Feeding and nutrition management of heat-stressed dairy ruminants

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ABSTRACT
Climate change, with a constant increase in the Earth temperature, negatively affects livestock production and health. This paper will focus on the possible nutritional and feeding strategies to mitigate the negative impact of heat stress (HS) on ruminants. The first nutrient interacting with hot weather conditions is fibre and its digestibility. It is well recognised how fibre may affect voluntary dry matter intake (DMI), chewing and ruminating activity. A possible dietary strategy to counteract the reduced DMI under hot environment is represented by the increased diet energy concentration, protein supply and essential amino acids. Mineral feeding under HS condition must cover the supply of each nutrient as consequence of altered turnover, and the needs related to buffer requirements. Particular interest is growing in the use of Se (Se-yeast), because of its role to support the animal antioxidative defences. Water is a pivotal nutrient to cope with HS in ruminants because it plays a role in animal thermoregulation. Recently, some vitamins and feed additives were studied for their action on the animal physiology to cope with HS. Among vitamins, niacin was tested for its action on the vasodilatation in the mammalian and for its role in lipid metabolism; among feed additives, yeasts and plant extracts may exert a positive action in rumen metabolism as well as in regulation of body temperature.

Introduction
Climate change is likely to have several impacts on feed crops and grazing systems (Hopkins and Del Prado 2007; Nardone et al. 2010). The main expected effects in new scenarios may be: (i) change in herbage growth as consequence of changes in atmospheric CO2 concentrations and temperatures; (ii) change in pasture composition, particularly in the grass:legume ratio; (iii) changes in herbage quality (i.e. changing concentrations of water-soluble carbohydrates and N at given dry matter yields); (iv) increasing drought that may reduce DM yield; (v) and finally, greater intensity of rainfall that may increase N leaching in certain systems.

Animals go through heat stress (HS) when the body temperature is higher than the optimal range specified for the normal activity because the total heat load is greater than the capacity for heat dissipation (Bernabucci et al. 2010). The thermal comfort zone for temperate-region adult cattle is in the range from 5 to 15°C as proposed by Hahn et al. (2003). McDowell (1972) revealed that significant changes in feed intake and physiological processes occur with temperatures greater than 25°C. However, the thermal comfort zone changes as a function of several other factors, including environmental humidity and air speed, genotype, physiological state, thermal susceptibility, acclimation and diet. Animals attempt to maintain the body temperature increasing heat loss and reducing heat production by physiological and behavioural responses.

Three management strategies have been identified to minimise the effects of HS: (1) physical modification of the environment, (2) genetic development of heat tolerant breeds and (3) improved feeding and...
nutritional management practices. The aim of the present review is to discuss the impact of hot weather on nutrient requirements and feeding practices with particular attention to domestic dairy ruminants.

**Metabolic acclimation under hot environment**

Reduced feed intake occurring in animals exposed to hot environment partly explains the biological mechanism by which HS impacts production and reproduction (Bernabucci et al. 2010). This also includes an altered endocrine status, reduction in rumination and nutrient absorption, and increased maintenance requirements (Collier et al. 2006) resulting in a net decrease in nutrient/energy availability. Reductions in energy intake coupled with increased maintenance costs during HS causes negative energy balance (NEBAL) in lactating cows (likely stage of lactation independent) and a bioenergetic state, similar (but not to the same extent) to the NEBAL observed in early lactation. Table 1 summarises the endocrine and metabolic acclimation related to energy metabolism during hot conditions in cattle.

Some studies demonstrated that despite reduced feed intake, heat-stressed mid-lactating cows do not have an increase in plasma NEFA (Abeni et al. 2007; Shwartz et al. 2009; Calamari et al. 2013; Rhoads et al. 2013). This agrees with the results obtained in other HS ruminant models (Sano et al. 1983; Itoh et al. 1998; Ronchi et al. 1999; Al-Dawood 2017). This is surprising as calculated energy balance is traditionally thought to be closely associated with circulating NEFA levels. The fact that heat-stressed cows fail to enlist this ‘shift’ in post-absorptive energetic metabolism (despite inadequate nutrient intake) may indicate that HS directly (not mediated by feed intake) impacts energetics (Ronchi et al. 1999). Despite marked reductions in nutrient intake, heat-stressed cattle exhibit increased basal insulin levels and stimulated insulin response (Itoh et al. 1998; Baumgard and Rhoads 2007; Wheelock et al. 2010) and this agrees with heat-stressed rodent experiments (Torlińska et al. 1987; Morera et al. 2012). The increased basal and stimulated insulin levels may explain the lack of an increase in basal NEFA levels in heat-stressed cows, as insulin is a potent antilipolytic hormone (Vernon 1992). The increase in basal insulin levels appears due to increased pancreas secretion, rather than reduced circulating insulin removal, because of the acute marked difference in insulin levels between heat-stressed and thermal neutral pair-fed cows following administration of an insulin secretagogue (Baumgard and Rhoads 2007).

In addition to adipose tissue, skeletal muscle is also mobilised during periods of inadequate nutrient intake (in thermal neutral conditions) to support lactation. Some studies demonstrated that mid-lactating heat-stressed cows (Baumgard and Rhoads 2007; Shwartz et al. 2009) and heifers (Ronchi et al. 1999) have increased plasma urea nitrogen levels compared to thermal neutral controls. Plasma urea nitrogen can originate from at least two sources: inefficient rumen ammonia incorporation into microbial proteins or from hepatic deamination of amino acids mobilised from skeletal muscle. A better circulating indicator of muscle catabolism is either 3-methyl-histidine or creatine, both of which are increased in heat-stressed poultry (Yunianto et al. 1997), rabbits (Marder et al. 1990) and lactating cows (Schneider et al. 1988).

Finally, the response of dairy cows to HS also depends on the interaction between physiological phase and ambient temperature. In a recent study, Basiricò et al. (2011) showed that transition heat-stressed cows had higher NEFA in agreement with changes of BCS, than their counterparts exposed to thermal-neutral conditions. Those results disagree to studies carried out on non-lactating or mid-lactating dairy cattle in which NEFA are usually reduced or did not change as reported before.

**Table 1.** Some endocrine and metabolic acclimation related to energy metabolism during hot conditions in cattle.

| Response                              | Reference                                      |
|---------------------------------------|------------------------------------------------|
| Reduced glucocorticoid secretion      | Collier et al. (1982)                         |
| Increased epinephrine secretion       | Alvarez and Johnson (1973)                    |
| Increased leptin secretion            | Bernabucci et al. (2006)                      |
| Decreased somatotropin secretion      | McGuire et al. (1991)                         |
| Decreased thyroxine secretion         | Collier et al. (1982)                         |
| Increased insulin secretion           | Baumgard and Rhoads (2007)                    |
| Decreased basal plasma glucose        | Ronchi et al. (1999); Abeni et al. (2007)     |
| Increased basal plasma NEFA           | Basiricò et al. (2011); Ronchi et al. (1999); |
| Decreased plasma NEFA                 | Wheelock et al. (2010)                        |
| Increased basal plasma BHBA           | Ronchi et al. (1999); Abeni et al. (2007)     |
| Decreased plasma cholesterol          | Ronchi et al. (1999); Abeni et al. (2007)     |
| Increased basal plasma urea           | Ronchi et al. (1999); Baumgard and Rhoads (2007) |

*Only in transition cows.*
Rumen and intestine health, passage rate and diet digestibility

The environmental HS determines a reduction in rumen motility either as contraction amplitude and frequency; this effect is direct and not mediated by a change in feed intake (Attebery and Johnson 1969; Bernabucci 2012). Additionally, the intraruminal temperature may affect rumen metabolism (Gengler et al. 1970). These authors showed that an increase in intraruminal temperature determines a reduction in feed and water intake (water intake increase only if necessary for the whole body thermoregulation), determining a decrease in volatile fatty acids (VFA) production and a shift in their composition with a significant decrease in the acetate to propionate ratio (Figure 1). Rumen temperature may be monitored by reticular temperature sensors (Ammer et al. 2016), as well as rumination time (RT) may be assessed and recorded by RT sensors (Soriani et al. 2013; Abeni and Galli 2016) available at commercial level. These recent studies evidenced how RT is dramatically reduced during the hottest-day period.

High environmental temperature adversely affects rumen health (Mishra et al. 1970) due to a variety of biological and management reasons (Bernabucci et al. 1999, 2009; Kadzere et al. 2002). Heat-stressed cows consume less feed and consequently ruminate less (Aganga et al. 1990; Soriani et al. 2013). Moallem et al. (2010) indicated that the primary negative effect of high temperature-humidity index (THI) is a depression of RT, which subsequently led to a reduction in DMI, followed by a decline in milk yield. The lower RT results in decreased buffering agents (ruminating is the primary stimulant of saliva production) entering the rumen. In addition, because of the redistribution of blood flow to the periphery (in an attempt to enhance heat dissipation) and subsequent reduction in blood delivery to the gastrointestinal tract, digestion-end products (i.e. VFA) are absorbed less efficiently and thus the total rumen VFA content may increase (and pH decreases). Furthermore, increased respiration rates also contribute to rumen acidosis because panting causes enhanced CO₂ to be exhaled. In order to be an effective blood pH buffering system, the body needs to maintain a 20:1 HCO₃⁻ to CO₂ ratio. Due to the hyperventilation-induced decrease in blood CO₂, the kidney secretes HCO₃⁻ to maintain this ratio. This reduces the amount of HCO₃⁻ that can be used (via saliva) to buffer and maintain a healthy rumen pH. In addition, panting cows often drool reducing the quantity of saliva available for the rumen. The reduction in saliva HCO₃⁻ content and the decreased amount of saliva entering the rumen make the heat-stressed cow much more susceptible to subclinical and acute rumen acidosis (Kadzere et al. 2002).

Changes in cow eating behaviour probably also contribute to rumen acidosis. High yielding dairy cows typically consume from 9 to 14 meals per day under thermal neutral conditions (Grant and Albright 2000). Under HS conditions, a shift of eating frequency during evening and night has been observed (West 1999; Pettera et al. 2006). Cows suffering from HS eat more

![Figure 1. Effects of environmental and ruminal temperatures on the rumen fatty acids production in cattle (adapted from Gengler et al. 1970). Treatments were: (18) ambient temperature 18.2 °C; (35) ambient temperature 35 °C; (18–43) ambient temperature 18.2 °C, intraruminal coil temperature 43.4 °C; and (18–51) ambient temperature 18.2 °C, intraruminal coil temperature 51.0 °C (Gengler et al. 1970).](image-url)
frequently, consume smaller meals, and spend less time lying than those not heat stressed (Petrea et al. 2006; Shiao et al. 2011). Steers eat more frequent but smaller meals under hot environment than under cool conditions (Hahn 1999). Furthermore, cows will typically gorge (over eat) the day following a heat wave (2006; Shiao et al. 2011). Steers eat more frequent but time lying than those not heat stressed (Petrera et al. 1999). Reduced rumen pH impairs fibre digestion efficiency: rumen fibrolytic bacteria are the most affected when decreased rumen pH drops (below 6.0). All of these factors may contribute to decreasing feed efficacy and acidosis is proven to affect the animals’ overall health status, fertility and longevity.

Intact intestinal epithelial tight junctions (TJs) are crucial barriers against paracellular penetration of pathogenic bacteria and toxic luminal antigens including endotoxins (Dokladny et al. 2006). The disruption of intestinal TJ barrier may result in a leaky TJ barrier favouring the permeation of toxic luminal substances. Various types of stresses, including HS, can cause increase of intestinal permeability to luminal endotoxins and lead to bacterial translocation (Lambert 2009). The gut derived endotoxins and pathogenic bacteria have been proposed to be responsible for the increased incidence of health problems such as heat stroke, sepsis, burn injury, ischemia-reperfusion injury, and in the critically ill.

The effect of HS on intestinal permeability has been studied in rat models. Hall et al. (2001) observed a significant increase in portal lipopolysaccharide concentration in rats heated to core temperatures of 41.5°C. Lambert et al. (2002) reported significant increases in intestinal permeability at core temperatures of 42.5°C in rats and at 41.5–42°C in everted rat intestinal sacs. Dokladny et al. (2006) showed Caco-2 cell monolayers maintained at 41°C over 24 h have significantly increased paracellular permeability and reduced epithelial resistance. The mechanisms underlying the effects of HS on intestinal barrier function is reported in a review paper by Lambert (2009). This author highlights that the combination of reduced intestinal blood flow and hyperthermia can cause loss of TJ integrity and likely enterocyte membrane damage. Reduced intestinal blood flow occurs with acclimation to HS as blood is diverted away from the splanchnic region to provide adequate perfusion of the skin (vasodilatation) for heat dissipation (Engelhardt and Hales 1977; Thatcher and Collier 1982; Hales et al. 1984; Lough et al. 1990). This can lead to intestinal hypoxia (Hall et al. 1999), which likely results in reduced cellular viability and increased paracellular permeability.

Reduced blood flow can also result in oxidative and nitrosative stress (Hall et al. 2001), which can damage cell membranes and open TJ (Hall et al. 1994). Furthermore, hyperthermia alone produces reactive oxygen and nitrogen species (Bernabucci et al. 2002; Lambert et al. 2002) leading to damaged cell membranes and TJ opening. Taken together, the dual effect of reduced intestinal blood flow and tissue hyperthermia during HS likely promotes significant intestinal mucosal damage (Lambert et al. 2002) leading to the passage of substances such as lipopolysaccharide into the internal environment.

Hyperthermia alone is responsible for enterocyte membrane damage. Intestinal barrier dysfunction with consequent increased permeability facilitates the penetration of endotoxins with consequent inflammatory response (Lambert 2009).

As reported before, the exposure to hot conditions is responsible for alteration of rumen functionality and changes in ingested diet with the increase in grain intake. Those factors are recognised to be responsible in a markedly greater concentration of rumen (Zebeli and Ametaj 2009) and serum (Emmanuel et al. 2008) lipopolysaccharide.

The alteration of gastrointestinal health together with changes in rumen functionality and increased risk of rumen acidosis make subjects exposed to HS conditions more susceptible to inflammation and oxidative stress and requires us to consider feeding adjustments to reduce the risk of serious problems these effects can produce.

Heat stress affects the nutrition of animals by altering the dynamic characteristics of the digestion processes (Beede and Collier 1986). After feed intake, the
second function in acquisition of nutrients is digestion. In a review, Kadzere et al. (2002) concluded that exposure to a hot environment is responsible for an increase of digestibility that may be explained by the reduction of DMI and prolonged retention of feed in the gastrointestinal tract. Nevertheless, results available in literature on the effects of hot exposure on diet digestibility are often conflicting. Some authors reported an increase in diet digestibility in cattle exposed to hot environments (Lippke 1975; National Research Council 1981; Christopherson 1985; Miron and Christopherson 1992; Weniger and Stein 1992; Bernabucci et al. 1999). In contrast, negative or no relationships between high ambient temperatures and diet digestibility have been reported in dairy cattle (McDowell et al. 1969; Mathers et al. 1989) and small ruminants (Silanikove 1985, 1992; Bernabucci et al. 2009). Some differences in responsiveness of sheep and cattle to thermal stress have been reported. Lippke (1975) found significant increases in digestibility of dry matter and fibre components of alfalfa pellets fed to steers housed at 32 °C compared with 21 °C. Goats adapted to a harsh environment (desert Bedouin goats) have higher digestion capacity of high fibre diet than non-desert goats (Saanan goats).

The increase in diet digestibility in heat-stressed ruminants was explained by increased mean retention time in the whole gastrointestinal tract (Coppock and West 1986; Miron and Christopherson 1992). Indeed, slower passage rate and longer mean retention time of digesta have been described in ruminants maintained under hot environments (Warren et al. 1974; Faichney and Barry 1986; Silanikove 1992; Bernabucci et al. 2009).

Reduction in DMI is generally associated with a decrease of rumen passage rate and an increase of diet digestibility in ruminants maintained in thermo-neutrality (Warren et al. 1974; Mulligan et al. 2001). Conversely, several authors have reported that, under hot conditions, diet digestibility and rumen passage rate were not affected by the changes of DMI (Attebery and Johnson 1969; Miron and Christopherson 1992; Silanikove et al. 1993; Bernabucci et al. 1999, 2009). In a previous study carried out in Holstein heifers (Bernabucci et al. 1999), rumen passage rate was not a determinant factor-influencing digestibility after prolonged exposure to hot conditions. A study, carried out on dairy sheep exposed to different periods to hot environment (Bernabucci et al. 2009), clearly demonstrated that diet digestibility of ewes chronically exposed to HS conditions is not related to changes in DMI or rate of passage of digesta into the gastrointestinal tract. Factors other than DMI or gut retention time may affect diet digestibility of heat-stressed ruminants. As reported before, HS is recognised to adversely affect rumen and intestine functionality. During HS, blood flow to rumen and intestine epithelium is reduced and the acid-base balance is altered. Heat stress induces a reduction in the amount of saliva produced and salivary HCO₃⁻ content, which may impair rumen functionality (Kadzere et al. 2002). Also dilution of rumen content due to higher water intake, reduction in rumen bacteria activity and decline in rumen motility may be responsible for digestibility changes when animals are chronically exposed to hot environment. The negative effect of a depression of rumen bacteria activity on diet digestibility might overcome the positive effects caused by the decline in DMI and digesta outflow rate, resulting in a net reduction of diet digestibility in chronically heat-stressed subjects. Other than alteration of bacterial activity, the reduction in digestibility might be related to the changes of ruminal and intestinal absorption of nutrients that might be dependent on an adaptive redistribution of cardiac output from the digestive system to peripheral tissues and respiration system to increase heat loss (Thatcher and Collier 1982; Christopherson 1985) as an acclimatisation response to hot environments (McGuire et al. 1991). A direct effect of HS on changes in blood flow that may alter the supply of nutrients may be involved (Beede and Collier 1986; West 1999).

**Nutrients and feeding management**

**Fibre**

Heat-stressed subjects show a decrease of their efficiency in energy utilisation. This is related to the higher maintenance requirements to alleviate excess heat load (Bernabucci 2012).

As reported by Chase (2006) using the Cornell Net Carbohydrate and Protein System model, the energy requirement for maintenance of a dairy cow (weighing 635 kg and yielding 36 kg of milk per day) at 32 °C is increased by 22%, when compared to the energy requirement at 16 °C.

Energy is generally the most limiting nutrient and a common approach aiming to increase energy density is to reduce forage and increase concentrate content of the ration. Digestion and metabolism may represent additional sources of heat production from the cow itself. High fibre diets may indeed increase heat production, as demonstrated by work showing that for diets containing 100, 75 or 50% of hay, the efficiency of conversion of metabolisable energy to milk was 54,
61 and 65%, respectively (Coppock and West 1986). As reported by Reynolds et al. (1991), diet containing 75% alfalfa resulted in greater heat production and reduced retained energy compared to a diet containing 75% concentrate. Moreover, the greater O₂ uptake by portal drained viscera and liver accounted for 44 and 72% of heat increment for the high concentrate diet and high alfalfa diet, respectively. Metabolism of acetate (related to high fibre diet) produces more endogenous heat than propionate (related to high concentrate diet; West 1999).

Therefore, the increased dietary fibre intake may increase heat load and then HS. West et al. (1999, 2003) reported that cows fed low fibre level (NDF = 30% of DM) during hot weather showed a higher daily milk production, lower body temperature and lower respiratory rates compared with those fed high fibre diets (NDF = 42% of DM). Thereafter, intake has a relevant effect on heat production and its role needs a great consideration in designing an effective nutritional and environmental management programme. Intake normally declines for high fibre diets, and West et al. (1999) demonstrated that in a diet with range of NDF concentration from 27 to 35%, the DMI decline was less severe, if NDF level was gradually increased during hot weather. Miron et al. (2008) evidenced how reducing NDF from roughage decreased the HS related symptoms, namely respiratory rate and rectal temperature, increasing DMI and milk production in lactating dairy cows. However, dietary fibre is necessary in adequate supply to ensure a correct rumen activity. Adin et al. (2009) observed that the reduction from 18 to 12% on DM basis of the amount of dietary NDF from roughage was responsible for a significant reduction of RT of a little bit less than 1 h.

The importance to avoid a dramatic drop in DMI is particularly crucial in the peripartum period, and this issue may be amplified under hot environment. Kanjanapruthipong et al. (2010) evidenced, that under hot and humid condition, is beneficial to reduce the level of NDF (from 21.0 to 17.4% on DM basis of dietary NDF from roughage) in the diet of the 3-week period before expected calving, with positive outcome on the postpartum metabolism and milk production (THI ranging between a minimum of 77.7 and a maximum of 86.8).

Sheep are more able to convert fibrous, low-quality feedstuffs into meat and other products than cattle (Hafez 1987). However, moderate HS decreases intake and growth in young sheep consuming a high diets containing high medium quality roughage (Marai et al. 2007).

Similar effects were also demonstrated for non-ruminant animals. Stahly and Cromwell (1986) reported a negative effect of dietary addition of alfalfa on daily gains, in pigs exposed to warm (22 °C) and hot (35 °C) conditions.

Under hot environments, nutrient requirements are altered during HS, which results in a need for reformulation of diets (Collier et al. 2006). In dairy cattle, a portion of the milk production lost (35–50%) during HS may be potentially recovered through nutritional management (Rhoads et al. 2013). The reduction in DMI and related efficiency of nutrient utilisation require a high dietary nutrient density if milk yield has to be maintained at a level comparable to that of thermo-neutral conditions (West 1999). In ruminants, diets formulated for low metabolic heat increments can help to improve feed intake and performance under HS conditions.

A high fermentable carbohydrate diets can be used under hot conditions to stimulate energy intake, but this positive effect must be balanced with the potential for rumen acidosis associated with high-grain diets (West et al. 2003). To avoid this disorder is important to maintain the optimal rumen function, with a level of ADF and NDF that should not be lower than 18% and 28% on dry matter basis of the diet, respectively (West 1999).

**Protein**

Lot of studies demonstrated that heat-stressed cattle were in negative nitrogen balance, as consequence of the reduction in feed intake (Kamal and Johnson 1970; Ronchi et al. 1999; Shwartz et al. 2009; O’Brien et al. 2010). The reduction in the feed intake can be counteracted by the increase of protein content of the diet, which can lead to an excess of nitrogen intake. The energy cost to metabolise the excess of N above requirements is 7.2 kcal/g of N (Tyrrel et al. 1979). Feeding excess of rumen degradable protein during HS was responsible of decreased DMI and milk production (Huber et al. 1994). Those authors explained this response with the extra energy required to convert protein into urea for excretion (Huber et al. 1994).

Endogenous heat production increases due to the metabolic utilisation of crude proteins and this is higher than that for starch or fat. The greater heat increment from crude proteins is partially related to urea synthesis and to greater protein turnover. Studies carried out in swine (Spencer et al. 2005; Silva et al. 2009) concluded that a decrease of crude protein content improves the finishing pig average daily gain under hot environments.
Other than the amount of protein fed, quality of protein source should be considered under heat-stress conditions. In a review paper, Huber et al. (1994) summarised that dairy cows fed diet containing 16.1% CP with low degradability (59% of total CP) had greater milk yield, than cows fed diet characterised by high protein content (18.5% CP) with medium degradability (65% of total CP). Belibasakis et al. (1995) reported that the level of ruminally undegradable protein had no effect on DMI during hot weather. However, milk yield increased and blood urea declined for diet containing high undegradable protein. Nevertheless, high level of rumen-undegradable proteins in diet did not improve the performance of dairy cows during unfavourable climatic conditions, as observed by Calamari et al. (2001). The same diet, richer in undegradable protein, including also calcium soaps of fatty acids (0.35 kg/d per head) and monopropylene glycol (0.3 kg/d per head), has allowed to obtain lower plasma urea, together with positive effect on milk yield and renneting milk clotting properties (Calamari et al. 2013).

Dietary essential amino acids may play a role in preventing risk of HS. During HS, transcription and translation of RNA are inhibited, with a reduction of milk protein synthesis (Sonn et al. 2002). Methionine is one of the major limiting amino acids for dairy cows (Lobley et al. 1987; Girard et al. 2005; Han 2009). Methionine supplementation improves milk production and antioxidant capacity, reduces lymphocyte apoptosis, promotes the expression of the Bcl-2 genes in lymphocytes, and inhibits the Bax gene (Nichols et al. 1998; Han 2009). Moreover, it was observed that the administration by perfusion of methionine can increase the synthesis of milk protein in lactating goats (Lin et al. 2013).

Also the supplementation of lysine showed similar effect. Huber et al. (1994) reported that the increase of lysine supplementation allowed the increase of milk yield (+11%). Moreover, cows under shaded environment and fed high lysine diet produced slightly more milk than those fed low lysine and receiving evaporative cooling plus shade, suggesting that dietary protein quality compensated for lack of cooling under hot weather (Huber et al. 1994).

In summary, it is necessary to increase the level of protein in the diet if DMI is depressed during HS. However, it is important to provide rumen undegradable proteins or improve protein quality, increasing the level of essential amino acids (particularly methionine and lysine), in diet with adequate energy availability. Despite these considerations, additional evaluations of the efficiency of dietary protein utilisation are needed.

### Fat

As previously discussed, the decrease in forage to concentrate ratio improves the efficiency of nutrients utilisation in animal exposed to hot environments. The addition of fat to the diet may be an optimal practice to obtain the same result.

Fat supplementation increases net energy intake in heat-stressed dairy cows thanks to its higher energy density and its lower metabolic heat, in comparison with fibre or starch (Baldwin et al. 1980; Morrison 1983; Beede and Collier 1986; Knapp and Grummer 1991).

Under thermo-neutral conditions, cows fed diets supplemented with protected tallow showed a more efficient utilisation of metabolisable energy for lactation, than those not receiving supplemental tallow (Kronfeld et al. 1980).

Literature reported conflicting results about the effect of fat supplementation under hot conditions (Table 2). Some studies showed beneficial effect for heat-stressed ruminants (O’Kelly 1987; Skaar et al. 1989), and other researches revealed ineffective (Knapp and Grummer 1991; Bunting et al. 1992, 1996) or detrimental (Huber et al. 1994; Gaughan and Mader 2009) effects.

Differences between studies carried out on ruminants are probably related to the effect of fat on

| Reference            | Fat Type        | RT | RR | DMI | FE | MY | MF | MP | Met     |
|----------------------|-----------------|----|----|-----|----|----|----|----|---------|
| Moallem et al. (2010)| CS(SFA/UFA)     | +  | -  | -   | +  | =  | +  | +  | =       |
| Wang et al. (2010)   | SFA             |    |    |     | +  | =  | +  | =  | =       |
| Warmjes et al. (2008)| SFA             | NM | NM | =   | =  | =  | =  | =  | NM      |
| Drackley et al. (2003)| LCFA           | =  | =  | =   | =  | =  | +  | =  | NEFA    |
| Calamari et al. (2001)| CS(SFA/UFA)  | =  | =  | =   | =  | =  | =  | =  | =       |
| Chan et al. (1997)   | SFA             | =  | =  | =   | =  | =  | =  | =  | NM      |
| Knapp and Grummer (1991)| LCFA/Tallow | =  | =  | =   | =  | =  | =  | =  | NM      |
| Skaar et al. (1989)  | SFA             | NM | NM | =   | =  | =  | =  | =  | =       |
| Moody et al. (1967)  | SFA             | =  | =  | =   | =  | =  | =  | =  | =       |

RT: rectal temperature; RR: respiration rate; DMI: dry matter intake; FE: feed efficiency; MY: milk yield; MF: milk fat; MP: milk protein; Met: metabolites; NEFA: non esterified fatty acids; SFA: saturated fatty acids; UFA: unsaturated fatty acids; CS(SFA/UFA): calcium soaps of SFA and UFA; LCFA: long chain fatty acids; NM: not measured; +: increase; -: decrease; =: No change.
ruminal fermentation (Van Nevel and Demeyer 1988). Diets with a fat supplementation lower than 5% have no toxic negative effects on ruminal microflora (Palmquist and Jenkins 1980).

Ruminally-protected fats in the diet lower metabolic heat increment significantly, improving the role of fats during heat-stress period. Holter et al. (1992) added 15% whole cottonseed, or 15% whole cottonseed plus 0.54 kg/d of a calcium salts of fatty acids and reported that heat production in excess of maintenance declined by 6.7 and 9.7%, and total heat loss declined by 4.9 and 7.0%, respectively. The high fat levels produced a measurable decline in heat production also under thermo-neutral conditions.

Despite the inconclusive results relative to the benefit of fat supplementation, it is possible to say that fats should be used with extreme caution in diets for heat stressed cows. Surely, the best option is the use of treated fat, which by-pass the rumen environment intact, thereby not affecting rumen microbial growth. Additional studies are needed to evaluate several levels and sources of fat supplemented over an extended period.

**Water**

Water is a basic molecule in the body of vertebrates because it is essential for the maintenance of some vital functions: tonicity of tissue (by electrolyte balance and osmotic regulation); lubrication and thermoregulation; nutrient transport; excretion. For the purpose of this review, it is pivotal the role of water in the homoeothermic subjects because it represents an important heat carrier for the regulation of thermal exchanges (Silanikove 2000). Water metabolism under heat stressful condition is closely linked to the thermoregulatory requirements of the ruminant.

High-producing dairy cows have higher metabolic rate than lower-producing ones; this implies they experience more difficulties to dissipate body heat during the hot season (Berman 2011), and this is also reflected in higher water requirement for thermoregulatory purpose. In dairy cows, the main water outputs are milk, urine, faeces, and various forms of evaporation (Murphy 1992), due to the needs for thermoregulation (Silanikove 2000; Berman 2006). A crucial role in high-producing dairy cattle is the carrier in the recycling of nutrients (mainly N and P) and in buffering action by saliva production.

Taking the example from Osborne (2006) for a lactating dairy cow of 640 kg of body weight (BW), the total body water (TBW) could be 422 L; with a water flux of 30% of TBW, it can be estimated a change of 140 L/d, and a complete potential water turnover every 3.3 days. In dairy cattle, Berman (2006) evidenced the increase in respiratory water loss (kg/h) due to the increase of air temperature. If under thermo-neutral conditions, the water excreted by milk yield corresponds to around one-third of the amount of drunk water (Woodford et al. 1984; Khelil-Arfa et al. 2014), during HS periods there is an increase in the water requirement for thermoregulation, as a result of an increased demand for evapotranspiration for heat dissipation (Berman 2006). This leads to a dramatic increase (polydipsia) in total water intake jointly with a shift in the partition of water across the different body compartments (Olsson et al. 1995). Table 3 summarises the main results on water fluxes under thermo-neutral and HS conditions in a French trial.

One important response is the great reduction of faecal water in lactating cow, a first attempt by the gastrointestinal tract to spare water to compensate the higher increase of evaporated water for thermoregulatory activity.

The changes in blood metabolites concentration due to water restriction should be separately considered in the acute and in the chronic restriction. During acute water restriction, serum protein and albumin increase due to the decreased blood volume; during chronic water restriction, both metabolites tend to decrease (Chedid et al. 2014). At the same time, acute water restriction induces the kidney to slow glomerular filtration and increase urea reabsorption; this induces the increase of plasma levels of creatinine and urea, whereas chronic water restriction induce a reduction of these plasma metabolites (Chedid et al. 2014). However, current knowledge seems not conclusive to explain if the reduction of plasma urea may be a result

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**Table 3. Comparison between water fluxes under thermo-neutral (TN) and hot (HT) conditions in lactating and dry cows (data adapted from Khelil-Arfa et al. 2014).**

| Item                        | Stage | TN   | HT   | Diff | Diff (% on TN) |
|-----------------------------|-------|------|------|------|----------------|
| Air temperature, °C         |       | 15.5 | 28.4 |      |                |
| THI                         |       | 59.4 | 73.2 |      |                |
| Free water intake, kg/d     | Lact  | 77.1 | 85.4 | 8.3  | +11            |
|                             | Dry   | 27.4 | 41.7 | 14.3 | +52            |
| Water ingested with feed, kg/d | Lact  | 30.9 | 27.4 | 3.5  | –11            |
|                             | Dry   | 20.6 | 20.6 | 0.0  |                |
| Urine, kg/d                 | Lact  | 17.8 | 20.4 | 2.6  | +15            |
|                             | Dry   | 13.5 | 13.8 | 0.3  | +2             |
| Fecal water, kg/d           | Lact  | 47.7 | 39.2 | –8.5 | –18            |
|                             | Dry   | 23.5 | 23.7 | 0.2  | +1             |
| Milk water, kg/d            | Lact  | 26.9 | 25.4 | –1.5 | –1             |
|                             | Dry   | 26.0 | 20.6 | –5.4 | –20            |
| Calculated metabolic water, kg/d | Lact  | 4.5  | 4.4  | 0.1  |                |
|                             | Dry   | 2.6  | 2.6  | 0.0  |                |
| Retained water, kg/d        | Lact  | 0.8  | 1.5  | –0.7 | –23            |
|                             | Dry   | 0.2  | 0.2  | –0.0 |                |
| Evaporated water, kg/d      | Lact  | 19.2 | 34.4 | 15.2 | +79            |
|                             | Dry   | 13.3 | 27.2 | 13.9 | +105           |

THI: temperature-humidity index.
of an increased recycling by rumen microflora as suggested by Chedid et al. (2014), rather than a secondary effect due to reduced DMI or an altered liver efficiency.

The dietary level of some nutrients may affect the water requirement, namely for an increased demand for urine excretion. This is the case for dietary crude proteins and K (Kume et al. 2010). Recently, a comprehensive model for water metabolism under thermo-neutral conditions gave a driving role to the requirement for N excretion in determining water loss by urine (Appuhamy et al. 2014).

Water restriction in lactating cows determines a reduction in feed intake, differently characterised by the extent and duration of water restriction. In trials with 48 h of water deprivation in cattle fed grass and corn pellet, the reduction of feed intake was mainly determined by a reduction of meal size for both grass and corn-pellet meals (Senn et al. 1996). Under thermo-neutral conditions, when the water restriction is at 50% of ad libitum intake, the decrease in water excretion in lactating cows amounts to 53% in water excretion by faeces, 43% in water excretion by urine, and 28% in water excretion by milk (Steiger Burgos et al. 2001). This essentially means that the first adaptation response is in the gastro-intestinal tract water reabsorption.

The ability to cope with water restriction under warm environment is affected by the evolutionary adaptation of species and breeds. From this point of view, it must be remembered that buffalo had its evolutionary adaptation under wet environments, and this led to a greater dependence on external water rather to be able to conserve internal water (Koga et al. 2002).

The main physic quality of interest for water during HS is its temperature. There is a general underestimation of the positive effect on thermal balance due to a low temperature of drunken water. Stermer et al. (1986) reported a positive effect of drinking water at 10°C compared to 28°C for the reduction of body temperature and breathing rate in lactating cows. To explain this effect by a thermal quantification, Lanham et al. (1986) summarised the results of three different trials evidencing the significant abatement of more than 250 kcal/d (371 vs. 718 kcal/d) by the ingestion of cold instead of ambient (28°C) temperature water by lactating dairy cows. Milam et al. (1986) reported how that extent of difference in drinking water temperature determined an increased dry matter intake (DMI) and, as consequence, increased milk yield.

One important feature of drinking water, namely in some geographic conditions, is its mineral content, especially as defined by salinity and total dissolved salts (TDS). In their review, Sanchez et al. (1994) evidenced how Cl could be considered less deleterious than SO4. Therefore, in those conditions, water desalination (by reverse osmosis) may represent an opportunity to maintain high water intake, DMI and milk yield (Sanchez et al. 1994). Another feature of drinking water is its degree of hardness, defined by its concentrations of Ca and Mg, but it seems less important than salinity to ensure cow performance during HS (Sanchez et al. 1994).

**Vitamins and minerals**

Recently, some vitamins were studied for their action on the animal physiology to cope with HS. The decreased feed intake observed under hot conditions also has repercussions on the intake of vitamins, which play an important role on immune function and performance. In order to reduce the negative effects of environmental stress, vitamins A, C and E are generally used in ‘hot diets’, particularly in poultry diet, because of their anti-stress effects, and also because their concentrations are lower during HS, as reported by West (1999) and Calamari et al (1999).

Dietary supplementation of vitamin A reduces the detrimental effect of HS on egg production (Lin et al. 2002). The supplementation is also favourable for the immunity of heat-stressed hens, and could alleviate the oxidative injuries induced by heat exposure and immune challenge as reported by Lin et al. (2006). Vitamin A, together to vitamin E and trace elements (in particular Se, Cu and Zn) are among the micronutrients playing important roles on mammary gland immunity and health, in particular during stress conditions (Sordillo et al. 1997). Khorsandi et al. (2016) observed the reduction of milk linear somatic cell score in dairy cows given multi-trace element/vitamin ruminal bolus under HS conditions, and concluded that the supplementation with trace elements and vitamins above National Research Council recommendation had positive effects on reproductive and lactation performance of high producing dairy cows kept under HS condition.

L-Ascorbic acid can be synthesised by many species and a supplementation in the diet is not required under normal conditions. McKee and Hurrison (1995) observed, in heat-stressed broiler chickens supplemented with ascorbic acid, a reduction of the respiratory quotient by emphasising a rise in fatty acid oxidation over the increase in protein-derived gluconeogenesis. Furthermore, because HS could cause an increase of oxygen free radicals (Calamari et al. 1999).
and could induce oxidative stress (Bernabucci et al. 2002), the supplementation with vitamin C could be relevant considering that ascorbic acid is one of the most important antioxidants in biological system. Also selenium and vitamin E are important constituents of antioxidant in biological system. Selenium protects tissues against oxidative stress (Surai 2006), as it is a component of the glutathione peroxidase, which destroys free radicals in the cytoplasm. Due to the antioxidant properties of tocophersols, the effect of α-tocopherol in the prevention of chronic diseases believed to be associated with oxidative stress has been examined and beneficial effects have been shown (Brigelius and Traber 1999). Selenium and vitamin E supplemented parenteral in dairy cows during hot weather can alleviate the effects of HS, particularly for night-time feeding (Tahmasbi et al. 2012). Nevertheless, injection of vitamin E did not ameliorate the pregnancy rate during HS in dairy cattle (Alan et al. 1994). In hens, vitamin E supplementation under hot conditions may have a positive effect on egg production (Lin et al. 2006). An improved resistance against HS in pigs supplemented with selenium and vitamin E was reported in the review of Renaudeau et al. (2012). More recently, Liu et al. (2016), in a study carried out on pig, demonstrated that the increase of dietary vitamin E and Se was able to reduce the negative impact of HS on intestinal barrier integrity.

Among vitamins, niacin was studied for its action on the vasodilatation in the mammalian and for its role in lipid metabolism. Niacin is a subcutaneous vasodilator in a many species and was studied in order to obtain a reduction of body temperature. However, niacin is rapidly metabolised in the rumen resulting in poor delivery to the small intestine (Campbell et al. 1994). Niacin helps to alleviate HS both by increasing evaporative heat loss from the body and also by reducing the effects of heat at the cell level (Lundqvist et al. 2008). Feeding protected niacin increased free plasma niacin levels, evaporative heat loss during peak thermal load and associated with a small but detectable reduction in rectal and vaginal temperatures in dairy cows experiencing a mild thermal load (Zimbelman et al. 2010), with variable effect on milk production (Zimbelman et al. 2013).

Mineral supplementation under hot climate must be viewed not only as a simple mean to cover the important (and increased) turnover of a specific nutrient, but also as a mean to buffer the effect of the diet and of climate (Calamari et al. 2007).

During HS, there is an increased demand for cations by the kidney, particularly Na\(^+\) and K\(^+\). Their urinary excretion under hot environment compared with excretion in a cooler environment may increase up to 80% and 18%, respectively (Sanchez et al. 1994).

As reviewed by Sanchez et al. (1994), NaHCO\(_3\) improves feed intake and performance of dairy cows under hot weather increasing milk yield and fat percentage. About K supplementation, supplementing K\(_2\)CO\(_3\) and KHCO\(_3\) during HS promoted greater feed intake and lactational performances (Sanchez et al. 1994).

During the cooler phase of the day, the respiratory alkalois induced by HS may lead to a compensated metabolic acidosis, characterised by a reduced blood and urine pH, and a reduced blood HCO\(_3^-\) concentration. Considering that during the summer about 80% of feed is generally consumed in the cooler part of the day, an increased rumen acid production post-feeding can exacerbate a condition of compensated metabolic acidosis (Sanchez et al. 1994).

There are also interactions between these minerals. For example, the interaction Na × K in summer diet is characterised by the ability of a cations to be spared for the other (Sanchez et al. 1994). The Na × Cl interaction leads to the greatest DMI and 4% fat corrected milk yield when the concentration of both minerals was increased (Sanchez et al. 1994).

Among trace minerals, Se is probably the most interesting to support antioxidative defence of the cow under HS (Calamari et al. 2011). The most effective form of dietary Se in cattle seems to be Se-yeast (Calamari et al. 2011). Experiment comparing Se-yeast, Na-selenite and no Se supplementation in heat stressed cows demonstrated a positive effect of Se-yeast (Sel-Plex Se yeast) on the reduction of circulating thiobarbituric acid reactive substances with lower plasma reactive oxygen metabolites, suggesting an improvement in the preventive antioxidant systems of cows fed Se-yeast (Calamari et al. 2011).

Chromium is a micronutrient that facilitates insulin action on glucose, lipid and protein metabolism. Little is known about actual dietary chromium requirements, however, because glucose use is predominant during HS, chromium supplementation could reduce the negative effects of HS. Dairy cows in early lactation supplemented with chromium under hot conditions have shown a reduction of weight loss, an improvement of milk production, a reduction of plasma NEFA concentrations, and an improvement of rebreeding rates (Mirzaei et al. 2011).

**Feed additives**

Fungal culture and plant extracts may exert a positive action in rumen metabolism as well as in regulation of
body temperature. Among fungal culture, yeast (Saccharomyces cerevisiae) was widely studied. Supplementation of live yeast could improve nutrient digestibility and feed efficiency (Piva et al. 1993; Ferraretto et al. 2012) and control ruminal pH (Bach et al. 2007). Improvements of DMI, feed efficiency and lactation performance have been observed in response to yeast supplementation in heat-stressed cows (Bruno et al. 2009; Moallem et al. 2009). Under HS, feeding strategies capable of increasing digestive efficiency, such as live yeast supplementation, may increase nutrient flow to the small intestine and dairy cow performance. Shwartz et al. (2009) observed that the supplementation of a mixture of exogenous enzymes and yeast culture reduced rectal temperature of heat-stressed dairy cows, suggesting an action on thermoregulatory functions. Higginbotham et al. (1993) observed a lower rectal temperature in heat-stressed cows supplemented with Aspergillus oryzae. Other studies did not confirm the effect of A. oryzae on body temperature in heat-stressed cows (Yu et al. 1997; Ominski et al. 2003).

Some suggestions to face to HS by a nutraceutical approach derive from Chinese researcher (Liu et al. 2013), sometimes with the aid of their folk medicine (Pan et al. 2014). Daidzein was assessed to be a useful promoter of antioxidant power in heat-stressed late lactating cows, essentially by an increased plasma activity of glutathione peroxidase (Liu et al. 2013). Extract from Radix bupleuri confirmed its antipyretic effect in heat-stressed lactating cows reducing rectal temperature, decreasing respiratory rate and increasing feed efficiency when fed at 0.25 g/kg DM. In a study carried out on dairy cows by using Ascophyllum nodosum to alleviate the negative effect of HS, Pompeu et al. (2011) observed a reduction of body temperature response to increasing ambient temperature and sustained milk production in supplemented cows.

It is clear that proper insulin action is one of the key components of successfully adapting to and surviving a heat load on dietary. Therefore, supplementing diet ingredients or pharmaceuticals that enhance insulin sensitivity may be an effective tactic to improve the likelihood of surviving an otherwise lethal heat load (Rhoads et al. 2013). Lipoic acid and its reduced form scavenge reactive oxygen and nitrogen species enhance cellular glucose uptake due to their insulin mimetic action (Diesel et al. 2007). It appears that lipoic acid enhances insulin action under thermo-neutral conditions, and, thus, the ability of lipoic acid to promote thermal tolerance during chronic heat-stress conditions is of obvious interest (Rhoads et al. 2013).

Also some drugs, such as thiazolidinediones (family of drugs that improve insulin sensitivity and are used to treat diabetes) could be interesting in heat stressed animals, also in relation to the upregulation of heat shock protein under heat-stress conditions (Rhoads et al. 2013).

As hyperthermia alone is responsible for enterocyte membrane damage and intestinal barrier dysfunction, some immunomodulatory nutrients (amino acids and fatty acids) and probiotics, that are able to modulate host immune and inflammatory responses and restore the intestinal barrier after injury could be used in diet during hot conditions. Recent studies reported by Andrade et al. (2015) have shown important therapeutic functions for certain amino acids such as glutamine, arginine, tryptophan and citrulline in gut-related diseases. Glutamine is a mediator of the cytokine chain reaction and is involved in transcription factors important for heat shock protein 70 expression (Hamiel et al. 2009). Caroprese et al. (2013) observed that rumen-protected glutamine is beneficial in dairy cows exposed to in hot climate by sustaining cow immune reactions in terms of a strengthening of cell-mediated immune response, which is weakened in heat-stressed cows (Lacetera et al. 2005).

**Conclusions, research needs, challenges and opportunities**

Heat stress is becoming a serious problem because of the negative impacts on ruminant performance. The negative effect of HS will become more severe in the future, as a consequence of global warming progresses and genetic selection for milk yield continues.

Heat-stressed animals change their metabolism and physiology in response to weather change. The direct and indirect effects of HS affect gastrointestinal health and functionality strongly influencing the efficiency of diet utilisation. The changes in nutrient partitioning and the alteration of rumen and intestine functionality should be taken into account when nutritionists will approach the formulation of ‘hot diets’.

Some studies showed that diets rich in starch and poor in fibre reduced HS in lactating dairy cows because the metabolised energy from high concentrate diets is used with greater efficiency than the metabolised energy from high forage diets. Recent advances in animal nutrition, like the feeding of ruminally-protected fats and protected proteins are other strategies to reduce metabolic heat production and supply the correct profile of nutrients to high producing cows in early lactation. However, dietary fibre is necessary in adequate supply to ensure a correct rumen activity, considering the negative effect of HS on RT, saliva
production and salivary HCO$_3^-$ content, decline in rumen motility, and altered acid-base balance.

More attention needs to be paid to some specific aspects of the physiological stage of the ruminant, considering the difference in the refractory response to lipolytic, adrenergic stimuli, and in the rate of amino acid oxidation among pre-partum, early-post-partum, and mid-lactating stage. The research must be further focussed on the possibility to match the evidenced preference for carbohydrates over lipid oxidation during HS, with more attention to the type of carbohydrate and its inclusion in the diet.

Continued advances in feeding are necessary as cattle are selected for high milk yield, but at the same time, are subjected to a reduction of DMI because of environmental stress. Developing nutritional strategies which support milk yield but which also address metabolic and physiologic disturbances caused by HS will help the cow to maintain a more normal metabolism which should promote performance.

Water availability and its temperature may represent a key tool to promote DMI and alleviate heat load subtracting heat from the body.

Further research is needed to understand the real extent of the effect of plant extracts and vitamins (or their metabolites) in improving cow ability to cope with hot climate.

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