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Serum haptoglobin and protein electrophoretic fraction modifications in buffaloes (*Bubalus bubalis*) around calving and during early lactation

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Abstract

Serum protein distribution and concentration can be affected by different physiological and pathological conditions. The aim of this study was to evaluate the changes in the concentration of serum protein fractions and haptoglobin in clinically healthy dairy buffaloes during late pregnancy and early lactation. Blood and milk samples were collected from 30 buffaloes at around 7 d before expected calving (blood only) and 7, 30 and 50 d after calving. In serum samples, the total protein, haptoglobin, albumin, α1-, α2-, β1-, β2-, γ-globulins, and albumin/globulin ratio (A/G) values were evaluated. In milk, fat%, protein%, lactose%, somatic cell score (SCS) were assessed, along with milk yield (MY) and daily milk production (DMP). The peripartum period significantly influenced ($P < 0.005$) total protein, albumin, haptoglobin, α2-, β2- and γ-globulins ($P < 0.005$). Milk yield, DMP and fat% changed significantly throughout the monitoring period ($P < 0.005$). Milk yield and DMP were positively correlated with total protein, albumin, β2-globulins and A/G ratio, and negatively correlated with haptoglobin and α2-globulins. These results provide new knowledge about the serum protein electrophoretic pattern in Italian Mediterranean Buffaloes during the last phase of pregnancy and early stages of lactation.

Laboratory medicine is an important approach that helps practitioners to monitor the animal's health at individual and herd levels. Serum biochemical analysis represents a useful tool to prove normality and to diagnose disease and physiological alterations (Kaneko *et al*., 1997). Pictorial representation of the protein profile, or proteinogram, is an important auxiliary exam, helpful to clinical biochemistry, and represents one of the most reliable methods for identification of blood proteins (Kaneko *et al*., 1997). The electrophoretic techniques most commonly used in veterinary medicine are made with cellulose acetate strips, agarose films and polyacrylamide gel (Kaneko *et al*., 1997).

Serum protein distribution and concentration can be affected by different physiological and pathological conditions. Therefore, an identifying and quantifying protein fraction enables the identification of animals with serum protein abnormalities, which may reflect responses to changes in homeostasis or disease. It has been suggested that acute phase proteins (APPs) may provide valuable diagnostic information in detection, prognosis and monitoring of disease in animal species (Tajik *et al*., 2012). During inflammation, protein production in the liver is switched toward increased synthesis of positive acute phase proteins such as haptoglobin (Tajik *et al*., 2012). Haptoglobin is synthesized in the liver and is a major acute-phase protein in numerous species of animals. It is believed that the circulating level of haptoglobin in ruminants is negligible in normal animals, but increases over 100-fold with immune stimulation (Tajik *et al*., 2012). The late gestation and early lactation period represents the most important and difficult time for high-yielding dairy cows as well as dairy buffaloes, connected with the change from a gestational non-lactating to a non-gestational lactating state (Fiore *et al*., 2017). Peripartum animals undergo serious metabolic and physiological changes during this period which may induce stress and affect metabolic processes (Fiore *et al*., 2017). To the best of our knowledge, literature concerning the evaluation of serum protein electrophoretic pattern and acute phase proteins and their variation during late pregnancy and early lactation in buffaloes is scarce. Since it is necessary to know the protein profile of healthy buffaloes for subsequent comparison with pathologic processes, the aim of this study was to evaluate the serum protein electrophoretic pattern in clinically healthy dairy buffaloes during late pregnancy and early lactation.

Materials and methods

Detailed materials and methods are provided in the online Supplementary File.

Farm conditions and animals

A total of 30 multiparous Italian Mediterranean Buffaloes (*Bubalus bubalis*) of mean age 114 ± 8 months and 540 ± 55 kg average body weight were randomly selected from a farm of 850 animals located in Southern Italy. The farm's average milk production was 2827.5 ± 654.6 kg per year with an average of 8.47% of milk-fat and 4.74% of milk protein. All buffaloes had a dry period of 120 d. Details of the total mixed ration (TMR) and the chemical composition of diets used during dry period and subsequent early lactation period are reported in online Supplementary Table S1. Water was available *ad libitum*. All animals enrolled in the study were clinically healthy and they were kept under natural photoperiod and an average environmental temperature of 14 ± 6 °C. Body condition score (BCS) was evaluated, by a single operator, on a scale from 0 to 5 at the same time points as blood sampling according to the method of Edmonson *et al.* (1989). The protocol of this study was reviewed and approved in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and Directive 2010/63/EU.

Blood sampling and chemistry analysis

Blood sampling was performed at 4 different time points; 7 ± 5 d before expected calving and 7 ± 5, 30 ± 5 and 50 ± 5 d after calving. Blood sampling was performed early in the morning, before daily delivery of ration, by jugular venipuncture into 10 mL vacuum tubes without anticoagulant agent (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK). Serum separation was carried out immediately and serum was stored at −18 °C until analysis. On serum samples, the concentration of total proteins, haptoglobin, albumin, α1-globulins, α2-globulins, β1-globulins, β2-globulins and γ-globulins was evaluated. Total protein concentration was measured by means of automated analyzer BT 1500 (Biotecnica Instruments S.p.a., Rome, Italy) using the Biuret method with commercially available kit (Gesan S.r.l, Campobello di Mazara, Italy). Electrophoresis for protein fractions assessment was performed using an automated system (Selvet 24, Seleo Engineering, Naples, Italy) according to the procedures described by the manufacturer. The major protein fractions were divided according to the recommendation by the manufacturer from cathode to anode into albumin, α1-, α2-, β1-, β2- and γ-globulins, respectively.

Serum haptoglobin concentration was determined by means of automated analyzer BT 1500 (Biotecnica Instruments S.p.a., Rome, Italy) using the turbidimetric method with commercially available kit (Gesan S.r.l, Campobello di Mazara, Italy).

Milk sampling and analysis

Daily milk production (DMP) was measured by means of a commercial milk meter Afimilk MCP (Afifarm, Kibbutz Afikim, Israel). For each animal two milk samples were collected, one for the determination of somatic cell count (SCC) and the other one for milk composition, at the 3 *post partum* sampling points. Collection was performed aseptically according to National Mastitis Council guidelines (Hogan *et al.*, 1999). Milk samples were stored at 2–6 °C and cultured until 24 h. The SCC obtained values were converted to SCS (Somatic Cell Score) by:

$$\mathrm{SCS} = \log 2(\mathrm{SCC}/100) + 3$$

where SCC was in units of cells per microliter (Wiggans and Shook, 1987).

For the determination of milk composition, milk samples were analyzed using a lacto-scan (Milkotronic Analyzer, MCC, Nova Zagora, Bulgaria). The Net Energy of Lactation ($\mathrm{NE_L}$) represents the energy contained in the milk produced and was calculated by the equation proposed by Tyrrell and Reid (1965):

$$\mathrm{NE_L}(\mathrm{Mcal/kg}) = 0.0929 \times \mathrm{Fat\%} + 0.0547 \times \mathrm{Crude\ protein\%} + 0.0395 \times \mathrm{Lactose\%}$$

Statistical analysis

Data, expressed as mean values ± standard deviation (±SD), were tested for normality using the Shapiro–Wilk normality test. All data were normally distributed ($P > 0.05$) and the statistical analysis was performed. One-way analysis of variance (ANOVA) was used to determine a statistically significant effect of peripartum period on serum total proteins, haptoglobin, albumin and globulin fractions, and in order to verify the effect of time after calving on productive parameters and milk constituents. Bonferroni's multiple comparison test was applied for post-hoc comparison. The Person test was performed in order to assess significant correlations between hematochemical parameters and productive parameters and/or milk constituents. P value <0.05 was considered statistically significant. Statistical analysis was performed using the STATISTICA 7 software package (Stat Software Inc., Tulsa, Oklahoma, USA).

Results

Statistical analysis showed a significant effect of peripartum period on the values of BCS ($P < 0.05$), serum total proteins ($P < 0.005$), albumin ($P < 0.005$), haptoglobin ($P < 0.005$), α2-globulins ($P < 0.005$), β2-globulins ($P < 0.005$), γ-globulins ($P < 0.05$) and A/G ratio ($P < 0.005$). In particular, as shown in Fig. 1, higher BCS values were found at 7 d before calving with respect to 50 d after calving and higher total protein values were found at 30 d after calving with respect to both 7 d before and 7 d after calving. Albumin and A/G ratio showed lowest values at 7 d after calving, whereas haptoglobin showed higher levels at 7 d before and 7 d after calving with respect to later time points. Figure 2 shows the decreasing trend of α2-globulins and the increasing trend of β2-globulins after calving. In particular, α2-globulins showed higher levels at 7 d before and 7 d after calving in comparison to the values measured later, whereas β2-globulins showed higher levels at 30 and 50 d after calving in comparison to the values measured earlier. The γ-globulins fraction showed lower values at 7 d before calving compared to 30 d after calving.

Statistical effect of time after calving ($P < 0.005$) was found on productive parameters and milk constituents. Milk yield showed an increasing trend throughout the postpartum period with the

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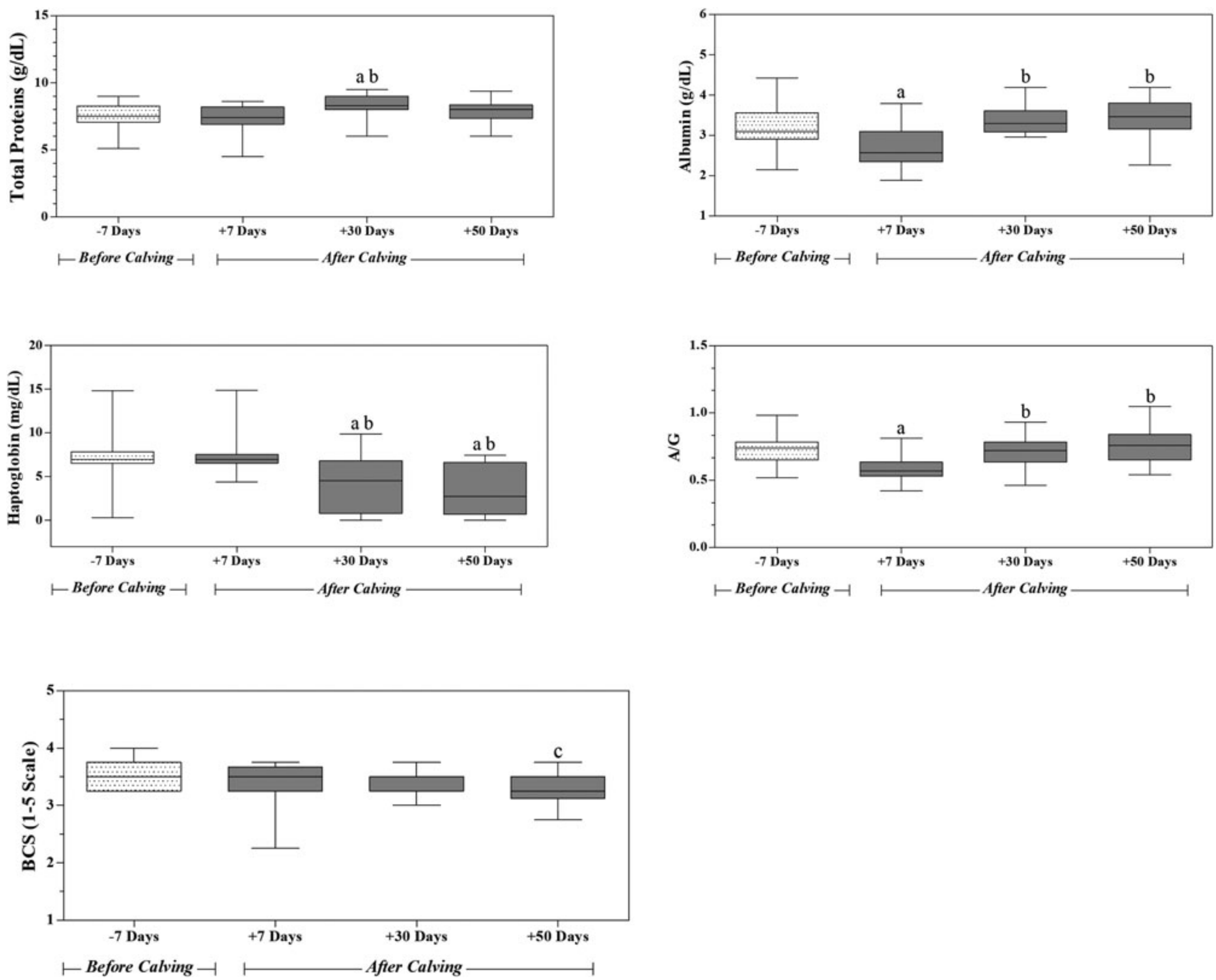

Fig. 1. Serum total protein, albumin, haptoglobin, A/G ratio and body condition score (BCS) measured in buffaloes throughout monitoring period(7 d before calving and 7, 30 and 50 d after calving). Values are mean ± SD with 25–75 percentile indicated by boxes. Superscripts report significant differences relative to 7 d pre-partum (a: $P < 0.01$, c: $P < 0.05$) or 7 d postpartum (b: $P < 0.01$).

highest values at 50 d after calving. DMP showed the lowest values at 7 d after calving. Fat% was significantly lower at 30 d with respect to 50 d after calving.

The values of serum total protein were positively correlated with milk yield ($r = +0.28$, $P = 0.01$) and DMP values ($r = +0.36$, $P = 0.0001$), albumin values were positively correlated with milk yield ($r = +0.52$, $P < 0.0001$) and DMP values ($r = +0.46$, $P < 0.0001$), β2-globulins were positively correlated with milk yield ($r = +0.30$, $P = 0.009$) and DMP values($r = +0.38$, $P = 0.008$), A/G ratio values were positively correlated with milk yield ($r = +0.54$, $P < 0.0001$) and DMP values ($r = +0.34$, $P = 0.003$). The values of serum α2-globulins fraction were negatively correlated with milk yield ($r = -0.55$, $P < 0.0001$) and DMP ($r = -0.46$, $P < 0.0001$) values. The haptoglobin levels were negatively correlated with the values of milk yield ($r = -0.47$, $P < 0.0001$) and DMP ($r = -0.36$, $P = 0.005$) values.

Discussion

In agreement with the findings of a previous study carried out on periparturient buffaloes (Deka *et al.*, 2014), the results obtained in the present study showed a lower BCS values of buffaloes after calving with respect to pre-partum, probably due to fat mobilization and energy requirement for milk production.

Buffalo milk is characterized by an elevated fat content, higher than that of cow milk. The results obtained in this study showed an increasing trend of milk yield throughout post-partum period. Highest DMP values and lowest milk fat% have been reported at 30 d after calving (Rosati and Van Vleck, 2002). In our study the protein% was stable during lactation with values similar to the ones reported by Rosati and Van Vleck (2002). The values of milk constituents obtained in the present study agree with those suggested for the Italian buffalo, officially registered by the Italian Breeders' Association (AIA, 2011).

The results obtained in this study showed dynamic changes in the concentration of serum total proteins, albumin, haptoglobin, α2-globulins, β2-globulins, α-globulins and A/G ratio in buffaloes during the last week of pregnancy and the early lactation. However, all considered serum parameters fell within the physiological reference values established for buffalo species (AbdEllah *et al.*, 2013). The lowest concentrations of serum total proteins were observed 1 week before and 1 week after calving followed

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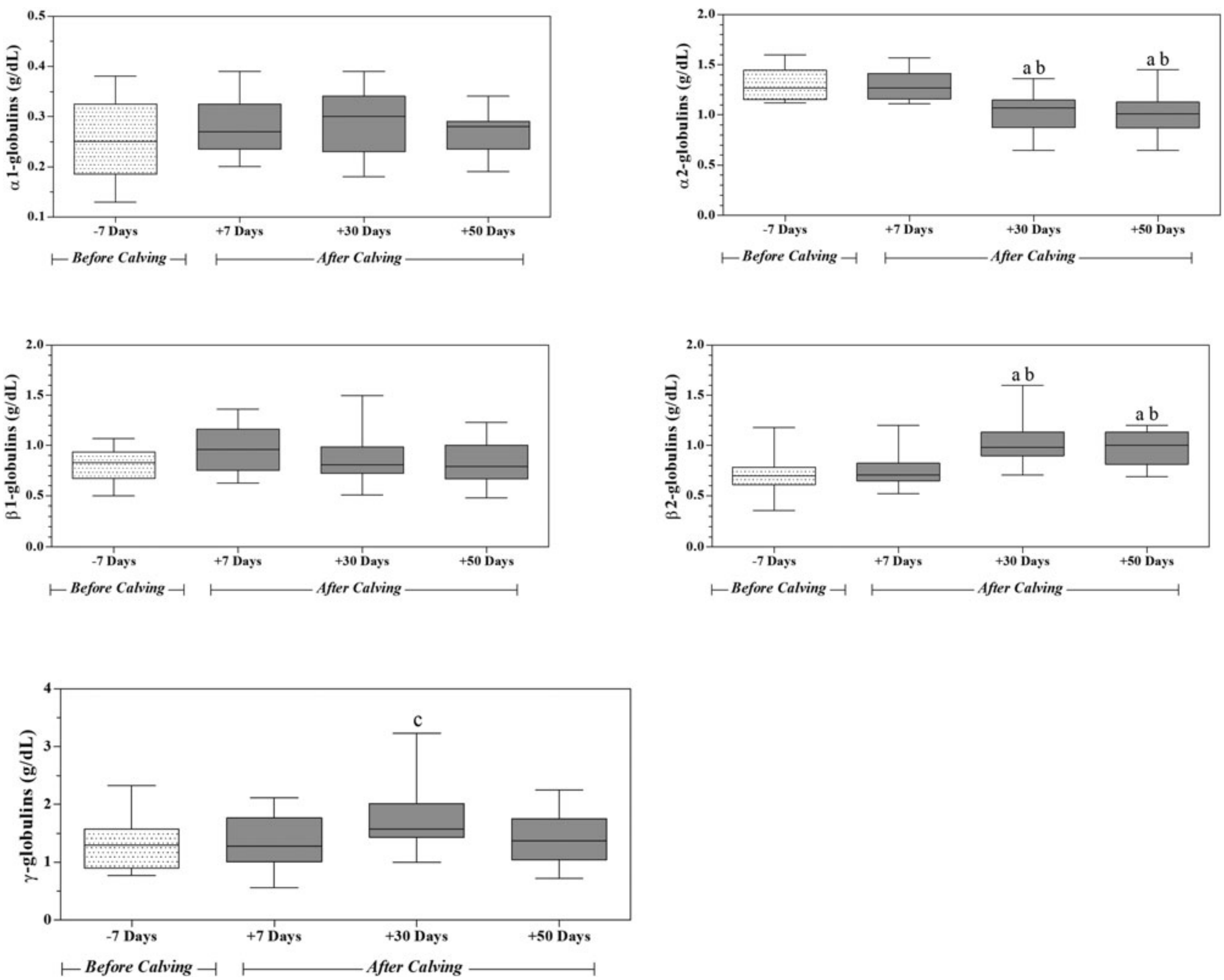

Fig. 2. Serum globulin fractions (α1-globulins,α2-globulins, β1-globulins, β2-globulins and α-globulins) measured in buffaloes throughout monitoring period (7 d before calving and 7, 30 and 50 d after calving). Values are mean ± SD with 25–75 percentile indicated by boxes. Superscripts report significant differences relative to 7 d prepartum (a: $P < 0.01$, c: $P < 0.05$) or 7 d postpartum (b: $P < 0.01$).

by an increase until the end of the monitoring period. The lower serum protein concentration observed at 7 d before calving might be caused by the utilization of amino acids from the maternal circulation for protein synthesis in the fetal muscles (Kaneko *et al.*, 1997). On the other hand, the lower concentrations of serum total proteins 7 d after calving may reflect the transfer of immunoglobulins from the bloodstream to the mammary gland for the synthesis of colostrum and milk (Kaneko *et al.*, 1997). Albumin showed lower values at 7 d after calving with respect to the other time points. The serum values of total proteins and albumin measured after calving correlated positively with milk yield and DMP. This finding seems to suggest that the diet administered to lactating buffaloes satisfied the increased protein demand during lactation period. Significant variations were found also for the concentrations of α2-globulins that showed highest values at 7 d before and 7 d after calving, thereafter showing a decreasing trend. The alpha fraction includes many diagnostically important acute phase proteins including haptoglobin. Effectively, in the current study haptoglobin followed the same trend of α2-globulins fraction throughout the monitoring period. These findings could be related to inflammatory processes occurring in the urogenital system and mammary gland in the period around calving. Although changes in the concentration of acute phase proteins were reported in diseased buffaloes (Tajik *et al.*, 2012), the modifications in the levels of these variables during the peripartum period should not be considered as a result of pathological processes. We believe that the increased secretion of α2-globulins, including haptoglobin, was a response to the traumatic experience during parturition and acute phase response preceding parturition, whereas it does not seem to depend on lactation. This supposition seems to be supported by the significant negative correlation found between milk productive parameters (milk yield and DMP) and α2-globulins and/or haptoglobin.

The β2-globulins fraction showed an opposite trend to that of the α2-globulins fraction. In particular, lower β2-globulins values were found in buffaloes at 7 d before and 7 d after calving in comparison to 30 and 50 d after calving. The lower concentrations of β2-globulins found in buffaloes before calving might be caused by the transfer of transferrin, which is one of the main proteins from this fraction, into the mammary gland for the synthesis of colostrum and milk. On the other hand, β2-globulins fraction includes some other proteins, such as complement, involved in the inflammatory and stress responses (Kaneko *et al*., 1997) and thus may be responsible for the increasing values of this globulin fraction after calving. The increase in the values of this protein fraction found at 30 and 50 d after calving highlights that early lactation period is especially critical and presents considerable physiological challenges to homeostasis in dairy buffaloes. This hypothesis seems to be supported by the significant positive correlation found between milk productive parameters (milk yield and DMP) and β2-globulins values. This study showed significant changes also in the γ-globulins fraction with lower values at 7 d before calving compared to postpartum. The lower concentrations of γ-globulins before calving could be associated with the transport of immunoglobulins from the blood stream across the mammary barrier and into the lacteal secretion during colostrogenesis (Kaneko *et al*., 1997). These shifts in the concentrations of albumin and globulin fractions resulted in changes of the A/G ratio that were positively correlated with milk yield and DMP. The ratio differences observed in buffaloes around the time of parturition may reflect the transport of immunoglobulins and some proteins to the mammary gland.

In conclusion, pregnancy and lactation are physiological periods that result in an increased metabolic demand that, if not satisfied, could provoke a threat to homeostasis. Because of the importance of various proteins to normal functions in the body, changes in serum protein electrophoresis, properly interpreted, can be one of the most useful diagnostic aids available to the clinician to monitor the health status of the buffaloes in order to promote well-being of the animal and to improve productivity (Kaneko *et al*., 1997). The results obtained in the present study showed that late pregnancy and early lactation periods are accompanied by dynamic changes in the levels of serum protein electrophoretic pattern in Italian Mediterranean Buffaloes. The changes found in the concentration of albumin, globulin fractions and haptoglobin seem to reflect a physiological response to the inflammatory processes occurring around calving, and to the beginning of lactation as well. Our results may contribute to the improvement of the current knowledge about the serum protein electrophoretic pattern in Italian Mediterranean Buffaloes during the last phase of pregnancy and early stages of lactation.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029919000438

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Conflict of interest. The authors declare no conflicts of interest.

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