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Short communication: Association between udder health status and blood serum proteins in dairy cows

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ABSTRACT

The aim of this study was to investigate the association between udder health (UH) status and blood serum proteins (i.e., total protein, albumin, globulin, and albumin-to-globulin ratio) in dairy cows. Blood and milk samples were collected from 1,508 cows of 6 different breeds (Holstein Friesian, Brown Swiss, Jersey, Simmental, Rendena, and Alpine Grey) that were housed in 41 multibreed herds. Bacteriological analysis was performed on milk samples with somatic cell count (SCC) >100,000 cells/mL and bacteria identification was confirmed by multiplex-PCR assays. Milk samples were grouped into 7 clusters of UH status: healthy (cows with milk SCC <100,000 cells/mL and not cultured); culture-negative samples with low, medium, or high SCC; and culture-positive samples with contagious, environmental, and opportunistic intramammary infections. Data of blood serum proteins were analyzed using a linear mixed model that included the fixed effects of stage of lactation, parity, breed, herd productivity (high or low production) and UH status, and the random effect of herd-date within herd productivity. Culture-negative samples with high milk SCC, which were most likely undergoing a strong inflammatory response and whose pathogens could not be isolated because they were engulfed by macrophages or because they had already cleared, and milk samples infected by contagious and environmental bacteria were associated with greater globulin concentrations (and lower albumin-to-globulin ratio) in blood. Variation in blood serum proteins seems to be associated with inflammatory status rather than infection, as serum globulin significantly increased in UH status groups with the highest milk SCC and no differences were observed among intramammary infections pathogens. Blood serum proteins can be a mammary gland inflammation indicator, but cannot be used to differentiate among different UH status groups.
Key words: subclinical mastitis, intramammary infection, blood serum proteins, dairy cattle

Short Communication

Udder health (**UH**) represents a critical issue for milk production, and monitoring the health status of the animals is of great importance for dairy farm economics. Bovine mastitis, an inflammatory status of the mammary gland in response to an infection, is the most prevalent production disease and strongly affects dairy herd income by decreasing milk yield and quality, cow welfare, fertility, and longevity (Seegers et al., 2003). Thus, a successful immune response of the animal to infection can improve milk production and reduce the treatment costs.

Blood serum proteins have been used as possible markers for assessing the immune status in dairy cows (Piccinini et al., 2004). In a previous study (Bobbo et al., 2017a), we characterized the variation in blood serum proteins [i.e., total protein, albumin, globulin and albumin-to-globulin ratio (**A:G**)] in dairy cattle housed in multibreed herds and concluded that environmental factors (e.g., herd productivity), breed, and individual cow factors (stage of lactation and parity) must be considered to appropriately interpret blood serum proteins as cow health indicators. After adjusting for those factors, a linear relationship between blood serum proteins and milk SCS was identified. Cows with high SCC in milk had greater total protein and globulin concentrations in blood serum, an expected result given that α- and γ-globulins are involved in the immune response of the mammary gland.

Besides the increased milk SCC related to the inflammatory status of the mammary gland, specific

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IMI pathogens can elicit differential immune responses (Bannerman et al., 2004) and differences in SCC variation (de Haas et al., 2002). To our knowledge, variation in blood serum proteins among various UH status groups has not been investigated yet. Therefore, the objective of the present study was to assess the association between UH status and blood serum proteins in dairy cows. Rather than simply focus on microbiologically positive infections, we evaluated both the IMI and the resulting inflammatory response.

This study is part of the Cowplus Project, described in detail in Bobbo et al. (2017a,b). Briefly, blood and milk samples were collected from 1,508 cows belonging to 3 specialized dairy breeds (Holstein Friesian, Brown Swiss, and Jersey) and 3 dual-purpose breeds of Alpine origin (Simmental, Rendena, and Alpine Grey), that were housed in 41 multibreed herds of Trentino region (northeast Italy). In a calendar year, 1 herd per day was visited once and only clinically healthy cows at the moment of the visit were sampled during an evening milking. Details on procedures used to collect and analyze blood samples for serum proteins determination can be found in Bobbo et al. (2017a). From each selected cow, a composite milk sample (40 mL) for bacteriological analyses and a second sample (50 mL) for SCC determination were collected. Details on milk samples collection, storage, and analyses were reported in Bobbo et al. (2017b). Cows with milk SCC <100,000 cells/mL were considered potentially healthy and were not subjected to microbiological examination. Bacteriological analysis was performed on milk samples with SCC >100,000 cells/mL according to the guidelines of National Mastitis Council (NMC, 1999). Briefly, each sample was plated twice by streaking 0.01 mL of milk onto Columbia Blood Agar containing 5% defibrinated sheep blood (Oxoid Ltd., Basingstoke, UK), incubated aerobically at 37 ± 1°C, and examined after 24 and 48 h. Grown colonies were characterized mainly according to their morphology, hemolytic features, Gram staining, catalase, oxidase, coagulase reactions, and biochemical properties (NMC, 1999). Gram-positive microorganisms were differentiated as staphylococci and streptococci by the catalase reaction. The coagulase tube test in rabbit plasma (bioMérieux Italia S.p.A., Grassina, Italy), was used to differentiate coagulase positive from coagulase-negative *Staphylococcus* spp.

The Christie, Atkins, Munch-Petersen (CAMP) test was used for the presumptive identification of *Streptococcus agalactiae*. *Enterococcus* spp. was confirmed plating suspected colonies onto Bile Esculin Agar (Oxoid Ltd.). *Lactococcus lactis* and *Aerococcus viridans* were identified by using API system (bioMérieux Italia S.p.A.). Gram-negative bacteria were identified by their morphological features on MacConkey agar, eosin methylene blue agar, Kligler iron agar, urea agar, and SIM agar (Oxoid Ltd.). Bacterial genus confirmation and species identification of *Staphylococcus*, *Streptococcus*, and *Escherichia coli* strains was confirmed by multiplex-PCR assays, as previously described by Shome et al. (2011) with minor changes related to Taq DNA polymerase (KAPA2G FastMultiplex PCR Kit, Kapa Biosystems, Wilmington, MA) and cycling protocol.

Contaminated plates presented 3 or more different colony types, with no prevalence of a single colony type (NMC, 1999). Samples were classified as culture-negative when no bacteria were isolated or no significant growth (<1,000 cfu/mL) was observed within 48 h of incubation, with the exception of potential contagious IMI cases, for which identification was performed even when 1 colony (≥100 cfu/mL) was isolated.

As reported in Stocco et al. (2017), farms were classified as high or low production. Briefly, the net energy content of milk (kcal/kg) yielded the day of sampling by each cow was estimated, converted to kilojoules per kilogram and multiplied by the individual daily milk production (kg/d) to obtain the individual daily milk energy production of each cow (kJ/d). Data were then analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC), including herd, breed, parity, and DIM of cows, to estimate the least squares means (**LSM**) of the average daily milk energy production of each herd. The 41 farms were ranked according to the estimated LSM of their average daily milk energy yield, and were classified as low- (n = 21) or high-producing (n = 20) based on the median value of their LSM.

Seven clusters of UH status were identified (Bobbo et al., 2017b): healthy (cows with milk SCC <100,000 cells/mL and not tested for the presence of bacteria); culture-negative samples with low (**No Growth_L**), medium (**No Growth_M**), and high (**No Growth_H**) SCC (divided on the basis of the SCS 25th and 75th percentiles); and culture-positive samples with contagious, environmental, and opportunistic IMI.

Data of blood serum proteins were analyzed using the MIXED procedure of SAS with the linear mixed model applied also in Bobbo et al. (2017b):

$$y_{ijklmno} = \mu + DIM_i + Parity_j + Breed_k + UH\ status_l + HP_m + HTD_n(HP)_m + e_{ijklmno},$$

where $y_{ijklmno}$ is the investigated trait (blood serum proteins); μ is the overall mean; DIM_i is the fixed effect of the ith class of DIM ($i = 6$ classes of 60-d intervals); $Parity_j$ is the fixed effect of the jth parity ($j = 1$ to ≥4); $Breed_k$ is the fixed effect of the kth breed ($k =$ Holstein