# Effect of exposure to heat stress conditions on milk yield and quality of dairy cows grazing on Alpine pasture

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Abstract: Milk yield, gross composition and fatty acid (FA) profile of 14 Aosta Red Pied (ARP) and 14 Aosta Black Pied-Aosta Chestnut (ABP-AC) cows were investigated in an Alpine pasture during the two years' grazing seasons: 2003 (heat stress conditions) and 2004 (thermoneutral conditions). The anomalous adverse weather settings (extreme dryness) occurred in summer 2003 determined a strong worsening in fresh grass quality at pasture. ARP cows suffered a significant drop in dry matter intake (P<0.01), milk production (P<0.01) and fat and protein yields (P<0.01) compared to summer 2004. ABP-AC cows showed lower milk yields and lower fat and protein yields compared to ARP ones (P<0.001), but they were able to maintain same feed intakes, production performances and fat and protein yields between years. In summer 2003 an improvement of the nutritional properties of milk associated to changes in the FA profile was observed: milk fat showed lower hypercholesterolemic (C12 to C16) and total saturated FA as well as higher total mono- and polyunsaturated FA with respect to summer 2004 in both ARP and ABP-AC cows (P<0.001). ABP-AC had a significant healthier milk FA composition than ARP in both years of analysis. The differences in milk FA between breeds were more evident in summer 2004, showing that thermoneutral conditions can better emphasize the genetic potential of different cattle breeds.

Keywords: dairy cows, milk yield, fatty acids, heat stress, summer pasture

# Introduction

Since the last decade of the 19th century, a marked average temperatures increase has taken place in the Alps (Böhm et al., 2001). A raise of about +1°C was observed in NW Italy only considering the period 1952-2002 (Hardenberg et al., 2007). Especially from 2002 to 2005 the climate was characterized by anomalous thermal values and in summer 2003, which was by far the hottest in the Alpine sites in the last half millennium (Luterbacher et al., 2004), persistent high temperatures and anomalous dryness conditions occurred.

Dairy cows are very sensitive to climatic variations, that greatly influence their welfare and their ability to produce milk. Heat stress (HS) conditions are normally associated with a decline of the production performances as they determine the activation of thermo-regulation mechanisms in order to avoid hyperthermia and maintain the vital functions of the animals (Nardone et al., 2006). The exposure to HS is also able to strongly modify ruminant milk gross composition. Milk protein levels have generally been shown to decrease under HS (Giustini et al., 2007; Kamiya et al., 2005; Bernabucci et al., 2002; Bouraoui et al., 2002). Results on milk fat levels differ among reports; however, declines of this parameter have been shown by many researchers (Giustini et al., 2007; Bouraoui et al., 2002; Sevi et al., 2001; Lacetera et al., 1996). Only a limited number of studies have focused on the effect of HS on ruminant milk fatty acid (FA) profile and the results obtained by different authors are sometimes conflicting (Lacetera et al., 2003).

The majority of the studies dealing with the effect of HS on milk yield and quality have been conducted in conditions of sustained moderate-severe HS under strictly defined experimental conditions (environmentally controlled chambers) on selected high yielding and intensively managed dairy ruminants. Information on the effects of mild-moderate HS is not broadly available; moreover further attention should be addressed to field situations (*in natura*, under the usual conditions of

extensively managed herds, e.g. grazing conditions), despite the greater difficulties in keeping the experimental conditions under control.

With respect to grazing ruminants, the stressful effects of a hot climate are moreover amplified if the animals are not protected from direct solar radiation. Such a condition, compared to that of ruminants protected by a shaded environment, can determine a reduction of ruminal contractions, an increase in rectal temperature and a decline in milk yield (West, 2003). Another direct negative effect related to the grazing activity is the further increase in energy requirements due to greater physical efforts and more time needed for eating (NRC, 2001). Additionally, an indirect negative effect has to be taken into account: high ambient temperatures and dryness conditions are, in fact, responsible for a quick ageing of the herbaceous biomass of the pasture lands due both to more intense lignifications processes and higher metabolic activities such as the conversion of photosynthetic products into structural matter (Van Soest, 1994), determining the fall of digestibility of forages and the consequent decrease in animal voluntary ingestion.

In the Autonomous Region of Aosta Valley (NW Italian Alps), the traditional breeding system is commonly oriented to autochthonous dual-purpose cattle breeds (Aosta Red Pied, Aosta Black Pied and Aosta Chestnut) that, despite of their medium levels of milk production, are characterized by better functional and conformation traits, and by a peculiar high adaptation degree to the mountain environment and to the exploitation of pasture lands during the grazing season (Bianchi et al., 2003). Aosta cattle breeding plays an essential role in the agricultural and socio-economical realities of this Region, contributing to the safeguard of ancient local traditions and typical food products. In Aosta Valley milk obtained from these breeds represents the main livestock productive objective for the particular interest linked to its almost total transformation into Protected Designation of Origin Fontina, a traditional cheese well known and appreciated in Italy and other European countries.

To our knowledge no studies are currently available on the effect of mild-moderate HS conditions on milk yield and quality obtained by local cattle breeds under natural grazing conditions in Alpine sites. Therefore, the aims of this trial were i) to examine the influence of a hot environment on yielding capacity, fat and protein levels, somatic cell count and FA composition of milk produced by Aosta cattle grazing on Alpine pasture and ii) to deepen if differences among Aosta breeds exist in response to variation of the climatic settings.

#### Materials and methods

#### Animals, feed and management

The trial was conducted in Aosta Valley and refers to data recorded during two summer grazing seasons - 2003 (HS conditions) and 2004 (thermoneutral - TN - conditions) - on 28 Aosta dairy cows (14 Aosta Red Pied - ARP - and 14 Aosta Black Pied-Aosta Chestnut - ABP-AC). Due to the high genetic similarity existing between ABP and AC breeds (Di Stasio and Dupont, 1983), they were considered as a single population group in the data set.

The breeding system for the Aosta cattle commonly emphasizes calving in autumn and winter months in order to bring cows at pasture in late lactation, with less nutritional requirements. At the beginning of the grazing seasons (experimental periods) days in milk (DIM) were 172  $\pm$  60 and 194  $\pm$  39 (mean  $\pm$  SD) for ARP cows and 166  $\pm$  56 and 193  $\pm$  40 (mean  $\pm$  SD) for ABP-AC cows in 2003 and 2004, respectively.

In both years of analysis, from October to May all the cows were kept indoor on a farm located in the valley bottom (Brusson; 45°46′N, 7°44′E, 1338 m a.s.l.) and were given the typical dietary regimen of the Fontina cheese disciplinary (on average 75% hay and 25% concentrates based diet). In 2004 the summer grazing season started the half of June while in 2003, it started one week earlier due to the precocious growth of the plants. The three months grazing periods provided for the exploitation of an Alpine pasture (experimental site) located in Aosta Valley as well (Challand Saint-Victor; 45°40′N, 7°45′E, 1300 to 1775 m a.s.l.). At pasture fresh water was provided *ad libitum* and the nourishment was characterized by *ad libitum* fresh grass plus a daily supplement of one kg of concentrate per

head (Table 1). The traditional daily grazing management allowed cows to pasture for six-seven hours per day in all, after moving indoors twice for hand milking (at 0400 and 1600 h, during which cows were also offered the concentrate supplement). The cows were returned to an enclosed shed during nighttime.

Table 1. Chemical and fatty acid compositions of the concentrate used as supplement to the pasture-based diet.

Chemical composition	
Dry Matter (%)	88.9
Ash (%DM)	6.1
Crude Protein (%DM)	17.4
Ether Extract (%DM)	3.2
Neutral Detergent Fibre (%DM)	26.2
NE <sub>L</sub> (MJ kgDM <sup>-1</sup> )	7.1
Fatty acid composition (% total fatty acids)	
Total SFA	16.98
Total MUFA	23.06
Total PUFA	59.96

Abbreviations: DM = Dry Matter; NE<sub>L</sub> = Net Energy for Lactation; SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids.

# Measurements, sampling procedure and chemical analysis

#### Milk

Before starting to collect the samples, a two-weeks pre-experimental period was provided at the beginning of the grazing seasons in both years of analysis to support the adaptation of the cows to the new conditions of diet and environment in the indoor-pasture transition.

Individual milk yield was recorded at morning and afternoon milkings once a week. For laboratory analysis, individual milk samples (a daily composite sample from the morning and the afternoon milkings) were collected monthly. One aliquot of each milk sample was stored at 4°C in a portable refrigerator and then immediately transported to the laboratory for the analysis of fat and protein contents and somatic cell count. A second aliquot was stored for four-five hours at 4°C before freezing at –20°C, until analysis for FA composition.

Milk fat and protein contents were determined by infrared spectroscopy (MilkoScan FT6000, Foss Electric, Hillerød, Denmark) and somatic cell count (SCC) analysis was assessed using an automatic cell counter (Fossomatic 5000, Foss Electric, Hillerød, Denmark).

Concerning FA analysis, after total lipid extraction FA were methylated in presence of BF<sub>3</sub> as catalyst (AOAC, 2000) and were determined by a gas chromatograph Shimadzu GC17A equipped with a HP88 capillary column (100 m x 0.25 mm ID, 0.2  $\mu$ m film thickness; J&W Scientific). The column temperature was held at 60°C for one min and then raised 20°C min<sup>-1</sup> to a final temperature of 190°C, where it remained for 40 min. Temperature of the injector and flame-ionization detector was maintained at 250 and 280°C, respectively; the injection volume was 0.1  $\mu$ l; nitrogen was set at 40 ml min<sup>-1</sup>. Peaks were identified by comparison of retention times with fatty acid methyl esters (FAME) standards (Matreya and Restek Corporation). Results were expressed as a percentage of each FA per total FA detected.

## Grass

Representative local fresh grass samples were hand-plucked at random transects twice a week during the studied periods, to approximate the height at which the cows grazed, and stored at  $-20^{\circ}$ C until analysis for chemical and FA composition.

The samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash and neutral detergent fibre (NDF) according to AOAC (2000).

Total lipids were extracted according to Folch et al. (1957) and FAME were prepared by methylation procedure (AOAC, 2000) and were separated and quantified by gas chromatography (Shimadzu GC17A) using a DB-Wax capillary column (60 m x 0.53 mm ID,  $1.0 \text{ }\mu\text{m}$  film thickness; J&W Scientific) with a temperature program of  $180^{\circ}\text{C}$  for one min, then raised  $5^{\circ}\text{C}$  min<sup>-1</sup> to  $225^{\circ}\text{C}$ , final isotherm for 30 min. Temperature of the injector and flame-ionization detector was maintained at 250 and 270°C, respectively; the injection volume was  $0.1 \text{ }\mu\text{l}$ ; nitrogen constant linear flow rate was set at 24 ml min<sup>-1</sup>. Peaks were identified by comparison of retention times with FAME standards (Restek Corporation). Results were expressed as a percentage of each individual FA per total FA detected.

### Meteorological data

Meteorological data (air temperature and relative humidity at one-hour interval) were obtained from data collected during the experimental periods by the nearest meteorological station (Saint-Vincent Salirod, latitude: 45°44′N, longitude: 7°40′E), located about six km as the crow flies far from the experimental site.

The Temperature-Humidity Index (THI) was used as indicator of thermal comfort for cattle. THI computation was performed by using air temperature (T, °C) and relative humidity (RH, %) values, according to the formula reported by Ravagnolo et al. (2000).

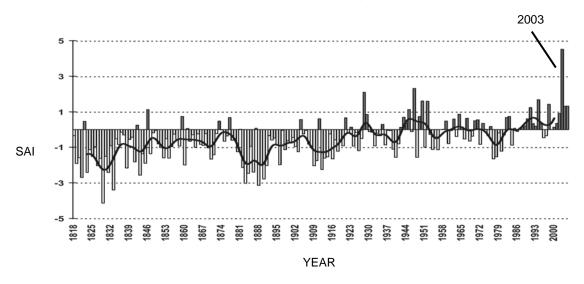
#### Statistical analysis

The statistical analysis of the data was performed using SPSS for Windows (SPSS, 2004). The effect of year on the chemical composition and FA profile of the pasture samples was measured by performing an independent sample t-test. Data about milk samples were submitted to the General Linear Model Univariate procedure; fixed effects were year and breed while days in milk were used as covariate. Significance was declared at P<0.05. Data are expressed as mean  $\pm$  standard deviation (SD).

# **Results and discussion**

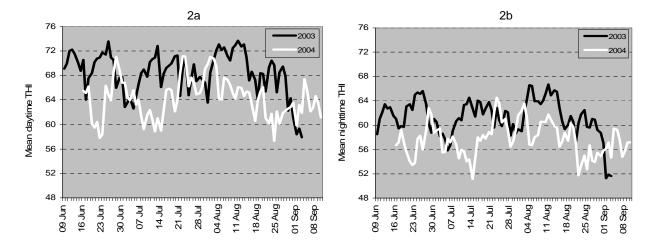
# **Environmental conditions during the experimental periods**

In summer 2003 a peculiar not favourable climatic combination occurred and the Standardized Anomaly Index (Giuffrida and Conte, 1989) of summer mean temperatures in the Aosta Valley Region was + 4.5 relative to reference mean values from 1818 to 2005 (Fig. 1; Regione Autonoma Valle d'Aosta, 2006).



**Figure 1**. Standardized Anomaly Index (SAI) of summer mean temperatures in Aosta Valley, calculated from 1818 to 2005. In summer 2003 an exceptional anomaly occurred, with regionalized SAI = + 4.5 (Regione Autonoma Valle d'Aosta, 2006).

During the summer grazing seasons, in the experimental site computed daily (0-24h) THI averaged  $65.2\pm3.2$  and  $61.2\pm3.0$  (mean  $\pm$  SD) in 2003 and 2004, respectively. Variations of mean daytime (0800 h -1900 h) and nighttime (2000 h -0700 h) THI during the experimental periods are presented in Fig. 2.



**Figure 2**. Mean daytime (0800-1900h; 2a) and nighttime (2000-0700h; 2b) THI during the summer grazing seasons of 2003 (June  $9^{th}$  - September  $3^{rd}$ ) and 2004 (June  $16^{th}$  – September  $10^{th}$ ).

Although the majority of researchers indicate as 72 the upper critical mean daily THI for dairy cattle, significant negative effects have been shown also with lower values of this index. Johnson et al. (1962) reported a significant decline in milk yield with THI exceeding 70. Ravagnolo and Misztal (2002) indicated 68 as the upper critical THI and more recently other authors showed that variations of THI values can have negative consequences on milk qualitative parameters also in winter (when THI values are normally lower than critical-considered ones) (Giustini et al., 2007).

In 2003 THI exceeded 68 on almost all (73 of the total 87) test days (30.6% of the total time over the experimental period), and exceeded 72 on 50 days (11.9% of the total time). On the contrary, in 2004 THI exceeded 68 on 39 of the total 87 test days and exceeded 72 on 9 days, covering respectively only 9.5% and 1.9% of the total time during the grazing season.

#### Forage quality at pasture

In 2003 fresh grass had an overall lower quality, showing significantly higher neutral detergent fibre (P<0.05) and lower crude protein levels (P<0.01) relative to values observed in 2004 (Table 2). It is well known that a negative relationship exists between ambient temperatures and dry matter digestibility of forages. High ambient temperatures determine a more rapid maturation of the plants, which tend to be higher in cell wall constituents and lower in soluble carbohydrates, such factors contributing to lower their digestibility. As digestibility of forages decreases, passage rate become slower, time for grazing increases and reduced dry matter intakes (DMI) by ruminants are observed (Barnes et al., 2007).

**Table 2.** Chemical and fatty acid compositions of the fresh grass in 2003 and 2004<sup>1</sup>.

	Year 2003	Year 2004	$P^2$
	n=22	n=23	г
Chemical composition			
Dry Matter (%)	$39.10 \pm 11.41$	$34.48\pm2.06$	ns
Ash (%DM)	$9.34 \pm 2.64$	$\textbf{9.28} \pm \textbf{2.54}$	ns
Crude Protein (%DM)	$10.73\pm4.68$	$14.65\pm4.12$	**
Ether Extract (%DM)	$2.72 \pm 0.51$	$2.73 \pm 0.57$	ns
Neutral Detergent Fibre (%DM)	$54.71 \pm 7.98$	$49.17\pm7.03$	*
Fatty acid composition (% total fatty acids)			
C16:0	$27.66 \pm 4.67$	$19.64\pm2.08$	***
C18:2n6	$18.78 \pm 5.25$	$\textbf{21.16} \pm \textbf{2.98}$	ns
C18:3n3	$30.71 \pm 8.49$	$42.30 \pm 4.95$	***
Total SFA	$39.08\pm6.96$	$\textbf{28.79} \pm \textbf{3.17}$	***
Total MUFA	$11.43\pm3.41$	$\textbf{7.75} \pm \textbf{1.50}$	***
Total PUFA	$49.49 \pm 8.54$	$63.46 \pm 3.45$	***

 $<sup>^{1}</sup>$ Values are expressed as mean  $\pm$  standard deviation of the mean.

Abbreviations: DM = Dry Matter; SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids.

Highly significant variations between the summer grazing seasons 2003 and 2004 were also observed in forage lipids. Alpha-linolenic (C18:3n3), linoleic (C18:2n6) and palmitic (C16:0) acids were the predominant detected FA in pasture plants (Table 2). Significant variations in the proportions of FA in forages have been previously observed during a grazing season by Mel'huchová et al. (2008), with the lowest levels of  $\alpha$ -linolenic and total polyunsaturated fatty acids (PUFA) and contemporarily the highest levels of total saturated (SFA) and monounsaturated (MUFA) fatty acids recorded during the summer months (advanced growth stage and maturity of the plants) relative to spring and autumn. In the current study higher levels (P<0.001) of palmitic acid, total SFA and total MUFA and contemporarily lower levels (P<0.001) of  $\alpha$ -linolenic and total PUFA were detected in summer 2003 compared to summer 2004, confirming a more rapid maturation of the plants caused by the high ambient temperatures and dryness conditions occurred in 2003.

#### Milk yield, gross composition and somatic cell count

Aosta Red Pied cows showed significantly higher levels (P<0.001) of individual milk yield compared to ABP-AC ones, during both years 2003 and 2004 (Table 3). This was expected as ARP cattle are characterized by higher production performances (about +900 kg of milk per lactation; AIA, 2008) relative to ABP-AC breeds.

**Table 3.** Milk yield, gross composition and somatic cell count of Aosta Red Pied (ARP) and Aosta Black Pied-Chestnut (ABP-AC) dairy cows in the summer grazing periods of 2003 and 2004.

	ARP			ABP-AC		
	Year 2003 n=42	Year 2004 n=42	Р	Year 2003 n=42	Year 2004 n=42	Р
Milk yield (kg h <sup>-1</sup> d <sup>-1</sup> )	$10.29 \pm 4.39 \text{ A}$	$11.14 \pm 3.64$ A	**	$7.83 \pm 3.06 \ B$	$7.28 \pm 2.15 \; \text{B}$	ns
$DMI^{2}$ (kg h <sup>-1</sup> d <sup>-1</sup> )	$13.73 \pm 1.23 \text{ A}$	$14.22\pm1.28~\textrm{A}$	**	12.95± 0.85 B	$12.79\pm0.80~\textrm{B}$	ns
4% FCM (kg h <sup>-1</sup> d <sup>-1</sup> )	$9.75\pm3.80~\textrm{A}$	$10.81\pm3.52~\textrm{A}$	**	$7.74\pm2.98~B$	$6.97\pm2.21~\text{B}$	ns
Fat (%)	$3.80 \pm 0.68$	$3.88 \pm 0.72$	ns	$3.89 \pm 0.69$	$3.71\pm0.67$	ns
Fat yield (kg h <sup>-1</sup> d <sup>-1</sup> )	$0.38\pm0.14~\text{A}$	$0.42\pm0.14~\text{A}$	**	$0.30\pm0.12\;\text{B}$	$0.27\pm0.10~\text{B}$	ns
Protein (%)	$3.27\pm0.39~\textrm{B}$	$3.46\pm0.48$	ns	$3.58\pm0.36~\textrm{A}$	$3.53 \pm 0.33$	ns
Protein yield (kg h <sup>-1</sup> d <sup>-1</sup> )	$0.33\pm0.13~\textrm{A}$	$0.38\pm0.11~\text{A}$	**	$0.27 \pm 0.09 \; B$	$0.26 \pm 0.07 \; B$	ns
SCC (n*1000 ml <sup>-1</sup> )	$215.06 \pm 80.76$	$190.07 \pm 104.79$	ns	$179.51 \pm 76.75$	$263.27 \pm 104.40$	ns

 $<sup>^{1}</sup>$ Values are expressed as mean  $\pm$  standard deviation of the mean.

Asterisks within rows indicate statistically significant difference between years (\*\*\*P<0.001; \*\*P<0.001; \*P<0.005; ns: not significant). Different letters within rows indicate, in the same year, statistically significant difference between breeds (A,B: P<0.001; a,b: P<0.01; a,b: P<0.05).

Abbreviations: FCM = Fat Corrected Milk; SCC = Somatic Cell Count.

<sup>&</sup>lt;sup>2</sup>Significance: \*\*\* P<0.001; \*\* P<0.01; \* P<0.05; ns: not significant.

<sup>&</sup>lt;sup>2</sup>Estimated according to NRC (2001).

In summer 2003 ARP cows suffered a statistically significant drop in milk yield relative to values observed in summer 2004 (-8%; P<0.01). In the current trial, the differences in both the vegetative stage and the nutritional quality of the fresh grass (e.g. -4% of CP in 2003 relative to 2004) could be probably enough per se to explain the observed significant variations in milk yield produced by ARP cows. Estimated DMI was found statistically significant lower (P<0.01) in ARP cows in summer 2003 compared to summer 2004 and this could be also attributed to the reduced quality of the available fresh forages at pasture. However, a direct negative effect of HS on the animals, thus not being distinguishable from the indirect negative effect of the low-quality feed, can not be excluded. In fact, depressing appetite and declining feed intake are considered the primary physiological responses of ruminants to HS, accounting for the majority of the reduced milk yield in dairy cows (Kadzere et al., 2002). Moreover, in heat-stressed ruminants feed intake decreases especially when low quality feed is offered or available (Costa et al., 1992; Goetsch and Johnson, 1999). As in this study THI values generally remained below 72 for the majority of the experimental period, it is possible to hypothesise that the negative effect of HS on plants quality, determining the worsening of their nutritional quality in 2003, exerted a stronger influence on ARP cows' ability to produce milk relative to the direct effect of HS on the animals.

It is interesting to note that ABP-AC, characterized by significantly lower yielding capacity than ARP, successfully maintained similar values of estimated DMI and milk yield in 2003 and 2004, suggesting a higher degree of heat tolerance and/or a lower impact of low-nutritional quality feed on feeding behaviour. Such results are not completely surprising as it is known that the negative effects of HS in dairy ruminants also depend on breed and yielding capacity (Bernabucci and Calamari, 1998; West, 2003): a higher genetic merit is normally associated to a greater extent of sensitiveness to heat stress.

In ARP cows, observed fat and protein yields were significantly lower in year 2003 than in 2004 (-10% and -13%, respectively; P<0.01). It has been proposed that the depressed fat levels in heat-stressed dairy cows could be attributed to the decrease in forage intake which may result in an inadequate fibre level in the diet to maintain normal rumen functions (Bouraoui et al., 2002). The reduction in milk protein seems to be attributed to the restricted feed intake as well (Kamiya et al., 2005). In both 2003 and 2004, ABP-AC cows showed lower fat and protein yields than ARP ones (P<0.001) but, as observed in milk production, they were able to maintain the same fat and protein yields between years.

Modest increases in milk SCC were observed in heat-stressed dairy cows (Igono et al., 1988; Wegner et al., 1976). In the current trial no significant variations between years were noticed. As commonly occurs in late lactation (Coulon et al., 1996) and in grazing periods (Lamarche et al., 2000), high SCC values were observed for both breeds, confirming previous results (Bianchi et al., 2003). However, the mean milk SCC was never >300.000 cells ml<sup>-1</sup>, showing no management difficulties and a good sanitary state of the cows (Coulon et al., 1998).

# Milk fatty acid composition

Concerning FA, remarkable differences were detected between years (Table 4). In summer 2003, milk from both ARP and ABP-AC cows showed significantly lower proportions of short and medium chain fatty acids (SMCFA, C10:0-C16:1) and significantly higher proportions of long chain fatty acids (LCFA, C17:0-C18:3) relative to the values observed in summer 2004. It has been hypothesized that the raise in the proportion of LCFA during HS could be ascribed to a reduced synthesis of SMCFA in the mammary gland caused by the reduction of feed intake and the consequent decrease in the production of short-chain fatty acid precursors in the rumen (Smith et al. 1983; Bernabucci and Calamari, 1998). Unsaturated fatty acids (both total MUFA and PUFA) were significantly higher and total SFA were significantly lower in summer 2003. The sum of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids (Hypercholesterolemic Saturated Fatty Acids, HSFA), universally considered detrimental for human health (German and Dillard, 2004), was significantly lower in 2003 compared

to the values observed in 2004. Such results point out that the exposure to HS was responsible for a significant improvement of the nutritional properties of milk fat in both breeds.

**Table 4.** Milk fatty acid composition (% of total fatty acids) of Aosta Red Pied (ARP) and Aosta Black Pied-Aosta Chestnut (ABP-AC) dairy cows in the summer grazing periods of 2003 and 2004.

	ARP			ABP-AC		
	Year 2003	Year 2004	Р	Year 2003	Year 2004	 Р
	n=42	n=42	Р	n=42	n=42	Р
HSFA	$37.99 \pm 3.05~\alpha$	$41.92\pm3.85~\alpha$	***	$36.50 \pm 3.22  \beta$	$40.03\pm3.19~\beta$	***
SMCFA	$43.25 \pm 3.29$	$47.24 \pm 4.04$ a	***	$41.84\pm3.71$	$45.00 \pm 3.48 \ b$	**
LCFA	$56.76 \pm 3.29$	$52.77 \pm 4.03 \ b$	***	$\textbf{58.17} \pm \textbf{3.72}$	$55.00 \pm 3.48$ a	**
Total SFA	$55.08 \pm 2.85$	$59.83 \pm 3.23$ a	***	$53.68 \pm 3.90$	$57.46 \pm 3.64 \text{ b}$	***
Total MUFA	$39.29 \pm 2.94$	$35.16 \pm 2.95  b$	***	$40.30 \pm 3.79$	$37.13 \pm 3.13$ a	***
Total PUFA	$5.63 \pm 0.73 \ b$	$5.01\pm0.70~\beta$	***	$6.03 \pm 0.80 \text{ a}$	$5.42\pm0.84~\alpha$	**
SFA/UFA	$\textbf{1.23} \pm \textbf{0.14}$	$1.51\pm0.20~\text{a}$	***	$\textbf{1.17} \pm \textbf{0.18}$	$1.37\pm0.20~\textrm{b}$	***

 $^{1}$ Values are expressed as mean  $\pm$  standard deviation of the mean.

Asterisks within rows indicate statistically significant difference between years (\*\*\*P<0.001; \*\*P<0.01; \*P<0.05; ns: not significant). Different letters within rows indicate, in the same year, statistically significant difference between breeds (A,B: P<0.001; a,b: P<0.01;  $\alpha$ ,B: P<0.05).

Abbreviations: HSFA = Hypercholesterolemic Saturated Fatty Acids; SMCFA = Short and Medium Chain Fatty Acids; LCFA = Long Chain Fatty Acids; SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; UFA = Unsaturated Fatty Acids.

Similar variations were obtained by other authors (Ronchi et al., 1995; Palmquist et al., 1993; Piva et al., 1993). A worsening of milk FA profile was instead achieved only in one trial in which the effect of high ambient temperatures and solar radiation on the FA composition of ewe milk were investigated (Sevi et al., 2002).

Notable variations in milk FA profile were observed between Aosta breeds in both years of analysis. The influence of breed on milk fat composition was previously reported by other authors (White et al., 2001; Pešek et al., 2005). In the current study, in 2003, no differences in total SFA and total MUFA between breeds were noticed, while HSFA (P<0.05) and total PUFA (P<0.01) contents were significantly better in terms of human health promotion in ABP-AC cows. The differences between ARP and ABP-AC breeds were more evident in the 2004 (TN conditions). In fact, variations between breeds clearly emerged and ABP-AC cows showed their aptitude to a better qualitative FA profile than ARP ones: lower total SFA (P<0.01) and HSFA (P<0.05) contents, higher MUFA (P<0.01) and PUFA (P<0.05) levels. Such results point out that climatic variations are able to influence strongly not only milk production and fat and protein yields, but also milk FA profile, causing the lipid fraction quality to improve. Moreover, TN climatic conditions seem to be able to better emphasize the genetic potential of different cattle breeds.

#### Conclusion

In summer 2003, HS conditions (high air temperatures coupled with extreme dryness) occurred during summer grazing periods in Alpine areas determined reductions in milk yield, and fat and protein yields in autochthonous ARP, but not ABP-AC, cows at pasture. The observed declines in milk yield and fat and protein contents were statistically significant, indicating a greater sensitiveness of ARP cows to environmental conditions that could impact feeding behaviour and thermoregulation.

Such influence of HS can be divided into: i) a direct component related to the negative effects of HS on animals' welfare and their production performances, and ii) an indirect component related to a quick ageing of the herbaceous biomass and the consequent worsening of its nutritional quality (climate-forage interaction which strongly contributes to animal distress, further worsening the direct depressive effects of HS on intake and grazing). It is quite hard to distinguish and quantify the relative weight of the first and of the second component due to the difficulties in keeping the experimental conditions under control. In the current trial, as computed THI values did not exceeded 72 for a long time during the whole experimental periods, it is likely to be plausible that the indirect effects of HS had a greater importance relative to the effects directly exerted on animal physiology.

Anyway, a direct negative effect of HS on the cows involved in this trial probably occurred also. In fact, in 2003 an improvement of milk nutritional properties due to a healthier FA profile both in ARP and ABP-AC was observed. Such result, as mentioned above, has been previously reported by other authors with dairy cows under HS conditions. Some differences in the lipid fraction were observed between Aosta breeds. ABP-AC cows showed a healthier milk FA profile especially in 2004, indicating that TN conditions occurring in the summer grazing months seem to be able to better highlight genetic differences among cattle breeds.

The possibility that harsher climatic conditions during the summer grazing periods may amplify the direct and indirect negative effects of HS and, as a result, the physiological and productive responses in local cattle breeds (with possible significant economic losses for farmers) cannot be discounted.

It must be remarked that recently other anomalous climatic events (exceptional warmth of autumn 2006 and winter 2007) occurred in Europe (Luterbacher *et al.*, 2007) and that climatologists agree on a more frequent recurrence, in the future, of exceptional heat waves similar to the one that occurred in summer 2003, especially from the second half of 21<sup>st</sup> century onwards (Meehl and Tebaldi, 2004). Consequently, it will become crucial to identify the levels of variation in milk yield and quality of different ruminant species and breeds reared in the Alpine regions directly and indirectly (feed quality) affected by the changes in the climatic pattern, and safeguard the presence of those local breeds that are able to tolerate hard climatic and environmental conditions as well as poor quality grasses available, such as Aosta Black Pied and Aosta Chestnut.

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