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The establishment of specific reference intervals (RIs) is advisable when different metabolic status in the patient population are present, or when existing RIs are not suitable for the laboratory's patient population. Healthy Holstein dairy cows, at the start of lactation, experience a state of negative energy balance and altered immune system function that can lead to different laboratory results compared with cows at peak lactation, when a more balanced metabolic state is present. RIs created taking into account these differences have not been published to date.

The aim of this study was the *a posteriori* determination of RIs of Holstein cows at 3 ± 1 and 30 ± 3 days in milk (DIM).

To this aim, data from 145 cows, from 4 herds, were selected from our database. Serum biochemistry and protein electrophoresis were performed with automated instruments (ILAB 300 plus, International Laboratory, Italy and Hydrasis, Sebia, Italy, respectively). Hematology was performed on an ADVIA 120 analyzer (Siemens, Italy). RIs were generated and the effects (regression analysis, $p < 0.05$) of stage of lactation, herd and day of sampling were analyzed with Reference Value Advisor and Analyse-it.

Data from 32/39 analytes (RBC, Hb, Ht, MCV, MCHC, PLT, neutrophil, lymphocytes, monocytes, total globulin, α 1-globulin, α 2-globulin, β 2-globulin, γ -globulin, A/G ratio, ALP, AST, creatinine, Cl, K, total bilirubin, NEFA, BOHB, Ca, GGT, Mg, Pi, total protein, urea, glucose, cholesterol and zinc) were significantly different according to lactation stage and specific RIs were adopted. On the contrary, 7/39 analytes (WBC, MCH, RDW, eosinophils, potassium, albumin, β 1-globulin) were not significantly different between the two groups, thus common RIs were adopted for these parameters. Creatinine at 3 ± 1 DIM was significantly different in the different sampling days. Statistical analysis revealed some herd-specific differences at 3 ± 1 DIM (MCHC, RDW, β 1-globulin, AST, total protein, glucose, and NEFA) or at day 30 ± 3 (MCHC, eosinophils, albumin, β 1-, β 2- and γ -globulin, ALP, Mg, total protein, glucose, zinc, NEFA, and BOHB). The adoption of day-specific or herd-specific RIs, however, has practical limitations and in routine practice it may be advisable to take into account the possible herd-specific peculiarities when results are close to the lactation-specific RI rather than generating additional RIs.

In conclusion, the use of RIs specific for the lactation stage is highly justified from both a statistical and a biological point of view. Preanalytical factors associated with day of sampling or management need to be considered in the evaluation of results from some analytes.

DISCLOSURES

No disclosures to report.