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# Effect of hot season on blood parameters, fecal fermentative parameters, and occurrence of *Clostridium tyrobutyricum* spores in feces of lactating dairy cows

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## ABSTRACT

High temperature influences rumen and gut health, passage rate, and diet digestibility, with effects on fermentative processes. The main aim of the study was to investigate the effect of hot season on hindgut fermentation, the occurrence of *Clostridium tyrobutyricum* spores in bovine feces, and on their relationship with metabolic conditions in dairy cows producing milk used for Grana Padano cheese. The study was carried out on 7 dairy farms located in the Po Valley (Italy), involving 1,950 Italian Friesian dairy cows. The study was carried out from November 2013 till the end of July 2014. Temperature and relative humidity were recorded daily by weather stations. Constant management conditions were maintained during the experimental period. Feed and diet characteristics, metabolic conditions, and fecal characteristics were recorded in winter (from late November 2013 to the end of January 2014), spring (from April to May 2014), and summer (July 2014) season. In each season, blood samples were collected from 14 multiparous lactating dairy cows per herd to measure biochemical indices related to energy, protein, and mineral metabolism, as well as markers of inflammation and some enzyme activities. Fecal samples were also collected and measurements of moisture, pH and volatile fatty acids (VFA) were performed. The DNA extracted and purified from fecal samples was used to detect *Clostridium tyrobutyricum* spores in a quantitative real-time PCR assay. The daily mean temperature-humidity index was 40.7 ± 4.6 (range 25 to 55), 61.2 ± 3.7 (range 39 to 77), and 70.8 ± 3.2 (range 54 to 83) in winter, spring, and summer, respectively. Total VFA concentration in feces progressively decreased from winter to summer. The seasonal changes of acetate and propionate followed the same trend of total VFA; conversely, butyrate did not show any difference between seasons, and its molar proportion was greater in summer compared with winter. A greater occurrence of *Cl. tyrobutyricum* spores in summer compared with the other seasons was observed. The plasma concentrations of glucose, urea, albumin, Ca, Mg, Cl, Zn, and alkaline phosphatase activity were lower in summer compared with winter, whereas the opposite occurred for bilirubin and Na. Our results show that summer season, through direct and indirect effect of heat stress, affected fecal fermentative parameters and hindgut buffering capacity, and was responsible for the increasing occurrence of *Cl. tyrobutyricum* spores in feces.
**Key words:** heat stress, dairy cows, *Clostridium tyrobutyricum*, metabolic conditions

## INTRODUCTION

Climate change is likely to be one of the main challenges of the current century. In many regions of Italy, the summer period is characterized by climatic conditions that can adversely affect the welfare of farm animals and in particular of the dairy cows. It is known that dairy cow, because it generates a lot of metabolic heat, is sensitive to high environmental temperatures to which it reacts by implementing various physiological responses. These involve reduction of feed intake, reduction of growth rate (Nardone et al., 2010; Das et al., 2016), alteration of gastrointestinal function (Bernabucci et al., 1999, 2009; Kadzere et al., 2002), reduction of reproductive performances (Hansen, 2009), changes in the endocrine-metabolic system (Abeni et al., 2007; Bernabucci et al., 2010; Baumgard and Rhoads, 2013), impairment of immune system (Lacetera et al., 2005), and even animal death in extreme cases (Vitali et al., 2015). Many studies reported that high air temperatures, coupled with high relative humidity, negatively affect milk yield and its composition and cheesemaking properties (Bernabucci et al., 2014, 2015; Bertocchi et al., 2014).

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Among these latter ones, the microbiological properties play an important role to determine physical, chemical, and biological processes of cheesemaking and ripening. Many of the defects of long-ripening cheese obtained with raw milk are related to microbial contamination (Dasgupta and Hull, 1989; Garde et al., 2011). Late blowing, caused by butyric acid fermentation, is one of the most feared defects of long-ripened hard and semi-hard cheeses. In particular, *Clostridium tyrobutyricum*, an anaerobe gram-positive and endospore-forming bacterium, was identified as the main spoiling agent responsible for this defect in cheese (Le Bourhis et al., 2005; Lopez-Enriquez et al., 2007; Doyle et al., 2015), and its occurrence in milk is closely related to the contamination of raw milk during the milking process, before cheese production.

*Clostridium* ssp. spores are highly resistant to environmental conditions and are abundant mainly in feces and in dust, colonize skin and hair of animals, and can easily contaminate raw milk at milking time (Barash et al., 2010; Ledenbach and Marshall, 2010). The concentration of spores in feces, and consequently the contamination of the environment, is strictly related to the content of spores in feeds (silage in particular). Also, diets different in forage/concentrate ratio and starch content, modifying the fermentative processes in the digestive tract, may influence the excreted/ingested spore ratio (Bani et al., 2001). A relationship was observed between excreted/ingested spore ratio and the pH measured in feces (Bani et al., 2001). Therefore, the climate change could affect digestive activity and *Clostridia* contamination in milk. The hot conditions influence rumen and intestine health, passage rate, and diet digestibility (Bernabucci et al., 2009), with effects on fermentative processes and potential changes of factors influencing germination of *Clostridia* spores in the gut.

Only few studies are available on the effect of season on hindgut fermentation, also considering that animal feeding often varies depending on season and climate. Therefore, the aim of the present study was to investigate the effect of hot season on fecal fermentative parameters and on the occurrence of *Cl. tyrobutyricum* spores in bovine feces and their relationship with metabolic conditions in dairy cows producing milk used for Grana Padano cheese.

## MATERIALS AND METHODS

### Animals and Management

The research and the animal care protocols were in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes (European Union, 2010).

The study was carried out on 7 dairy farms located in the Po Valley (Mantova and Brescia provinces). Only 7 farms were selected based of budgetary limitations and study complexity, using a convenience sample. The milk yielded by all herds was used to produce Grana Padano cheese. The herds were selected because they were representative of Grana Padano production area in terms of production system adopted (intensive), average milk yield per cow, and barn design and management (total confinement free barn housing with no time at pasture, TMR, and feeding practices based on corn silage). The herd size was 110, 330, 600, 320, 210, 150, and 230 lactating and dry cows (herds A, B, C, D, E, F, and G, respectively). All herds were freestall barns equipped with axial flow fans and sprinklers in the feeding area. Lactating cows were fed TMR once a day, in the morning. To ensure cows had ad libitum access to the TMR, the amount offered to the cows was assessed on a daily basis with the aim of producing a 3 to 8% refusal. Cows were milked twice daily (0300–0500 h, and 1500–1700 h).

Constant management conditions within farm (operators, similar batches of feed, milking frequency, and working routine) were maintained thorough the experimental period.

The study was carried out from November 2013 till the end of July 2014. Measurements on microclimatic conditions, feed and diets, metabolic conditions, and fecal characteristics were carried out in winter (from the end of November 2013 to the end of January 2014), spring (from April to May 2014), and summer (July 2014). Individual blood and fecal samples were taken in each season from 14 cows per farm. The cows were selected according to parity and lactation stage [7 cows in early lactation: 30–90 d in milk (DIM), and 7 cows in mid lactation: 160–240 DIM]. The parity number and DIM, as well as milk yield of the selected cows are showed in Table 1. The sampling days in winter season were distributed in a wider period of time because climate conditions are quite stable, in spring the sampling days were mainly concentrated in May (5 farms), and finally in summer they were concentrated during the hottest period (July).

### Measurements and Analyses

***Microclimatic Conditions.*** The weather stations providing the climatic data belonged to the following institutions: Regional Environmental Protection Agency of the Lombardy Region and the Research Unit for Agricultural Climatology and Meteorology. The distances between farms and weather stations were calculated