever. Given the increase in plasma lactate in response to LPS infusion, non-oxidative mechanisms were presumably operative under these conditions. A reduction of heat loss could have resulted from peripheral vasoconstriction (Elin and Wolff, 1976) due to an increased plasma norepinephrine concentration (Boosman et al., 1990).

The significant increase in plasma TNFα with LPS infusion reflects the systemic and local activation of monocytes/macrophages and endothelial cells by LPS (Beutler and Cerami, 1986; Mathison et al., 1988). Although TNFα is considered to be one of the first cytokines in the cascade of immunomodulators that mediate acute phase reactions (Fong and Lowry, 1990), the plasma concentration of TNFα increased after the onset of most metabolic responses to LPS. This indicates that these metabolic effects of LPS are initiated independent of an increase in circulating TNFα, and are therefore presumably triggered by local cytokine production (Martinet et al., 1988; Miller et al., 1997).

The steep rise in plasma cortisol that characterized the initial stress-like reaction in response to LPS infusion is consistent with previous findings (Boosman et al., 1990; Werling et al., 1996) and presumably reflects a stimulation of the hypothalamo-pituitary-adrenal axis. In addition to ACTH and glucocorticoids, an activation of the sympathetic nervous system may contribute to the LPS-induced changes in carbohydrate and fat metabolism observed during this period of time (Boosman et al., 1990; Nonogaki and Iguchi, 1997). The initial hyperglycemia in response to LPS presumably results from enhanced glycogenolysis (Filkins and Buchanan, 1977; Wolfe et al., 1977; Casteleijin et al., 1988) and gluconeogenesis (Filkins and Buchanan, 1977; Mizock, 1995). The higher RQ with LPS infusion compared to saline infusion with restricted feeding suggests that LPS initially stimulated carbohydrate oxidation. This is in line with previous findings in rats (Filkins and Buchanan, 1977). However, RQ values have to be interpreted cautiously in particular in ruminants because the RQ is also affected by other processes like rumen fermentation, metabolic acidosis or the observed changes in gluconeogenesis and ketogenesis, as well as ketone body utilization (Dale, 1984; Ferrannini, 1988). Although LPS may enhance carbohydrate utilization at least to some extent, there was obviously a stimulation of lipolysis during

this first phase of responses to LPS, as reflected by the profound initial increase in plasma concentrations of FFA and glycerol. Increases in plasma ACTH, cortisol, and catecholamines, as well as enhanced sympathetic nervous system activity, are the most likely mediators of this lipolytic response. In addition to enhanced lipolysis, a decrease in muscle FFA utilization may have contributed to the LPS-induced increase in plasma FFA (Romanowsky et al., 1980).

Despite the transient, initial LPS-induced increase in the plasma concentration of FFA, plasma BHB decreased with LPS infusion far more than with saline infusion and restricted feeding and reached a nadir between 3 and 4 h. This indicates that the decrease in plasma BHB in response to LPS reflects an inhibition of hepatic ketogenesis rather than a reduction of ruminal ketogenesis (Huhtanen et al., 1993) due to a reduction in food intake. The present data do not reveal whether a stimulation of ketone body utilization (Memon et al., 1992) also contributed to the decrease in plasma BHB with LPS infusion. The LPS-induced inhibition of ketogenesis may be mediated by TNFα and interleukin-1 (IL-1), which are both known to inhibit hepatic ketogenesis (Memon et al., 1992). An additional mediator of the inhibition of ketogenesis is insulin (Neufeld et al., 1980; Lang and Dobrescu, 1989). The exact coincidence of the dramatic, short-term rise in plasma insulin and the transient decrease in the plasma concentrations of BHB, FFA and glycerol in fact strongly implicates insulin in this metabolic change. The failure of LPS to increase the plasma triglyceride concentration is interesting because hypertriglyceridemia and increased very-low-density lipoprotein (VLDL) production in response to LPS have often been observed in non-ruminants (Wolfe et al., 1985; Meraïhi et al., 1991; Memon et al., 1992), and are attributed to a TNFα-mediated inhibition of lipoprotein lipase and a stimulation of hepatic triglyceride synthesis (Feingold and Grunfeld, 1987). In contrast, FFA did not seem to be diverted away from the oxidative pathway and incorporated into triglycerides with LPS in the present study. The reason(s) for this discrepancy and whether this is somehow related to the normally low plasma concentration of VLDL in ruminants (Bell, 1981) remain to be investigated.

Enhanced glycogenolysis and a reduced capacity of extrahepatic tissues to utilize lactate are considered to be major contributors to the increase in plasma lactate in response to LPS administration (Harada et al., 1994; Mizock, 1995). In addition, LPS decreases cardiac output and increases peripheral vasoconstriction (Elin and Wolff,

1976). As a result, poor tissue perfusion may have promoted anaerobic glycolysis and, hence, also contributed to the observed increase in plasma lactate (Hurtado et al., 1992). High plasma concentrations of lactate enhance TNFα production and may therefore have contributed to the increase in plasma TNFα together with LPS and other endogenous mediators (Mohri et al., 1990; Pettipher et al., 1996).

The second phase of the reactions to LPS infusion (after 4 h) presumably reflects the beginning lack of energy. With glycogen stores near depletion (Lohuis et al., 1988; Naylor and Kronfeld, 1985), an animal depends increasingly on lipolysis and fatty acid oxidation, and the RQ decreases. With saline infusion and restricted feeding, which also limited the availability of exogenous energy, a transient increase in carbohydrate oxidation was observed after the presentation of 3 kg food at 6 h. This increase in RQ was probably due to an enhanced utilization of glucose that was postprandially synthesized from propionate. With LPS infusion, there was no increase in carbohydrate utilization at that time although food intake was, by definition, close to the saline infusion plus restricted feeding condition, despite the elevated plasma concentrations of lactate and glycerol, which are efficient gluconeogenic substrates. Therefore, LPS obviously inhibited gluconeogenesis during this second phase (Mizock, 1995), an effect that presumably also contributed to the concomitant hypoglycemia (Naylor and Kronfeld, 1985). Surprisingly, the plasma concentration of triglycerides was very high with LPS treatment at 22 h, a time when the other metabolic effects of LPS (with the exception of the hyperlactemia) had already dissipated. This observation requires further investigations.

In conclusion, with the notable exception of the observed decrease in heat production, the metabolic effects in response to a pathophysiologically relevant dose of LPS were independent of the concomitant reduction in food intake. The induction of fever without a concomitant increase in heat production indicates that anaerobic mechanisms or a reduction of heat loss contributed to the fever. The latter possibility contrasts with findings in laboratory animals (Jennings and Elia, 1987) and calculations for humans, in which a 1°C increase in body temperature has been related to a 10 – 15% increase in heat production (Kluger, 1979). Moreover, the present findings implicate insulin in some of the metabolic reactions to LPS. The pathophysiological relevance of this observation has yet to be determined. Finally, the results suggest that the

presumably enhanced local production of cytokines is sufficient to trigger most of the observed responses.

4.5. Implications

There are profound, complex endocrine and metabolic reactions in response to bacterial infections. Local cytokine production may trigger many of these reactions and affect how the immune, endocrine, and neural systems interact. Insulin may also be involved and this relates to the interesting question of cytokine – pancreatic-β-cell interactions. The lack of an increase in plasma triglyceride concentrations in response to lipopolysaccharide (LPS) in heifers may be related to the usually low plasma lipoprotein concentrations in ruminants and could translate into a high LPS sensitivity of ruminants because plasma lipoproteins usually bind LPS and reduce their activity. Together, this information enhances our knowledge of the pathophysiology of systemic Gram-negative bacterial infections in ruminants.

5. EFFECT OF WATER RESTRICTION ON FEEDING AND METABOLISM IN DAIRY COWS

based on:

Steiger Burgos, M., M. Senn, F. Sutter, M. Kreuzer and W. Langhans. *Am. J. Physiol.*, in press.

5.1. Introduction

Restriction of water intake has often been shown to reduce food intake in man (Engell, 1988) and various animal species (e.g. Adolph, 1947), including ruminants (e.g. Bianca, 1966; French, 1956; Little et al, 1976). Ruminants differ from monogastric animals because much more saliva is secreted during eating (Bailey, 1961) and because they have a large fluid reserve in the rumen, which can buffer osmotic changes in the rumen derived from digesta. In previous studies of ours, pygmy goats (Langhans et al., 1991) and lactating cows (Senn et al, 1996) progressively reduced food intake during water deprivation and did not compensate for the dehydration-induced weight loss by increasing food intake during the subsequent rehydration period. These results contrast findings in rats, which are known to compensate for dehydration-induced body weight loss by markedly increasing food intake during the subsequent rehydration (Adolph, 1947). This different response suggests that ruminants are better able to cope with dehydration than rats, i.e. a similar degree of dehydration presumably provokes a smaller energy deficit in ruminants than in rats. Two mechanisms may contribute to the limitation of the dehydration-induced energy deficit in ruminants: 1) the digestibility of forage-based diets may be higher during dehydration (Balch et al, 1953; Brosh et al, 1987; Silanikove, 1985) and 2) resting metabolic rate may decrease with dehydration (Brosh et al, 1986). Such adaptive mechanisms have mainly been shown in non-lactating, mostly small and desert-adapted ruminants (Balch et al., 1953, Brosh et al., 1986; Brosh et al., 1987, Silanikove, 1985). Whether similar mechanisms or other compensatory changes in digestion and metabolism are activated by dehydration in lactating dairy cows, which are more susceptible to water shortages than desert goats, is unknown. The present study addressed this question by trying to

identify and quantify such mechanisms in addition to the food intake suppression during graded levels of water restriction in lactating dairy cows.

In one experiment we characterized the feeding behavior of lactating cows in response to different levels of water restriction, to see whether the cows are able to reach and maintain a new equilibrium under these conditions. In a second experiment we determined water, energy, and nitrogen balances as well as plasma metabolite and hormone concentrations when cows had ad libitum access to water and when they were subjected to the higher water restriction level of the first experiment.

5.2. Material and methods

Animals and experimental procedures

In Experiment 1, 17 Brown Swiss cows (268 ± 14 [mean ± SEM] days *post partum,* all pregnant [137 ± 8 days of pregnancy], 644 ± 13 kg body weight) were used. The cows were housed in a tying stable with ad libitum access to water. After recording food and water intake as well as milk yield for 5 days (baseline), the cows were distributed into 2 groups (matched for baseline food intake, milk yield, and water intake) which were subjected to 25% (n=9) and 50% (n=8) water restriction relative to individual ad libitum drinking water intake (mean baseline) for 8 days. These treatments are subsequently referred to as 25 and 50% water restriction, respectively. Note, however, that the total water intake (drinking water plus water in food) restriction level was somewhat less (see Results) because food intake was not reduced by 25 and 50%. The restriction period was followed by a 4 day rehydration period with ad libitum access to drinking water. During the restriction period, the cows had access to drinking water every day from 06.00 hr, at the same time when food was first presented in the morning, until the allotted amount of drinking water was consumed. The cows were weighed once during baseline and every day during the restriction and rehydration periods.

In Experiment 2, 6 lactating Brown Swiss cows (191 ± 17 [mean ± SEM] days *post partum*), 4 of them pregnant [59 ± 5 days of pregnancy], 601 ± 23 kg body weight) had ad libitum access to water for 5 days (baseline) and were then subjected to 50% water restriction relative to individual baseline intake for 9 days (restriction period).

During the restriction period, animals had access to water from 09.00 hr, at the same time when food was first presented in the morning, until the allotted amount of water was consumed. The 5 baseline days and the 5 last days of the restriction period (days 5-9 of restriction) were used for data collection, assuming steady-state according to the results of Experiment 1. During these days feces, urine and milk were collected. For respiratory measurements on days 3 and 4 of the baseline and on days 7 and 8 of the restriction period, 2 cows each were randomly placed into 2 respiration chambers. During all other days they were housed in a tying stable with slatted floor designed for balance trials. On the last day of the baseline and restriction periods the cows were weighed (at 08.00 hr) and a blood sample was taken (at 15.00 hr).

Feeding and meal patterns

During both experiments the cows had ad libitum access to a mixed diet of grass silage (57%), corn silage (33%) and hay (10%). Diet composition was as follows: 51.9% dry matter (DM), 6.1 MJ/kg DM net energy for lactation, 78.1 g/kg DM digestible protein in the intestine, 124 g/kg DM crude protein, and 257 g/kg DM crude fiber. In Experiment 1 the diet was provided three times a day (at 06.00, 10.30 and 15.30 hr). In Experiment 2 the diet was provided at 09.00 and 15.30 hr. The animals were adapted to the diet for at least 2 weeks prior to the experiments. Food intake and meal patterns were continuously recorded on line by a computerized feeding system similar to the one described previously by Senn et al. (1996). Meals were defined as weight changes $\geq$ 50 g following electronic identification of a cow at the feeding trough, lasting $\geq$ 1 min (minimum meal duration), and separated by $\geq$ 8 min (minimum intermeal interval) from other weight changes, as described previously (Senn et al., 1996). Meals defined and recorded this way accounted for 99.9 % of total daily food intake.

Drinking and water restriction

The drinking troughs were controlled by computer-assisted water meters and water valves (Bürkert, Fluid control systems, Ingelfingen, Switzerland). This system allowed to continuously record ($\pm$ 1%) and to automatically restrict individual 24 hr

water intake when a preset maximum volume of water was consumed, i.e. the water valve for a particular cow's drinking trough was automatically closed until the next morning when this cow had consumed the amount allotted for the day.

Milk recording

Milk yield was recorded automatically throughout both experiments (Metatron, Westfalia Separator AG, Oelde, Germany).

Respiratory measurements

During Experiment 2, oxygen consumption and the output of carbon dioxide and methane were measured by open circuit calorimetry (Steiger et al., 1999) in the respiration chambers for 2 x 24 hr in each data collection period (baseline and restriction). The 2 chambers were air conditioned to match the climate in the tying stable (21.8 ± .1 °C [mean ± SEM] temperature and 59.8 ± .4% relative humidity). Air flow was maintained at 28.6 ± .6 m^3/h and recorded with in-line electronic flow meters (Swingwhirl DV 630, Flowtec AG, Reinach, Switzerland). Gaseous composition of the air flowing into and out of the chambers (internal volume: 20 m^3 each) was measured using infrared analyzers (Binos, Leybold-Heareus, Zurich, Switzerland) for carbon dioxide and methane, and a paramagnetic analyzer (Oxymat 3, Siemens AG, Dietikon, Switzerland) for oxygen. The whole system was manually calibrated each day prior to the onset of the respiratory measurements. Heat production of each animal was estimated according to the following equation (Brower, 1965): heat production [kJ] = 16.179 x O_2 [L] + 5.022 x CO_2 [L] – 2.168 x CH_4 [L] – 5.989 x N in urine [g].

Collection and processing of samples in Experiment 2

During the 5 day baseline period and during the 5 data collection days of the restriction period in Experiment 2, samples were taken either daily (feces, urine, milk) or every second day (food). The food samples were dried (60°C, 48 hr) and milled for later analysis of total ash, crude fiber, gross energy, carbon, and nitrogen content as described below. The daily samples of feces were stored at 4°C until the end of the respective collection periods, when they were mixed to an aliquot for each period and

cow. One part of each aliquot was dried and milled for the analysis of total ash, crude fiber and gross energy, the other part was frozen and stored at –20°C until later analysis of carbon and nitrogen content.

Twenty-four hour urine was collected in 2 containers via a flexible urinal fixed on Velcro® tape which was glued onto the body with special adhesive (Cyanolit, 3M AG, Rueschlikon, Switzerland) . The urine in one container was mixed with 60 mL sulphuric acid (5M) to avoid N-losses for further analysis of nitrogen. The urine in the second container was used for carbon analysis. The daily samples of acidified urine were stored at 4°C, whereas the non-acidified samples were frozen until the end of the collection periods. At the end of each collection period, the daily samples were pooled, frozen, and stored at –20°C until analysis of carbon and nitrogen.

For milk composition analysis, two samples per milking were taken automatically in the stable and manually in the respiration chambers. One of these samples was conserved with sodium acid (Bromopol, BSM2, D&F Control, San Ramon, CA) and stored at 4°C for the weekly analysis of fat, protein and lactose by the Swiss Brown Cattle Breeders' Federation (Zug, Switzerland). The other sample was frozen and stored at –20°C until the end of the collection period and then also pooled. One part of this pooled sample was then frozen in liquid nitrogen and lyophilized for the analysis of dry matter and gross energy. The other part was used for the carbon and nitrogen analysis.

At 15.00 hr on the last day of the baseline and restriction periods, about 35 mL blood were taken from the jugular vein. The blood was collected into EDTA (for hormones analysis), heparin, and NaF-tubes (for analysis of metabolites, osmolality and electrolytes). One mL of the EDTA-blood was mixed with 500 Kallikrein Inactivating Units (Aprotinin, Böhringer Mannheim, Rotkreuz, Switzerland) for later analysis of glucagon. All blood samples were immediately centrifuged (1600 g, 4°C, 15 min), and the plasma was then frozen at –20°C until analysis.

Laboratory analyses

The Weende method was used to analyze total ash and crude fiber in food and feces (Naumann and Bassler, 1997). Carbon and nitrogen in food, feces, urine and milk

were analyzed using an automatic C/N-analyzer (Leco-analyzer, Type FP2000, Leco Intruments, St. Joseph, MI): the samples were oxygenized at 950°C and the carbon was then measured by an infrared cell; nitrogen was measured by a detector for heat conductivity with helium. The gross energy content of food, feces and milk was measured with an adiabatic bomb calorimeter (IKA – calorimetry system C 700 T, IKA – Analysentechnik GmbH, Heitersheim, Germany). The gross energy content of urine was calculated by the following equation (Hoffmann and Klein, 1980): gross energy in urine [kJ] = 33.1 x C in urine [g] + 9.2 x N in urine [g]. Fat, protein, and lactose in milk were measured by an infrared-photospectrometer (Milkoscan 4000, Foss Electric, Hillerød, Denmark). Plasma glucose, lactate, free fatty acids (FFA), β-hydroxybutyrate (BHB), triglycerides, glycerol, urea, and protein were measured according to standard enzymatic procedures (Drewes, 1974) using an automatic analyzer (Cobas-Mira, Roche Diagnostics, Basel, Switzerland). A freezing point osmometer (Multi-osmette, Precision Systems Inc., Natwick, MA) was used to measure plasma osmolality. Plasma sodium, potassium, and chloride were determined by flame photometry (FLM3, Radiometer Copenhagen, Instrumenten-Gesellschaft AG, Zurich, Switzerland). Commercially available radio-immunoassay kits were used for the determination of plasma cortisol and glucagon (Diagnostic Products Corp., Los Angeles, CA) and insulin (Pharmacia, Uppsala, Sweden). EDTA-blood was used to measure the hematocrit (Hettich Hematocrit-centrifuge, 5 min).

Calculation of energy and nutrient balances

Intake was opposed to excretion for calculation of water, nitrogen, carbon and energy balance. Metabolizable energy is defined as gross energy intake minus fecal, methane and urinary energy. Retained energy comprises energy in milk and body energy balance. Body fat balance was calculated from carbon and nitrogen balances (Brower, 1965).

Statistics

In Experiment 1, a repeated measures ANOVA was performed to test for the effects of day and group, and for day x group interactions. The data were analyzed in 2

steps: first baseline and restriction periods were compared, then baseline and rehydration. When the day effect was significant, pair-wise comparisons of selected days were made with the paired t-test. In Experiment 2, mean values were calculated for further analysis when more than one value was obtained for a given parameter during the data collection periods (e.g. daily milk production). Due to the small number of animals (n=6) the data were often not normally distributed. Therefore, the non parametric Wilcoxon-test was used for the comparison of baseline and restriction period values of the same animals. All analyses were done with SYSTAT 7.0 (Systat Inc., Evanston, IL). Data are presented as means ± SEM. P-values < .05 were considered significant.

5.3. Results

Experiment 1

Baseline drinking water intake in Experiment 1 was 59.8 ± 1.9 (5 day individual means ± SEM) and 59.7 ± 3.3 L/day for the cows subsequently subjected to 25% and 50% drinking water restriction, respectively. Note, however, that total water intake (drinking water plus water in food) was reduced only about 22 and 44% with 25 and 50% water restriction because food intake was reduced less than 25 and 50%, respectively. During the 8 days of water restriction body weight decreased in cows with 50% restriction, but not in cows with 25% restriction (interaction group x day: $P < .05$, Fig. 14). Body weight stabilized around 99% (25% restriction) and 95% (50% restriction) of baseline values after 4 days of water restriction. Upon rehydration, the body weight of both restriction groups immediately increased above the baseline level and was higher ($P < .05$) than during baseline on all rehydration days.

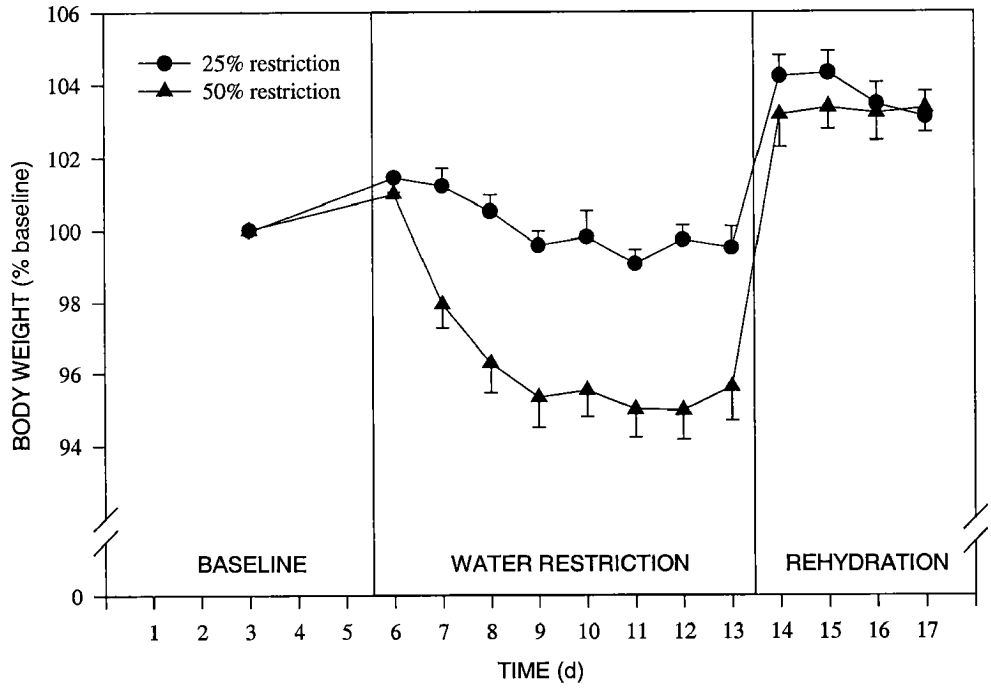

Fig. 14: Body weight changes (relative to the initial weight) of lactating dairy cows during 25% and 50% water restriction (relative to baseline intake), and rehydration (Experiment 1). Data are means ± SEM of 9 (25% restriction) and 8 (50% restriction) cows. See text for further details.

Daily food intake decreased in relation to the water restriction level (interaction group x day: $P < .05$, Fig. 15) during the first 3-4 days of water restriction, and fluctuated around an 11% and 21% (with 25 and 50% water restriction, respectively) lower level for the remainder of the restriction period. Food intake immediately returned to the baseline level upon rehydration.

Meal pattern analysis revealed a particularly strong effect of water restriction (50 and 25% restriction) on the size of the first meal after the presentation of fresh food in the morning (Fig. 16). The first meal during water restriction started at about the same time as during baseline, but, from the third restriction day onwards, it was more than 50% smaller (effect of day: $P < .05$) and shorter (effect of day: $P < .05$, data not shown) than baseline meals. Note that the size of the first meal was not affected on the first

water restriction day because at that time the cows did not experience any water restriction yet. On the first restriction day they run out of water (reached their allotted amount) at 12.00 (50% restriction) and 17.00 h (25% restriction), respectively. From the third day of 50% water restriction onwards, the cows consumed all the allotted drinking water within the first 60 minutes of access. With 25% water restriction they consumed about 65% of the allotted amount within the first 60 minutes. A more detailed analysis of feeding and drinking patterns during the first 60 minutes of access to drinking water showed that the cows irregularly alternated between drinking and feeding. Upon rehydration, the size of the first meal rapidly reached the baseline level again. The mean size of the second and of all following meals decreased only by about one third with 50% water restriction (effect of day: $P < .05$) and scarcely so with 25% water restriction.

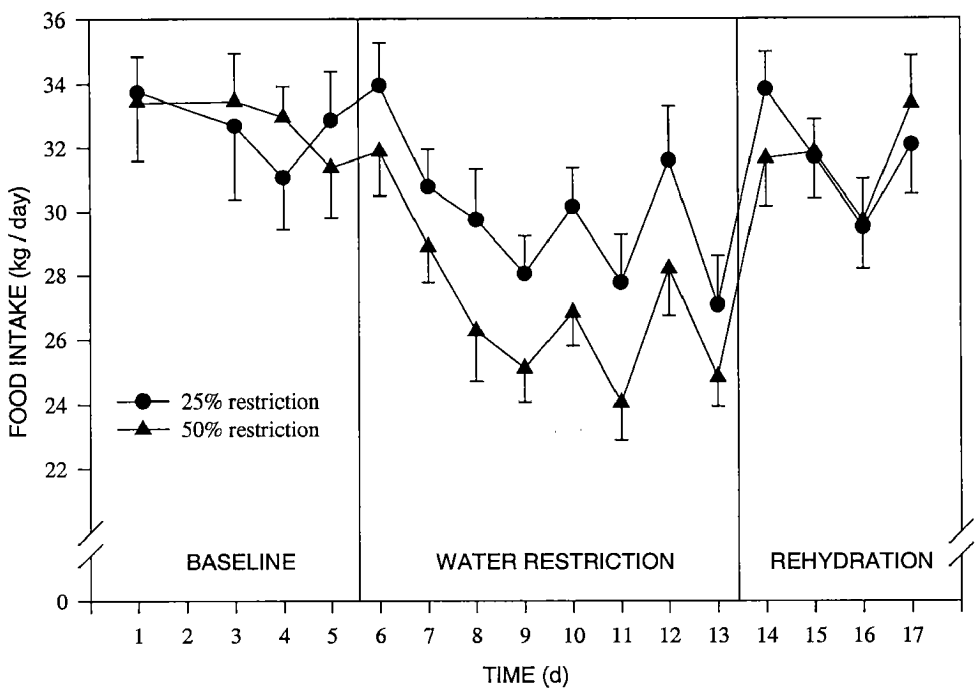

Fig. 15: Food intake of lactating dairy cows during baseline, 25 and 50% water restriction (relative to baseline intake), and rehydration (Experiment 1). Data are means ± SEM of 9 (25% restriction) and 8 (50% restriction) cows. See text for further details.

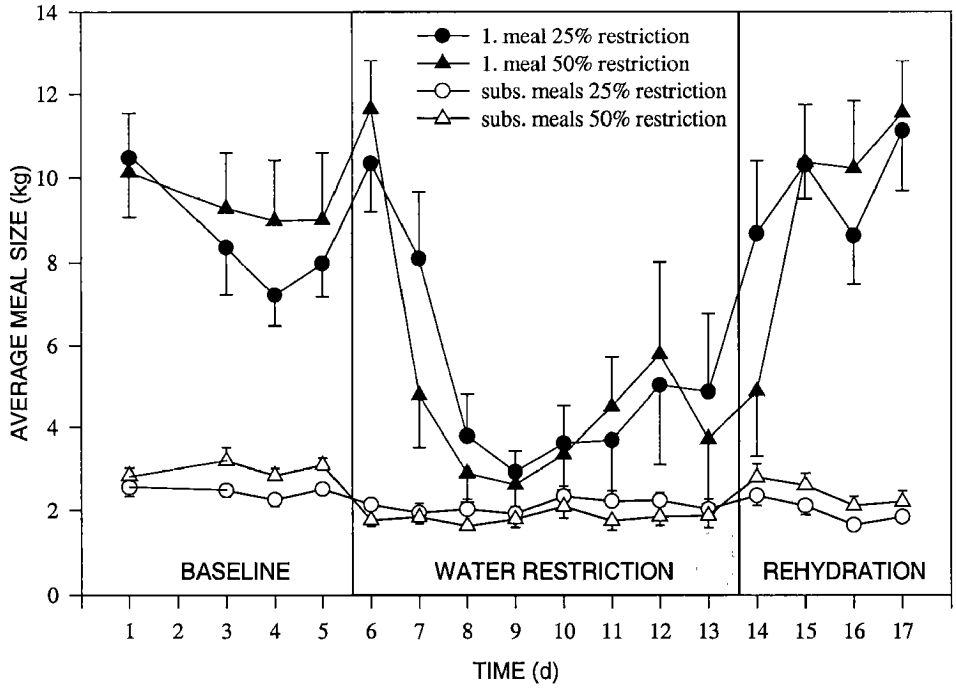

Fig. 16: Size of the first meal and of all subsequent meals during baseline, 25 and 50 % water restriction (relative to baseline intake), and rehydration (Experiment 1). Data are means ± SEM of 9 (25% restriction) and 8 (50% restriction) cows. For the subsequent meals values individual means were calculated first. See text for further details.

Meal frequency increased with 25 and 50% water restriction to reach a maximum on the third day of restriction (day 8, significantly higher than all baseline days, Fig. 17). On the first day of the rehydration period after 50% water restriction meal frequency decreased (day 13 vs day 14: $P < .05$) to approximately the baseline level. Following 25% water restriction meal frequency stayed as high as at the end of restriction. Milk yield was lower with 25 and 50% water restriction (effect of day: $P < .05$) than during baseline, decreasing with a delay of one day (Fig. 18). Milk yield also recovered with a one day delay during the subsequent rehydration period.

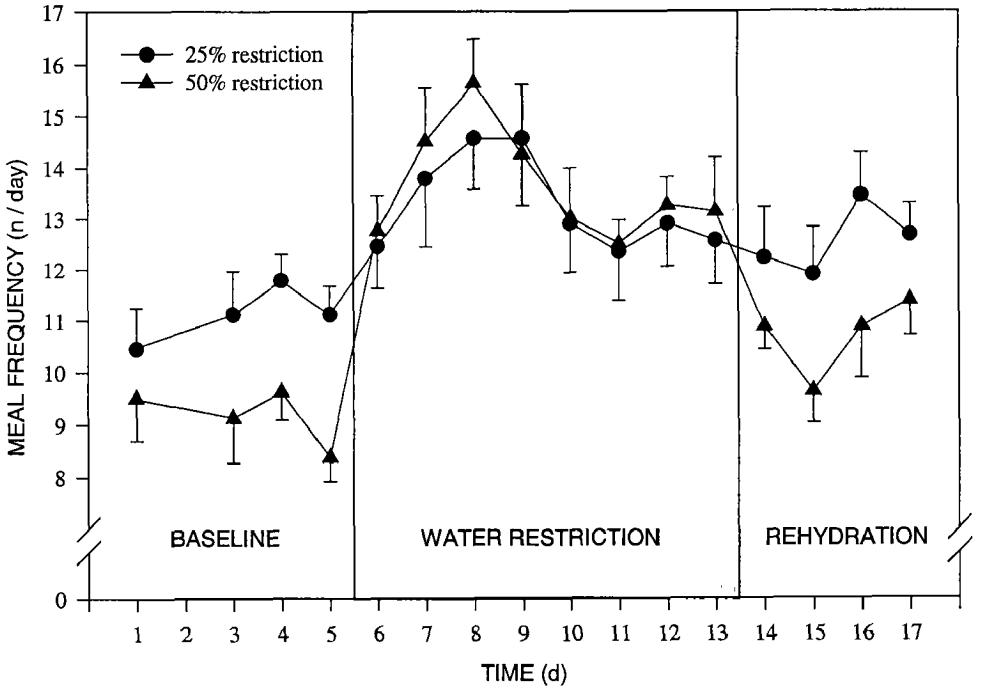

Fig. 17: Meal frequency of lactating dairy cows during baseline, 25 and 50 % water restriction (relative to baseline intake), and rehydration (Experiment 1). Data are means ± SEM of 9 (25% restriction) and 8 (50% restriction) cows. See text for further details.

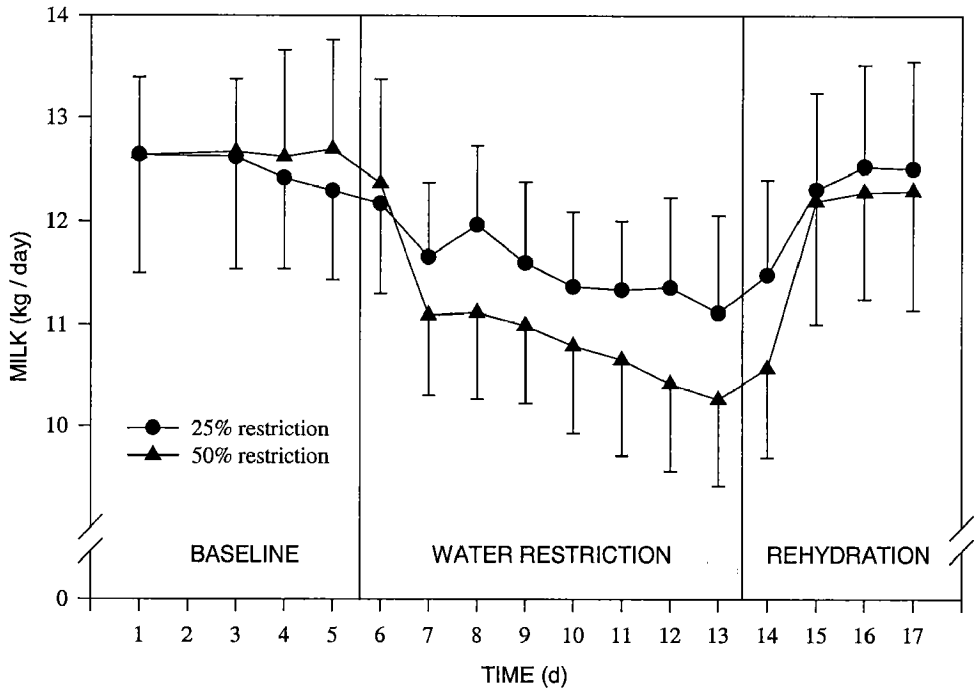

Fig. 18: Milk production of dairy cows during baseline, 25 and 50% water restriction (relative to baseline intake), and rehydration (Experiment 1). Data are means ± SEM of 9 (25% restriction) and 8 (50% restriction) cows. See text for further details.

Experiment 2

With 50% drinking water restriction (37.7 ± 1.8 L vs. 75.4 ± 4.5 L, $P < .05$), food intake during the five last days of the restriction period was about 20% lower than during the water ad libitum (baseline) period (25.7 ± 1.2 kg/day vs. 32 ± 1.2 kg/day, $P < .05$). The total water/food ratio ([intake of drinking water + water in food] / food DM [kg/kg]) decreased from 5.6 ± .2 (mean ± SEM) in the baseline period to 4.3 ± .1 ($P < .05$) in the water restriction period. Body weight, measured on the last day of each period, decreased from 601 ± 23 kg to 535 ± 18 kg ($P < .05$). Compared to baseline, about 50% fewer feces ($P < .05$) were produced in the water restriction period, but their DM content was markedly ($P < .05$) higher (Table 2). Similarly, the total volume of urine produced decreased ($P < .05$) during the water restriction period by 43%, but the

urine was more concentrated ($P < .05$, Table 2). Milk production decreased ($P < .05$) during the water restriction period by about 27% from 18.7 kg/day to 13.6 kg/day, but milk composition did not change significantly except for lactose and urea content, which were higher during the water restriction than during the baseline period (Table 2). The total water/milk ratio ([intake of drinking water and water in food] / milk [kg/kg]) decreased from 4.9 ± .2 (baseline) to 3.8 ± .1 (restriction period) ($P < .05$).

Table 2. *Composition of feces, urine, and milk of 6 lactating dairy cows with ad libitum access to water (Baseline) and with 50% water restriction (Restriction) in Experiment 2*

	FECES		URINE		MILK	
	Baseline	Restriction	Baseline	Restriction	Baseline	Restriction
WW kg/d	41.5 ± 3.1	20.6 ± 1.2*	20.2 ± 1.0	11.5 ± .4*	18.7 ± .6	13.6 ± .4*
DM g/kg	126.4 ± 5.4	170.0 ± 3.5*	35.5 ± 1.6	48.5 ± 3.1*	131.5 ± 2.1	130.9 ± 2.9
GE MJ/kg DM	19.0 ± .2	19.0 ± .03	.5 ± .01	.7 ± .02*	24.8 ± .3	24.6 ± .4
N g/kg WW	3.6 ± .2	4.9 ± .1*	3.0 ± .1	4.9 ± .4*	5.1 ± .2	5.3 ± .2
C g/kg WW	60.4 ± 2.2	79.6 ± 1.0*	13.6 ± .3	19.9 ± .7*	73.6 ± 1.5	77.0 ± 2.2
Fat g/kg	ND	ND	ND	ND	46.2 ± 1.7	47.8 ± 2.2
Protein g/kg	ND	ND	ND	ND	33.7 ± 1.0	34.1 ± 1.0
Lactose g/kg	ND	ND	ND	ND	48.3 ± .8	49.9 ± 1.1*
Urea mg/dL	ND	ND	ND	ND	21.6 ± 1.8	34.2 ± 2.4*

Values are means ± SEM of 6 cows throughout the 5 day baseline and water restriction collection periods. DM: dry matter, GE: gross energy, ND: not determined, WW: wet weight. *Significantly ($P < .05$) different from baseline (Wilcoxon test). Urine DM was estimated with the following formula (Hinsberg, 1953): DM [%] = (urine density – 1) x 2.6 x 100.

Water balance did not change between baseline and restriction periods (Table 3). The decreases ($P < .05$) in water excretion via urine, feces and milk paralleled the reduced intake so that the water excretion/intake ratio remained < 1 since water loss via body surface and lung was not considered in the balance calculation. Organic matter digestibility was higher during the restriction than during the baseline period (.74 ± .01 vs .70 ± .01, $P < .05$). Digestibility of the crude fiber tended to be higher in the restriction than in the baseline period (.70 ± .01 vs .66 ± .02, $P > .05$).

Table 3. *Water balance of 6 lactating dairy cows with ad libitum access to water (Baseline) and with 50% water restrition (Restriction) in Experiment 2*

	Baseline	Restriction
Intake via drinking water, L/d	75.4 ± 4.5	38.7 ± 1.8*
Intake via food, kg/d	15.7 ± .6	13.5 ± .7*
Excretion via urine, kg/d	19.4 ± 1.0	11.0 ± .4*
Excretion via feces, kg/d	36.3 ± 2.9	17.1 ± 1.0*
Excretion via milk, kg/d	16.3 ± .6	11.8 ± .3*
Ratio excretion/intake	.80 ± .02	.77 ± .01

Values are means ± SEM of 6 cows. *Significantly ($P < .05$) different from baseline (Wilcoxon test). Water losses through skin and lung were neglected.

The nitrogen balance became negative during the water restriction period ($P < .05$, Fig. 19). Expressed as percent of nitrogen intake, the proportion of nitrogen excreted with milk and especially with urine was higher ($P < .05$) during the restriction than during the baseline period (Fig. 19). Therefore, nitrogen utilization was less efficient during water restriction than during the baseline period (.53 vs .65), and the cows had 44.6% less nitrogen available for production (retained N) than during the baseline period ($P < .05$, Fig. 20).

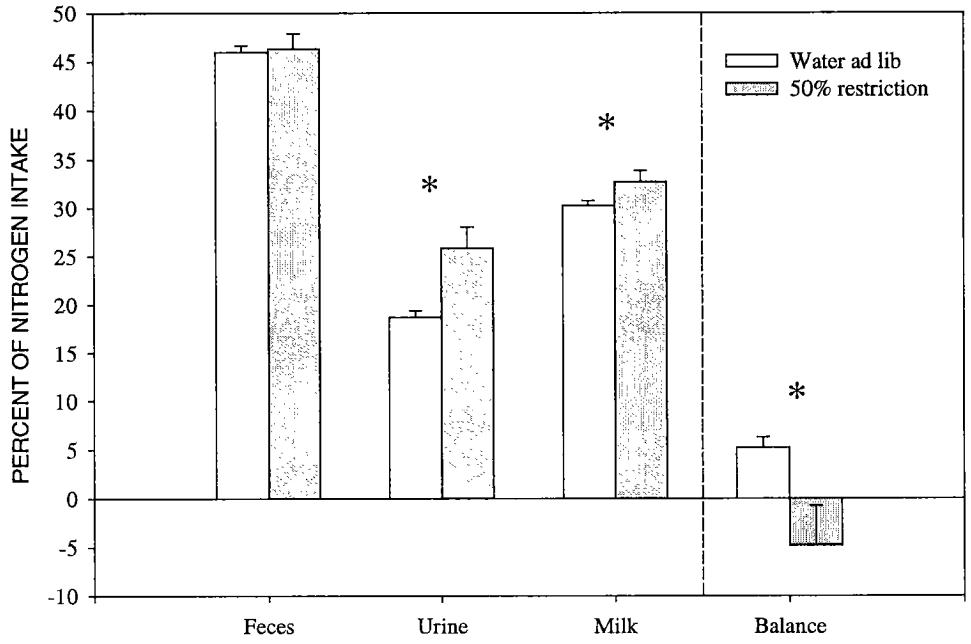

Fig. 19: Nitrogen balance of 6 lactating dairy cows with ad libitum access to water and 50% water restriction (Experiment 2). Data are means ± SEM. *Significant (P< .05) difference between water ad lib and 50% restriction values. See text for further details.

The energy balance did not change significantly with 50% water restriction (Fig. 21) because the decrease in absolute energy excretions with water restriction largely paralleled the decrease in energy intake. Furthermore, a reduction ($P < .05$) in the proportion of ingested energy lost through feces compensated for the increase ($P < .05$) in the proportion of energy excreted in urine during the same period (Fig. 21).

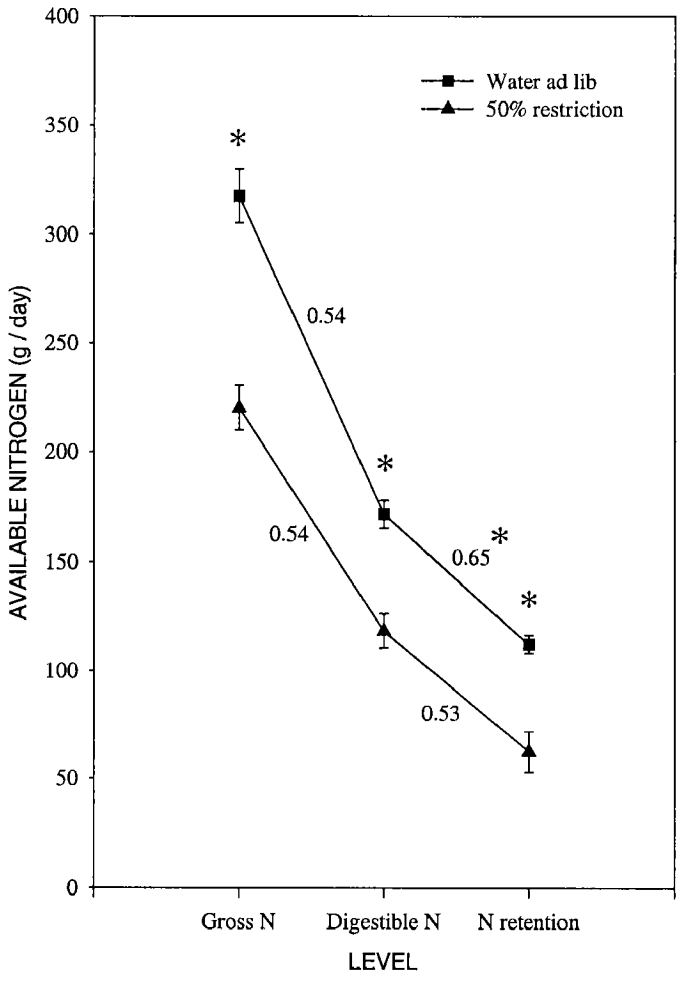

Fig. 20: Nitrogen utilization levels of 6 lactating dairy cows with ad lib access to water and 50% water restriction (Experiment 2). Data are means ± SEM. The numbers represent the coefficients of utilization from one level of nitrogen utilization to the next. *Significant ($P < .05$) difference between water ad lib and 50% restriction values. See text for further details.

Plotting the various stages of energy utilization during the baseline and restriction periods (Fig. 22) reveals that the differences ($P < .05$) between both periods at the level of gross energy, digestible energy, and metabolizable energy became gradually smaller and disappeared at the level of retained energy. The coefficients of energy utilization tended to be higher during the restriction than during the baseline period, but this difference reached significance ($P < .05$) only for digestibility (.71 vs

.67). Based on the reduced ($P < .05$) gaseous exchange during the restriction period, absolute heat production decreased ($P < .05$) by 21.7% (Table 4), but again, the proportion of heat production relative to intake did not (Fig. 21). Heat production can be divided into heat related to milk production (calculated with $k_L = .6$ [Menke and Huss, 1987]) and heat related to maintenance needs. Under the assumption of an unchanged efficiency of utilization of metabolizable energy (k_L), heat production for maintenance was reduced ($P < .05$) by 17.8% during the restriction period (Table 4). In line with the trend observed in energy balance, the cows mobilized less ($P < .05$) fat during water restriction than during baseline (Table 4).

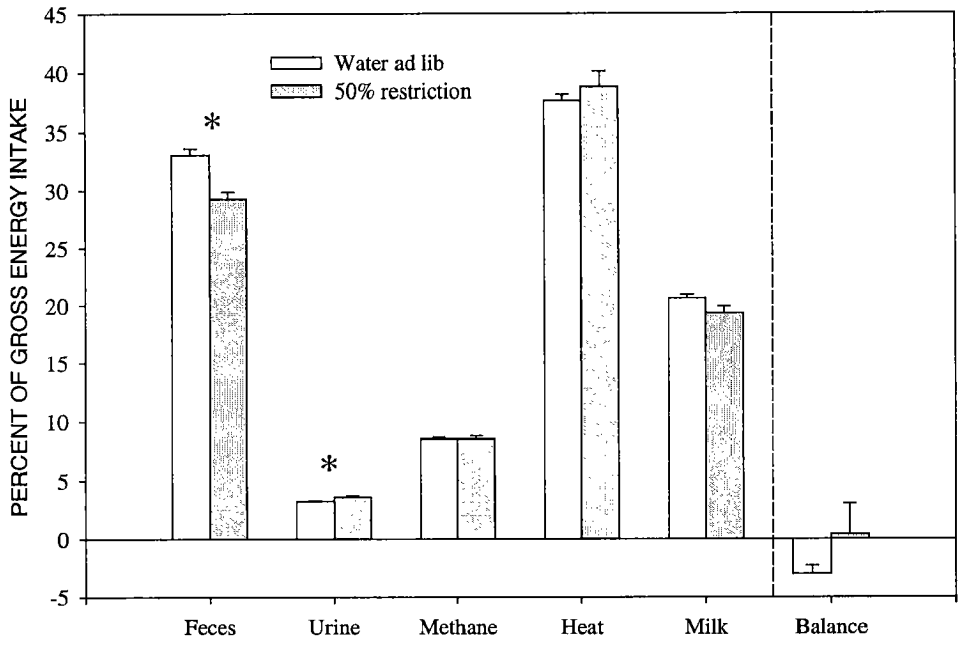

Fig. 21: Energy balance of 6 lactating dairy cows with ad libitum access to water and 50% water restriction (Experiment 2). Data are means ± SEM. *Significant ($P < .05$) difference between water ad lib and 50% restriction values. See text for further details.

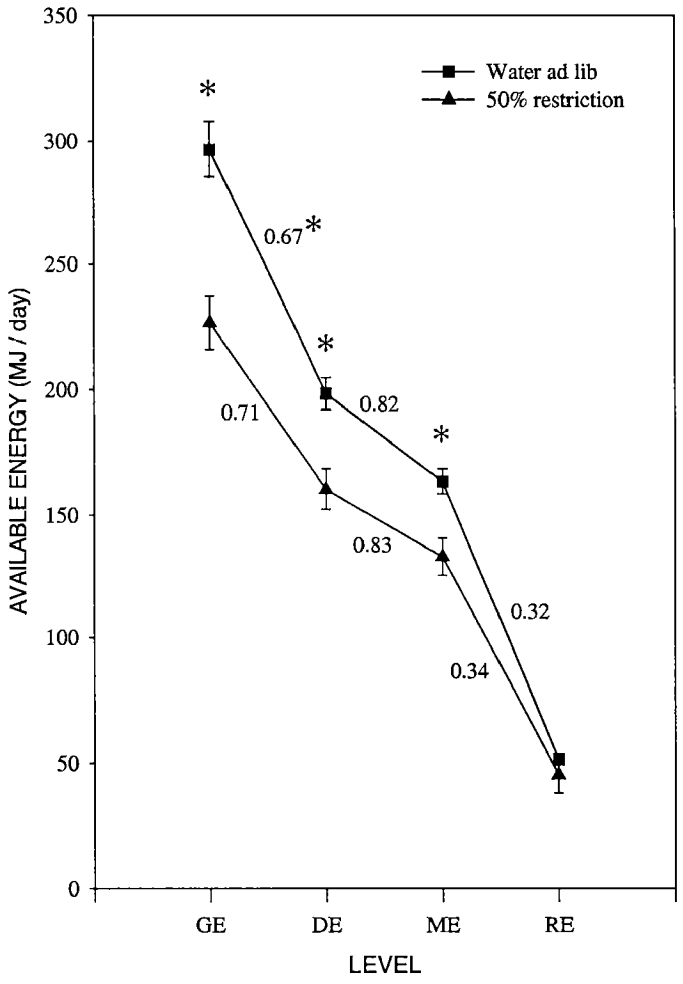

Fig. 22: Energy utilization levels of 6 lactating dairy cows with ad libitum access to water and 50% water restriction (Experiment 2). Data are means ± SEM. The numbers represent the coefficients of utilization from one level of energy utilization to the next. GE: gross energy, DE: digestible energy, ME: metabolizable energy, RE: retained energy. *Significant (P< .05) difference between water ad lib and 50% restriction values. See text for further details.

Table 4. *Gaseous exchange, heat production and body fat balance of 6 lactating dairy cows with ad libitum access to water (Baseline) and with 50% water restriction (Restriction) in Experiment 2*

	Baseline	Restriction
O_2-consumption, L/d	5212 ± 174	4125 ± 120*
CO_2-production, L/d	5758 ± 214	4375 ± 132*
CH_4-production, L/d	640 ± 27	488 ± 20*
Heat production, MJ/d	111.5 ± 3.8	87.3 ± 2.5*
Extra heat for milk production (k_L= .6)	40.8 ± 1.5	29.2 ± 1.4*
Heat spent for maintenance	70.7 ± 2.4	58.1 ± 1.7*
Body fat balance, kg	- .62 ± .09	- .08 ± .17*

Values are means ± SEM of 6 cows. *Significantly ($P < .05$) different from baseline (Wilcoxon test). k_L: efficiency of utilization of dietary metabolizable energy for milk production

The plasma concentrations of urea and sodium as well as the hematocrit were higher ($P < .05$) during the water restriction than during the baseline period (Table 5). Plasma concentrations of lactate, BHB, and chloride, as well as plasma osmolality, tended to be higher during the water restriction period; plasma glucose, FFA, and glycerol tended to be lower (Table 5), but all these differences did not reach significance. Plasma concentrations of insulin, cortisol, glucagon, triglycerides, protein, and potassium, did not change during water restriction (Table 5). The plasma concentrations of some metabolic hormones and metabolites showed great individual variations due to one animal which was in estrous during the baseline period.

Table 5. *Composition of the blood plasma of 6 lactating dairy cows with ad libitum access to water (Baseline) and with 50% water restriction (Restriction) in Experiment 2*

	Baseline	Restriction
Insulin, μU/mL	8.9 ± .6	8.5 ± .6
Cortisol, nmol/L	21.5 ± 3.2	17.3 ± 2.1
Glucagon, pg/mL	126.5 ± 11.7	128.8 ± 11.6
Glucose, mmol/L	3.47 ± .23	3.17 ± .14
Lactate, mmol/L	.58 ± .04	.61 ± .02
BHB, mmol/L	.73 ± .06	1.11 ± .17
Triglycerides, mmol/L	.20 ± .01	.20 ± .01
FFA, mmol/L	.12 ± .05	.05 ± .00
Glycerol, μmol/L	19.3 ± 1.2	18.5 ± 1.1
Proteins, g/L	75.3 ± 2.2	75.3 ± 2.3
Urea, mmol/L	3.0 ± .1	5.5 ± .1*
Na, mmol/L	135.2 ± .5	139.3 ± .7*
K, mmol/L	4.0 ± .1	4.0 ± .1
Cl, mmol/L	99.5 ± 2.0	103.0 ± 1.8
Osmolality, mosm/kg	275.3 ± 4.4	282.8 ± 1.5
Hematocrit	29.6 ± .8	31.5 ± .6*

Values are means ± SEM of 6 cows. *Significantly ($P < .05$) different from baseline (Wilcoxon test).

5.4. Discussion

This study shows that even lactating dairy cows are able to cope with a sustained 50% restriction of drinking water intake (approximately 44% of total water intake), and identifies some of the mechanisms involved. In demonstrating that water restriction primarily reduces meal size, the results of the first experiment confirm and extend previous studies of our group in which ruminants were completely deprived of drinking water for shorter periods of time (Langhans et al., 1989; Senn et al., 1996). The new finding is that this reduction in meal size was also present with sustained water restriction, and that it was particularly pronounced for the first meal on every day. This is surprising because the cows consumed substantial amounts of water around the time of this first meal. It is unlikely that meal size was somehow limited by the competing drinking behavior or by rumen space, because on the first day of rehydration after 25% water restriction the size of the first meal was much bigger than during water restriction (see Figure 16) although the cows ingested 38 L of water during the first hour of access, i.e. exactly the amount that was consumed in the same time with 50% restriction. Despite the substantial reduction in its size, the first meal was still the biggest meal of the day during water restriction. As rumen hypertonicity has been proposed to be a major control of meal size in ruminants (Carter and Grovum, 1990a), it is possible that the intake of a substantial amount of water prior to and together with the first meal allowed the cows to eat more in this meal before a level of rumen fluid or perhaps systemic osmolality was reached that limited meal size. In particular with 50% water restriction, this level would be reached much sooner during subsequent meals, when no more water was available. Results from another study in which we measured rumen fluid osmolality in relation to spontaneous meals in cows with unlimited access to water and during water restriction (Steiger Burgos et al., in press) are consistent with the idea that an abnormal prandial increase in rumen fluid osmolality contributes to the meal size reduction during water restriction. Other possible factors include systemic hypertonicity (Steiger Burgos et al., in press-a) and cardiovascular and vascular volume effects of feeding related to the copious production of saliva in ruminants (Bailey, 1961; Carter and Grovum, 1990a; Silanikove and Tadmor, 1989), which could not be detected with the design of the present study. Despite the marked reduction of the first meal's size, it should be noted that the food intake suppression which occurred with 50%

drinking water restriction was mainly due to the consistent reduction of subsequent meals' sizes, because this effect, similar to the feeding suppressive effect, was not observed with 25% water restriction.

Most of the parameters measured in the first experiment, in particular body weight, reached a new and remarkably constant level after 3-4 days of water restriction. Moreover, when water was offered ad libitum again, there was no sign of a compensatory increase in food intake. Thus, the cows appeared to reach a new equilibrium after 3-4 days of water restriction. The second experiment was based on the assumption that the cows were in stable energy balance during the balance measurement periods, and that representative mean values could be calculated over the last 5 days within the water restriction period. The unchanged water excretion/water intake ratio during the baseline and restriction periods in Experiment 2 confirmed that a new equilibrium was reached.

The observed increases in hematocrit and plasma osmolality are signs of a reduced plasma volume (El-Hadi, 1986) in response to water restriction. Also, the rumen water volume was certainly reduced, and this presumably accounted for a major part of the total water loss. Utilization of gut water helps to attenuate the rise in blood plasma osmolality during dehydration (Silanikove and Tadmor, 1989). Active sodium absorption from the rumen (Holtenius and Dahlborn, 1990), which usually drives fluid absorption, presumably contributed to the observed hypernatremia. Increased sodium retention in the kidney also helps to conserve water. It reflects the activation of compensatory endocrine mechanisms (renin-angiotensin-system, aldosteron, vasopressin) by the reduction in plasma volume and the increase in plasma osmolality (Ben-Goumi et al., 1993; Blair-West, 1979; Langhans, 1988). With an increase in plasma sodium, renal sodium excretion eventually increases again (McKinley et al., 1983; Michell and Moss, 1995) and stabilizes plasma sodium at a higher level. Plasma sodium is also recycled and concentrated in the saliva (Silanikove, 1994).

Water restriction caused an increase in the apparent digestibility of organic matter and energy, which helped to maintain energy balance. It is unlikely that the better digestibility during water restriction was an artifact of the short collection period of only 5 days because others reported similar results with longer adaptation and collecting periods (Balch et al., 1953; Silanikove, 1985). One reason for the better digestibility is

probably a longer mean retention time of the digesta in the gastrointestinal tract, in addition to a decrease in the size of particulate matter in the rumen (Choshniak et al., 1988). So, the marked osmotic changes that presumably occurred every morning when the cows quickly consumed the allotted amount of water did not seem to inhibit the fermentation capacity of the rumen microorganisms (Bergen, 1972; Brosh et al., 1983). In another similar experiment of ours (Steiger Burgos et al., in press-a) in which drinking water intake was restricted by 65% of ad libitum intake, rumen fluid osmolality in fact never reached 400 mosm/kg, i.e. a level above which cellulose degradation appears to be inhibited (Bergen, 1972).

The apparent digestibility of nitrogen was not improved and, in fact, the nitrogen balance became negative when water was restricted. It is not clear why the apparent digestibility of nitrogen did not change. Brosh et al. (1987) found the dehydration-induced increase in nitrogen digestibility to be inversely related to the quality of the diet. But the quality of our ration was not high enough for such an explanation. Perhaps enhancing effects of water restriction on nitrogen digestibility at the site of the rumen were compensated by the incomplete digestion of the rumen microbial protein synthesized from the additional ruminally fermented organic matter. It is of course also possible that changes in endogenous nitrogen excretion masked changes in true nitrogen digestibility. Further studies are necessary to clarify this question. The negative nitrogen balance was due to the relative increase in nitrogen excretions (in % of intake) in form of urinary urea and, partly, milk urea as a result of the elevated plasma concentration of urea. Tissue protein breakdown and the subsequent increase in amino acid catabolism was the most likely source of the increase in plasma urea concentration. Recycling urea in the kidneys helps to reduce urinary volume and, hence, to conserve water (Maltz et al., 1981). The increased plasma urea content was unlikely to be a consequence of the reduced nitrogen needs for milk production because water restriction increases plasma urea also in non-lactating animals (e.g. Qinisa and Boomker, 1999)

During the water restriction period the cows still had about 81% of the baseline metabolizable energy available. This energy was used for maintenance and milk production (neglecting the needs for early pregnancy). When water is limited, milk production declines according to the water and nutrient shortage (Dahlborn, 1987), thus

decreasing the energy needs for milk production to a certain extent. Interestingly, the results suggest that the energy needs for maintenance also declined. A similar observation was reported by Brosh et al. (1986) in non-lactating, infrequently watered, desert-adapted goats. These authors based their conclusion on the reduced O_2 consumption under these conditions, and on the fact that the goats did not loose body mass during dehydration except for body water. In the dairy cows of our study the absolute heat production for maintenance was reduced by water restriction. According to the nitrogen and fat balance calculations, the cows may have lost about 60 g proteins and 80g fat per day during water restriction. This appears to be at odds with the numerically positive energy balance, but all these changes were very small and are probably within the error range of the methods employed. Therefore, the present data do not allow us to judge whether the cows lost some solid substances during water restriction or not. In Experiment 1, the cows completely regained their body weight during the first day of rehydration, suggesting that they had mainly lost water during the water restriction period. Silanikove and Tadmor (1989) showed that body water loss accounted for about 89% of the total weight loss of non-lactating beef cows during 3 days of water deprivation. If a similar relationship held for the lactating dairy cows in the present study, they would have lost about 59 kg of water and 7 kg solid substances. This would have negligible effects on net energy for maintenance when using the following equation (AFRC, 1990): $NE_M = (.53 \times \text{body weight} / 1.08)^{.67} + .0091 \times \text{body weight}$. The energy requirements for maintenance include the energy needed for gut metabolism (eating, food processing and absorption), which was found to decrease with the decrease of food intake in non desert-adapted animals (Webster et al., 1975). This could contribute to the decrease in energy requirements for maintenance in the cows of the present study. All in all, it remains unsolved whether and to which degree reduced maintenance requirements and/or an increased efficiency of utilization of metabolizable energy for milk production was responsible for the slight increase in overall utilization of metabolizable energy during water restriction (.34 vs .32).

The results of the blood metabolite measurements fit the roughly stable energy balance. The lack of a change in plasma fat and carbohydrate metabolites indicates that there was no fat mobilization and, hence, no energy deficit during water restriction. The lack of changes in the plasma levels of metabolically active hormones is also consistent with this interpretation. In addition, the constancy of the plasma cortisol concentration

across the baseline and water restriction periods suggests that the cows were not continuously stressed by the water restriction. This is also interesting from an animal welfare point of view. In line with our findings, others (Qinisa and Boomker, 1999) recently reported that also in South African indigenous goats several blood parameters changed only during the first few days of a 50% water restriction period and returned to baseline levels thereafter. Thus, in principle, it could be possible to extend the water restriction period for a longer period of time without additional effects, as did Little et al. (1978) during 3 weeks, but with a lower level of water restriction (40%).

To our knowledge, this is the first report in which calorimetry measurements in respiration chambers were used to critically examine the changes in metabolism as well as energy and fat balance during water restriction. The results reveal that dairy cows are able to cope with a sustained restriction of total water intake to almost 50%, i.e. the decrease in milk yield and food intake, with the concomitant decrease in heat production (metabolism), and improved water retention combined to save sufficient water to reach a new equilibrium at a lower water turnover level. The body weight and food intake data show that the cows reached a new balance after 3-4 days of water restriction. The food intake suppression was primarily due to a reduction in meal size, which fits the idea that a an abnormal prandial increase in rumen fluid osmolality contributes to the food intake suppression during water restriction (Steiger Burgos et al., in press-a). The lower energy intake was compensated by a lower milk production, a higher digestibility and, apparently, a more efficient metabolic energy utilization. Further studies should also critically examine nitrogen metabolism during water scarcity because a loss of nitrogen, which might occur based on our nitrogen balance calculations, could become limiting for long-term adaptation to water scarcity.

6. ROLE OF FLUID HYPERTONICITY IN THE DEHYDRATION-INDUCED HYPOPHAGIA OF COWS

based on:

Steiger Burgos, M., W. Langhans and M. Senn. *Physiol Behav.*, in press.

6.1. Introduction

Ingestion of food in ruminants causes a rise in rumen fluid osmolality (Ternouth, 1967; Warner and Stacy, 1965), and rumen fluid hypertonicity may be an important contributor to satiation (the process that terminates a meal) in euhydrated animals (Carter and Grovum, 1990b; Phillip et al., 1981). Consistent with this idea, intraruminal infusions of hypertonic or hypotonic solutions were found to inhibit and stimulate food intake, respectively (Bergen, 1972; Phillip et al., 1981; Ternouth and Beattie, 1971). Phillip et al. (1981) found a correlation (r^2) of .71 – .84 between 30 min food intake and rumen fluid osmolality in sheep. Osmolality may be registered by osmosensors in the reticulo-ruminal wall (Bergen, 1972). The signal from these sensors appears to affect food intake directly, and not through a reduction of rumen motility (Carter and Grovum, 1990b).

Dehydration reduces food intake in ruminants (Bianca, 1966; French, 1956; Little et al., 1976) mainly by decreasing meal size (Senn et al., 1996). Without drinking water, rumen fluid osmolality increases during eating more than with ad libitum access to water (Ternouth, 1967). This raises the possibility that an abnormal increase in rumen fluid osmolality during eating is a major contributor to dehydration-induced hypophagia in ruminants.

The present study addressed this possibility. We first recorded the course of rumen fluid and plasma osmolality before and after a test meal when water was restricted or offered ad libitum. Then we investigated whether intraruminal infusion of water normalized food intake in dehydrated cows. In the last experiment a local anesthetic was given into the rumen in an attempt to inactivate the osmosensors potentially involved in dehydration-induced hypophagia.

6.2. Material and methods

Animals and housing conditions

Non-lactating, non-pregnant, rumen-fistulated Brown Swiss cows were used for all experiments. They were fed hay ad libitum. The mean composition of the hay across all three experiments was as follows: 5.0 ± .1 MJ/kg dry matter (DM) net energy for lactation, 72.5 ± 2.4 g/kg DM protein digestible in the intestine, 82.3 ± 8.9 g/kg DM crude protein and 866 ± 4 g/kg DM. The hay was freshly provided 4 times a day at 06.00, 10.00, 15.30, and 17.30 hr in an amount bigger than what the animals would eat before the next provision of food. Each time, the leftovers as well as the provided amount of fresh hay were weighed. In Experiment 1, food intake and meal patterns were continuously recorded on line by a computerized feeding system similar to the one described previously by Senn et al. (1996).

The drinking troughs were controlled by computer-assisted water meters and water valves (Bürkert-Contromatic AG Schweiz, Hünenberg, Switzerland). This system allowed us to continuously record (with a 1% maximum margin of tolerance) and to automatically restrict individual 24 h water intake (in Experiment 1). When an individually allotted volume of water was consumed, the water valve for this particular cow's drinking trough was automatically closed until the next morning.

Experimental procedures

In the first experiment 8 cows weighing 745 ± 37 kg (mean ± SEM) received the following 2 treatments in a cross-over design: water ad libitum for 5 days (baseline) and 65% water restriction for 5 days (restriction). The 65% water restriction was calculated individually based on each cow's mean ad libitum water intake recorded on 3 separate control days prior to the experiment. During restriction, the cows had access to water from 06.00 hr, i.e. beginning at the same time when food was first presented in the morning, until the allotted amount of water was consumed. On the last day of each treatment blood and rumen fluid samples were taken shortly before the second provision of fresh food (10.00 hr) and after the end of the first spontaneous meal after 10.00 hr (test meal). To standardize the conditions somewhat, no food was available from 09.00 to 10.00 hr. The size of the test meal was recorded using a meal definition (minimum

meal size 50 g, mimimum meal duration 1 min, mimimum intermeal interval 8 min) previously established under comparable conditions (Senn et al., 1996). After the test meal rumen fluid samples were taken in 30 min intervals until the next individual spontaneous meal. The last one of these samples was considered to be the pre-meal value of the second meal. The time between the end of the test meal and the onset of this second meal (intermeal interval) was measured. It must be noticed that on the last day of restriction all cows had consumed the allotted amount of water before 09.00 hr, i.e. no water was available during the test meal of the restriction treatment.

In the second experiment, 8 cows (769 ± 34 kg body weight) received the following 3 treatments in a within subject design: water deprivation for 24 h (deprivation), water deprivation for 24 h with intraruminal water infusion (infusion), and continuously water ad libitum (control). The cows had at least 48 h ad libitum access to water for recovery between treatments. With the deprivation and infusion treatments the water troughs were closed at 17.30 hr before feeding. The infusions started the next day at 10.00 hr and lasted until 14.00 hr. During these 4 hours the individual mean amount of water (32.8 ± 5.3 L) consumed from 17.30 hr to 14.00 hr during 3 control days with ad libitum access to water was infused intraruminally in 15 min intervals. Thus, about 2.1 L tap water at room temperature was infused every 15 min (Trial 1). After establishing the treatment effects on food intake, a second trial was performed under similar conditions to take rumen fluid and blood samples. Seven cows (753 ± 20 kg body weight) were used. The mean amount of water infused was 42.3 ± 4.4 L in 4 hours, i.e. about 2.6 L every 15 min. Blood and rumen fluid samples were taken at 17.30, 09.30, 12.00, 14.00, 15.00, and 17.00 hr. Food intake was measured from 17.30 hr (begin of water deprivation) for 24 h in both trials.

In the third experiment the same 7 cows (760 ± 20 kg) as in Experiment 2 (Trial 2) received the 2 following treatments in a cross-over design: 29 h water deprivation with intraruminal administration of a local anesthetic (mepivacaine HCl 2% (d,1-N-methyl pipecolic acid 2,6' dimethylanilide hydrochloride, Kantonsapotheke, Zürich, Switzerland), and 29 h water deprivation with administration of 1 L distilled water (control). The administrations were given at 10.00 hr, after 22 h of water deprivation. Two different doses of mepivacaine (2 and 4 g) were tested in two separate cross-over trials with a minimum intermittent period between trials of at least 48 h with water ad

lib for recovery. In the first trial 100 mL mepivacaine were administered with 900 mL of distilled water. In the second trial 200 mL mepivacaine were administered with 800 mL of distilled water. Rumen fluid samples were taken at 12.00 hr and at 17.00 hr on the day before the infusion, and at 09.30 (shortly before the infusion), 12.00, 14.00, 15.00, and 17.00 hr (shortly before the end of the deprivation period). Food intake was measured from 17.30 hr for 24 h.

Blood and rumen fluid samples

Blood samples were taken either by jugular vein puncture (Experiment 1) or through a jugular vein catheter (Experiment 2). The catheters (Cavafix Certo, B. Braun AG, Melsungen, Germany) were inserted about 3 h before the first blood samples were taken (at 17.30 hr), to allow the animals time for recovery . Ten mL blood were drawn into sterile tubes (after discarding the first 5 mL when taken from the catheter) and mixed with one droplet of heparin solution (Liquemin, Roche Pharma AG, Reinach, Switzerland). The tubes were immediately placed on ice, centrifuged (1600 g, 4°C, 15 min), and the plasma was frozen at –20°C until analysis. In Experiment 2 the catheters were filled with heparin solution (100 U/mL) after each blood sampling.

The rumen fluid samples were taken with a flexible pipe equipped with a sieve at one end to avoid obstruction by food particles. The pipe was inserted deep into the rumen, and a syringe was used to aspirate the fluid. Ten mL of rumen fluid were drawn in a tube, centrifuged (5100 g, 4°C, 15 min) and frozen at –20°C until analysis. Shortly before analysis, the samples were ultracentrifuged (centrifuge 5415C, Eppendorf Scientific, Westbury, NY). Occasionally, sterile filtration (.2 µm, Chromafil, Macherey-Nagel, Düren, Germany) in addition to ultracentrifugation was necessary to remove all suspended particles from the rumen fluid samples which interfered with the osmometer analysis. A freezing point osmometer (Multi-osmette, Precision Systems Inc., Natwick, MA) was used to measure plasma and rumen fluid osmolality.

Statistics

As the data were often not normally distributed, the Friedman two-way analysis of variance by ranks test was used for the paired, non parametric evaluation of overall

treatment and time effects on all variables measured. This test allows for the analysis of repeated observations on the same experimental unit with a small n-value. For pair-wise comparisons between treatments and for judging changes over time within treatments, the Wilcoxon signed rank test was used. All analyses were done with SYSTAT 8.0 (Systat Inc., Evanston, IL). P-values < .05 were considered significant. Data in the text and in graphs are presented as means ± SEM.

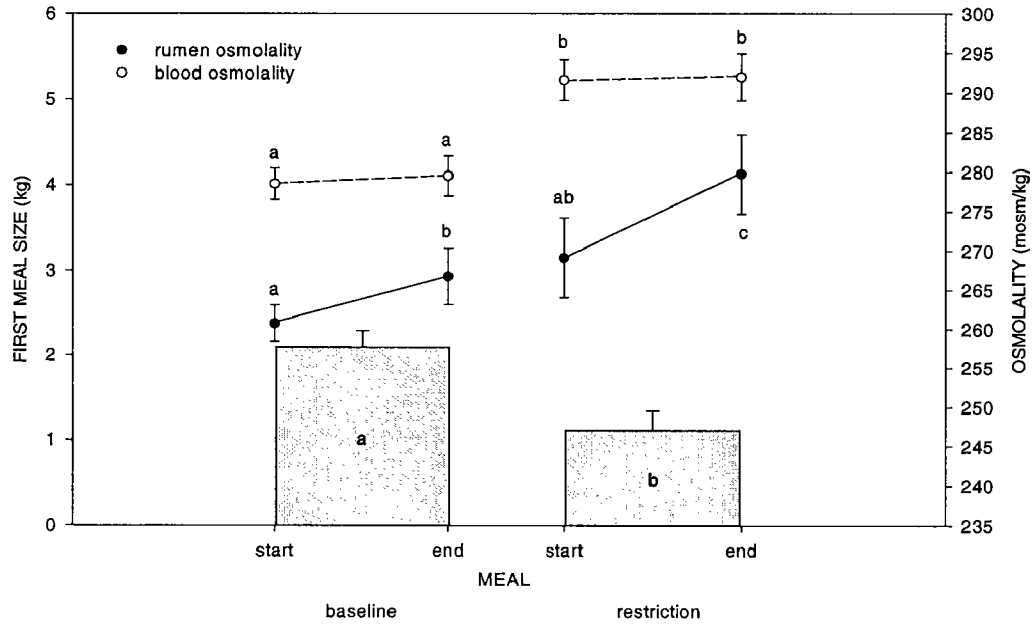

Fig. 23: Test meal size and prandial changes in rumen fluid and plasma osmolality of cows with ad libitum access to water (baseline) and with 65% water restriction. Data are means ± SEM of 8 cows (Experiment 1). [a, b, c] Corresponding values with different superscripts are significantly different (P< .05). See Text for further details.

6.3. Results

The test meal on the fifth day of water restriction in Experiment 1 was about 50% smaller (P < .05) than the corresponding meal during baseline (Fig. 23). During the test meal rumen osmolality increased during baseline (P < .05) and restriction (P < .05, Fig. 23), but the increase during restriction tended to be more pronounced despite

of the smaller meal (Fig. 23). Rumen fluid osmolality increased by 2.9 ± .9 mosm/kg food during baseline and by 11.1 ± 3.3 mosm/kg food during restriction (P = .069). Already before the test meal rumen fluid osmolality tended to be higher during restriction than during baseline (P = .058, Fig. 23). There were no significant changes in rumen fluid osmolality between the end of the test meal and the onset of the subsequent meal under both conditions. Yet, rumen fluid osmolality tended to decrease in that period during baseline (from 266.8 ± 3.6 to 263.9 ± 4.4 mosm/kg) and to increase during restriction (from 279.8 ± 5.0 to 285.8 ± 6.3 mosm/kg). Plasma osmolality was markedly higher during restriction than during baseline (P < .05), but unaffected by the test meal under both conditions (Fig. 23). Plasma osmolality was generally higher than rumen osmolality (P < .05, Fig. 23). The interval between the first and the second meal (intermeal interval) was shorter during restriction than during baseline (76 ± 31 vs 153 ± 16 min, P = .05).

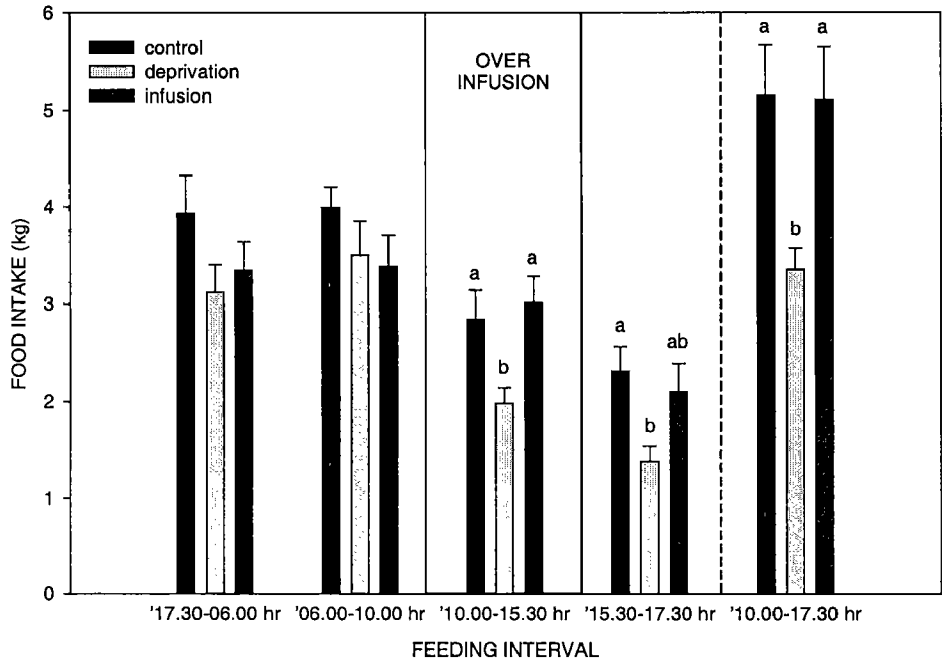

Fig. 24: Food intake of cows with ad libitum access to water (control), during water deprivation (deprivation), and during deprivation with intraruminal infusion of 32.8 ± 5.3 L of water during 4 hours (infusion). Data are means ± SEM of 8 cows (Experiment 2, Trial 1). [a, b] Corresponding values with different superscripts are significantly different (P< .05). See Text for further details.

In the first trial of Experiment 2, food intake started to decline with water deprivation but was not yet significantly decreased between 17.30 hr and 10.00 hr (Fig. 24). Yet, water deprivation decreased food intake compared to control conditions from 10.00 to 15.30 hr and from 15.30 to 17.30 hr ($P < .05$). Intraruminal water infusion increased food intake from 10.00 to 15.30 hr to the level observed under the control condition ($P < .05$, Fig. 24). Cumulative food intake from 10.00 to 17.30 hr was about 2 kg higher ($P < .05$) with deprivation and intraruminal water infusion than with deprivation alone. Similar changes in food intake were observed when rumen fluid and blood samples were taken in the second trial, i.e. the 39% suppression of food intake with water deprivation was blunted by the intraruminal water infusion.

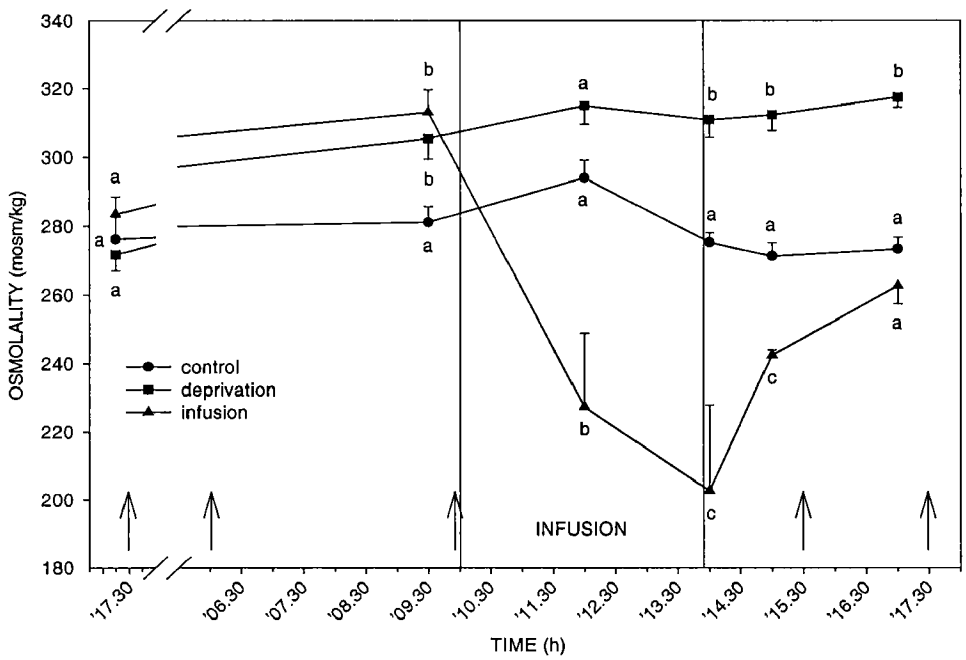

Fig. 25: Rumen fluid osmolality of cows with ad libitum access to water (control), during water deprivation (deprivation), and during deprivation with intraruminal infusion of 42.3 ± 4.4 L of water during 4 hours (infusion). Arrows indicate the feeding times. Data are means ± SEM of 7 cows (Experiment 2, Trial 2). $^{a, b, c}$ Corresponding values with different superscripts are significantly different ($P< .05$). See Text for further details.

In addition, water deprivation caused a gradual increase in rumen fluid osmolality compared to the control treatment ($P < .05$ at 09.30, 14.00, 15.00 and 17.00 hr, Fig. 25). During water infusion rumen fluid osmolality decreased below the control value until 15.00 hr ($P < .05$ at 12.00, 14.00 and 15.00 hr, Fig. 25); after infusion end, it increased again and was not different from the control value at 17.00 hr. The big variations during and at the end of infusion were presumably due to bad mixing of the infused water with the rumen content at sampling time in 2 animals. Rumen fluid osmolality remained constant under control conditions except for a transient small increase at 12.00 hr ($P < .05$, Fig. 25).

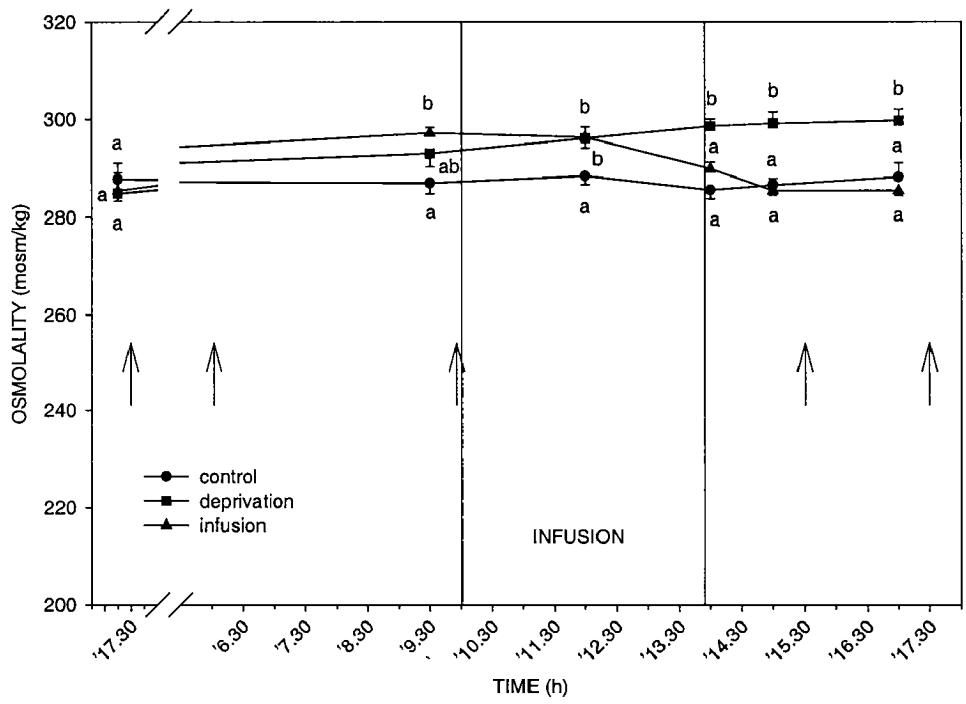

Fig. 26: Plasma osmolality of cows with ad libitum access to water (control), during water deprivation (deprivation), and during deprivation with intraruminal infusion of 42.3 ± 4.4 L of water during 4 hours (infusion). Arrows indicate the feeding times. Data are means ± SEM of 7 cows (Experiment 2, Trial 2). [a, b] Corresponding values with different superscripts are significantly different ($P < .05$). See Text for further details.

Plasma osmolality changes were also visible, but they were smaller than the changes in rumen fluid osmolality (Fig. 26). During deprivation, plasma osmolality slowly increased above the control level from 12.00 hr until the end of the measuring period ($P < .05$, Fig. 26). With a short delay, intraruminal water infusion caused plasma osmolality to decrease towards the control level (Fig. 26), which was reached one hour after the end of infusion (Fig. 26). Plasma osmolality did not change with the control treatment (Fig. 26).

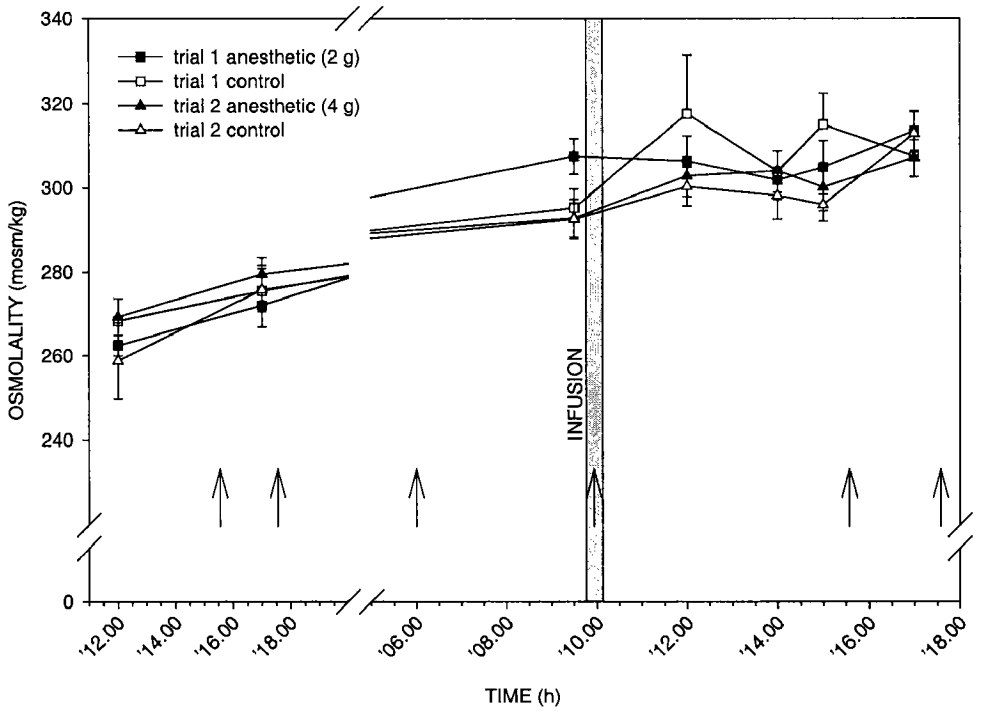

Fig. 27: Rumen fluid osmolality of cows during water deprivation with injection of 2 g (Trial 1) and 4 g (Trial 2) of the local anesthetic mepivacaine HCl (anesthetic) and during water deprivation with control infusion (1 L distilled water, control). Arrows indicate the feeding times. Data are means ± SEM of 7 cows (Experiment 3). There was no significant within trial difference except at 9.30 hr in Trial 1 ($P < .05$). See Text for further details.

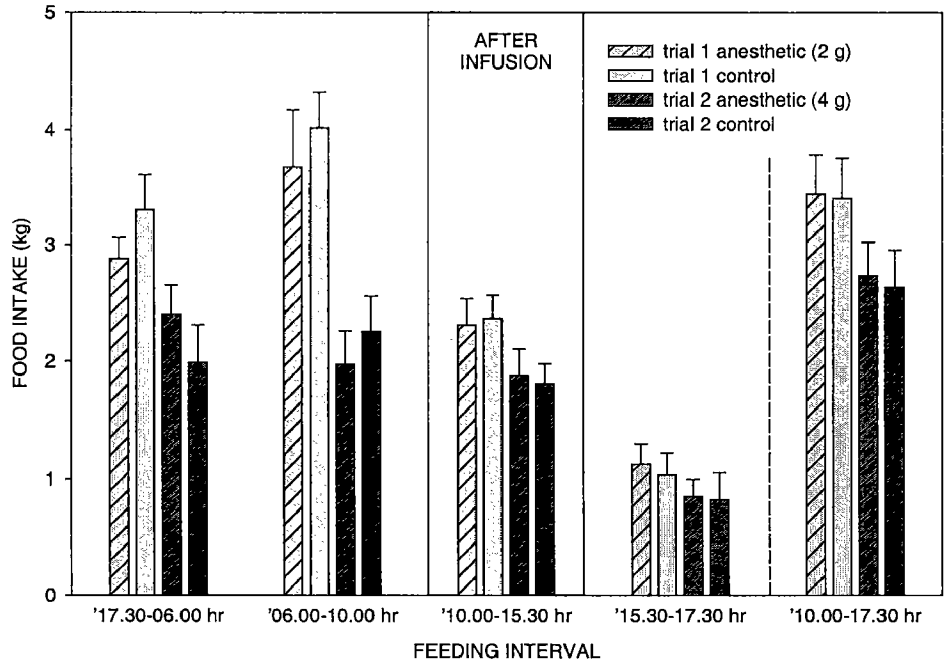

Fig. 28: Food intake of cows during water deprivation with injection of 2 g (Trial 1) and 4 g (Trial 2) of the local anesthetic mepivacaine HCl at 10.00 hr (anesthetic) and during water deprivation with control infusion (1L distilled water, control). Data are means ± SEM of 7 cows (Experiment 3). See Text for further details.

In the first trial 1 of Experiment 3, the intraruminal administration of 2 g mepivacaine per cow did not affect rumen fluid osmolality, except for a transient increase ($P < .05$) at 9.30 hr, shortly before the anesthetic was injected (Fig. 27). Rumen fluid osmolality gradually increased with increasing duration of the deprivation period, independent from the treatment (Fig. 27). Both doses of mepivacaine (2 and 4 g/cow) had no effect on food intake. It should be noted, however, that food intake appeared to be generally lower in the second trial than in the first trial (not statistically tested, Fig. 28)

6.4. Discussion

Despite the 50% smaller test meal, the observed prandial rise in rumen fluid osmolality was bigger (P = .069) with water restriction (when in fact no more water was available at the time of the test meal) than during baseline (with ad libitum access to water). This confirms and extends previous similar findings of others (Ternouth, 1967). The important difference between the two studies is that in Ternouth's experiment (1967) the sheep were trained to consume a certain amount of food, whereas in our study the animals could freely choose how much they ate. Our experimental condition therefore mimicked natural conditions in that food intake could be adapted to the lack of water.

The prandial rise in rumen osmolality in cows is equivalent to the increase in gastrointestinal osmolality in monogastric animals such as pigs (Houpt, 1984). In ruminants this rise is mainly due to an increase in the concentrations of volatile fatty acids produced during and after ingestion of food (Bennink et al., 1978). Theoretically the prandial rise in rumen fluid osmolality can be attenuated by fluids that enter the rumen and are derived from (i) prandial drinking (Senn et al., 1996), (ii) enhanced saliva secretion (Bailey, 1961), and (iii) inflow of extracellular fluid across the ruminal wall (Ternouth, 1967; Warner and Stacy, 1965). The latter two mechanisms cause a transient decrease in the extracellular fluid volume (interstitial and vascular compartments) and could therefore cause a reduction of plasma volume and an increase in plasma osmolality. Yet, without drinking water salivary secretion is reduced and the direction of the net water flow through the rumen wall is altered, i.e. rumen fluid is partly used to attenuate the rise in blood plasma osmolality (Silanikove and Tadmor, 1989). In the first experiment the rumen fluid and blood samples were taken on the 5th day of water restriction. At this time the water saving mechanisms were certainly activated (Steiger Burgos et al., in press-b). So there was probably a net outflow rather than a net inflow of water across the rumen wall. Evidence for rapid fluid absorption from the rumen during dehydration was provided by Dahlborn and Holtenius (1990), who reported that at least 1 L water was rapidly absorbed from the rumen in dehydrated sheep 20 min after a voluntary intake of 9 L water. A net water absorption from the rumen is also visible in Experiment 2: The increase in rumen fluid osmolality after the end of the water infusion coincided with the gradual decrease in plasma osmolality

towards the control level. Some of the infused water presumably passed the reticulo-omasal orifice and was absorbed in the omasum. But due to the comparatively small volume capacity of the omasum this was probably not a substantial amount. The seemingly opposite changes in rumen fluid osmolality between the end of the test meal and the onset of the following meal during baseline and restriction are also consistent with the assumption of an opposite water flow across the rumen wall under these conditions.

If rumen fluid hypertonicity is involved in the early satiation of dehydration-induced hypophagia, an intraruminal water infusion should inhibit the hypophagia. Consistent with this hypothesis, the results of Experiment 2 show that the water-deprived cows ate during and after the water infusion as much as under control conditions (water ad libitum). Yet, although water infusion decreased rumen fluid osmolality far below the value observed under control conditions, the cows did not eat more. Thus, although an increase in rumen fluid osmolality may well suppress eating, an abnormal decrease in rumen fluid osmolality obviously did not increase food intake above the control level. This is in line with the observation that food intake in ruminants does also not increase above the control level in response to dehydration after water deprivation (Senn et al., 1996), i.e. at a time when substantial oral water intake temporarily causes rumen fluid hypotonicity. The intraruminal water infusion simultaneously normalized plasma osmolality and food intake, suggesting that systemic hypertonicity also contributes to dehydration-induced hypophagia. As there were no prandial changes in plasma osmolality, however, systemic hypertonicity alone can scarcely provide the fast acting signal that reduces meal size during dehydration. Given the importance of bidirectional fluid exchanges between rumen and plasma for osmoregulation and fluid balance in ruminants (Silanikove and Tadmor, 1989), perhaps the difference between rumen fluid and plasma osmolality or some other integrator of both parameters plays a role in dehydration-induced hypophagia. It is interesting in this context that the difference between rumen and plasma osmolality at the end of the test meal during baseline and restriction was similar (about 13 mosm). Plasma osmolality could be registered by osmosensors located in liver (Forbes and Barrio, 1992) or brain.

The intraruminal infusion of water through a fistula does not allow to fully exclude possible oro-pharyngeal sensory effects, as boluses are regurgitated during

rumination (Forbes and Barrio, 1992). Nevertheless, it seems unlikely that an intraruminal water infusion stimulates food intake in dehydrated animals mainly through oro-pharyngeal effects because rumination usually occurs after eating. It appears more likely that the inverse relation between rumen osmolality and food intake that is known to exist in euhydrated ruminants (e.g. Carter and Grovum, 1990b) also holds for dehydrated ruminants, and thus plays a role in the short-term regulation of food intake during dehydration, perhaps in concert with plasma osmolality (see above). The influence of rumen fluid osmolality also appeared to be more important than any effect that the water infusion perhaps had on rumen distension. This assumption is in line with previous observations (Ternouth and Beattie, 1971).

The feeding inhibitory effect of rumen fluid hypertonicity is supposed to be mediated through osmosensors in the reticulo-ruminal wall (Bergen, 1972). But intraruminal infusion of a local anesthetic failed to stimulate feeding in water-deprived cows in Experiment 3. It should be noted, however, that in similar experiments in euhydrated small ruminants (Bergen, 1972); Martin and Baile, 1972; Carter and Grovum, 1990b) only intraruminal infusion of carbocaine reversed the suppression of feeding in response to intraruminal infusion of hypertonic salt solutions, whereas other local anesthetics (Xylocaine, Oxethazaine and Lidocaine) had no effect. The mepivacaine hydrochloride that we used is the same substance as carbocaine. Nevertheless, none of the 2 doses tested attenuated the hypophagia in water restricted cows. Preliminary studies, which are not reported here, revealed that the 1 L volume of fluid administered with the anesthetic or the control infusion was not large enough to stimulate feeding and, hence, to obscure a possible effect of the anesthetic. Some authors question the existence of reticulo-ruminal osmosensors. Forbes and Barrio (1992) for instance speculated that the osmotic changes in the rumen may act indirectly on the concentration of sodium and potassium in the extracellular fluid of the rumen mucosa, and may therefore affect the frequency of afferent impulses in the vagus nerve. In general, the failure of the anesthetic to increase feeding in dehydrated cows is not consistent with a role of reticulo-ruminal osmosensors in dehydration-induced hypophagia, but it does also not imply that rumen fluid hypertonicity is not involved. The infused doses of the anesthetic were perhaps not sufficient to reach and inactivate all the receptors, or it was not distributed fast and well enough to do so. This may be particularly true for cows because so far the intraruminal infusion of a local anesthetic

was shown to stimulate feeding under certain conditions only in small ruminants. Carter and Grovum (1990b) also mentioned the dilution of the anesthetic in rumen contents and its breakdown by the rumen microorganisms as potential problems. In Trial 2 of Experiment 3 the cows ate generally less than in Trial 1. As a preliminary experiment and the two trials were performed within 1 month, it is possible that by the time of the second trial the animals had learned that water would be available again at 17.30 hr, and that they adapted their feeding behavior to this schedule.

In conclusion, several signals presumably interact to reduce food intake during dehydration in ruminants (Langhans et al., 1995). According to the results of the present study, rumen fluid hypertonicity, perhaps in concert with plasma hypertonicity, may play a role in this behavioral phenomenon.

7. CONCLUSIONS

The results of the first part of this thesis show that intravenously infused bacterial lipopolysaccharides (LPS) reduced food intake and induced pronounced metabolic changes that were largely independent of the concomitant food intake reduction. The water restriction experiments (second and third part) show that dairy cows were able to cope with a sustained water restriction and reached a new balance after 3-4 days of restriction. Rumen osmolality seems to be an important factor involved in the food intake reduction in response to water restriction.

The LPS-induced anorexia and the food intake suppression by water deprivation, both, are examples of the impressive adaptation ability of the ruminant organism. They are an integral part of complex physiologic and behavioral reactions after a modification of the internal/external environment. The organism triggers this cascade of reactions in response to a new situation and in order to maintain homeostasis. The complexity of these physiologic reactions is reflected by the different changes in the various parameters measured (for example all the blood parameters examined in the first study). Nevertheless, to fully comprehend this complexity still more parameters would have to be measured, which could not be done due to financial and technical constraints. For instance, in the water restriction studies salivary secretion was not considered, although it largely contributes to the complexity of fluid homeostasis in ruminants (Silanikove, 1994). In a way, saliva provides an additional connection between the blood compartment and the gastro-intestinal tract. For a complete understanding of the mechanisms of dehydration-induced hypophagia it will be necessary to consider the changes in saliva production in relation to water restriction. This was originally planned as part of the present thesis, but had to be abandoned.

Intravenous infusion of LPS and lack of water, both, induced a marked reduction in food intake. This reaction appears surprising at first glance, considering that its own survival and the survival of the species have highest priority for the organism.

In the case of an infection, some authors experimentally demonstrated that hypophagia is beneficial for the survival of the host, at least in the short term (Murray

and Murray, 1979; Wing and Young, 1980). Hypophagia reduces mortality directly or indirectly in several ways (Hart, 1990). From the behavioral point of view, a decreased motivation to eat reduces the possibility of predation whilst the animal is less vigilant (Hart, 1990). Interestingly, the animal adopts the archetypical ‚sickness behavior', i.e. a sleeping posture that is only infrequently interrupted by ambulation (Exton, 1997). This behavior reduces heat losses and thus promotes fever production (Hart, 1990). The elevated body temperature enhances immune functions (Jampel et al., 1983), what is clearly a benefit for the infected organism. Hypophagia also contributes to hypoferremia by removing the dietary source of iron (Exton, 1997). This is of course particularly relevant for carnivores, but also for omnivores and, to some extent, for ruminants. Iron is an essential element required for many cellular mechanisms involved in the growth of diverse pathogens (Exton, 1997). Furthermore, the decrease in plasma iron level is thought to act synergistically with fever development as a host defense mechanism. When bacteria are grown in an iron deficient environment, their growth rate is considerably lower at febrile temperatures (Kluger and Rothenberg, 1979). Hypophagia has also effects on the immune system in increasing the proliferation of macrophages, natural killer cells and T lymphocytes (Exton, 1997). All these cell types play a crucial role in the host defense (production of cytokines, pathogen recognition and elimination). Hypophagia during infection has therefore many positive effects for the organism, at least initially. On the other hand, there is no doubt that longer lasting undernutrition compromises the defense reactions by depleting energy and protein stores and ultimately delays recovery.

The whole reaction to LPS must also be considered as part of an homeorhetic mechanism, i.e. a shift in the priorities of the organism. Spurlock (1997) has recently referred to the possibility that the proinflammatory cytokines could also be responsible for the slowdown of growth observed in growing ill animals. In this case the nutrients and in particular the amino acids would be directed away from tissue growth by the cytokines and provided for the acute phase reaction. This shows that the highest priority is given to host defense at the time of an infection.

Also dehydration-induced hypophagia serves the interest of the organism, at least in the short term. When the animal does not have the possibility to drink, eating less

helps to longer maintain an osmotic balance. Hypophagia prevents the rapid disturbance of this balance by an osmotic load (food) unbalanced by concomitant fluid intake. Radin et al (1996) showed in an experiment with chickens that serum osmolality rose faster in the birds that had no water but food available than in the birds without food and water. In dehydrated ruminants the failure to decrease food intake in response to water scarcity may even compromise the osmotic buffer function of the rumen. It may increase rumen fluid osmolality so much as to prevent the use of the ruminal fluid to alleviate the systemic hypertonicity that develops during dehydration. Unchanged food intake under these conditions might even trigger a water flow from plasma into the rumen and, hence, aggravate the dehydration. Therefore, the hypophagia during dehydration must be considered to be an overall beneficial compromise between the needs to ingest nutrients and to maintain osmotic balance.

The results of the second paper also provide yet another example for the preference that nature gives to the survival of the offspring over the survival of the maternal organism. Drinking water intake was reduced by 50%, but milk production decreased only by 27%. So in nature the suckling calf with access to no other source of nutrients and water than maternal milk would be submitted to a less severe water restriction than the cow and, hence, would have a better chance to survive. In a way, this phenomenon therefore represents also an homeorhetic mechanism, i.e. the priority is shifted from the functioning of the own organism to securing the survival of the offspring. In camels an even more extreme reaction is known: dehydrated camels have the ability to dilute their milk (Wilson, 1989). This dilution involves the production of milk with a higher water content when the animal is dehydrated than when it is fully watered. In that milk the concentrations of fat, lactose, protein, calcium and magnesium decreased, whereas sodium, phosphorus and chloride increased with no changes in urea level. The dilution of milk under dehydration was considered to be a physiological adaptation to ensure an adequate supply of fluid to the young. Yagil et al. (1986) reported the same ability in cattle, but we were not able to confirm that in our study. These different results may be related to the breed, to the climatic conditions, to the duration and severity of the water restriction, and to other factors. Nevertheless, the less severe decrease in milk production compared to the water restriction observed in our study shows that even European, non desert-adapted dairy cows have retained the

ability to protect their offspring from dehydration during times of water scarcity to some extent.

In conclusion, the present thesis describes a reduction of voluntary food intake as part of complex homeostatic mechanisms in two very different situations, i.e. in a model of bacterial infection and during dehydration. The mechanisms that lead to hypophagia are presumably different in both situations. Cytokines are at the core of the hypophagic reaction in the case of an infection, and rumen fluid hypertonicity presumably plays a role in the hypophagia during dehydration. Yet, in both situations the hypophagia has short term beneficial effects for the organism, and both situations represent good examples of homeorhetic mechanisms giving the best chances for survival.

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