ORIGINAL ARTICLE

The Association Between Plasma Natural Antibodies and Inflammatory Biomarkers Two Weeks After Calving in Cows with No Dry Period

Novi Mayasari[1], Henk K. Parmentier[2], Erminio Trevisi[3], Bas Kemp[2], Ariette van Knegsel[2]

[1] Laboratory of Physiology and Biochemistry, Faculty of Animal Science, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM 21, Jatinangor, 45363, Indonesia,
[2] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands
[3] Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy

ABSTRACT

Introduction: Improved energy balance, metabolic status, and natural antibodies (NAb) has been shown in cows with no dry period, however these cows showed increased inflammation status in early lactation. The aim of this study was to determine the association between plasma natural antibodies and inflammatory biomarkers in cows with no dry period during the first two weeks postpartum. **Methods:** Holstein-Friesian dairy cows (n=55) were selected. Before enroll to the experiment, cows were clinically healthy. Plasma samples were collected at week 1 and 2 after calving and were analyzed for NAb binding megantura-keyhole limpet hemocyanin and inflammatory biomarkers. **Results:** Cows with no dry period in this study had an improved energy balance and maintain NAb titers but increased ceruloplasmin (inflammatory biomarkers) in early lactation. In this study we found a significant correlation between NAb IgG binding KLH and haptoglobin in plasma ($P<0.01$). However, there were no correlations between albumin, cholesterol and NAb (IgG and IgM) binding KLH. **Conclusion:** This study demonstrate that cows with no dry period have an improved energy balance and maintained the level of natural antibodies in plasma. Moreover, IgG titers in plasma might be correlated with haptoglobin due to inflammation during calving until 2 wk postpartum.

Keywords: Continuous milking, Inflammation, Antibodies

Corresponding Author:
Novi Mayasari, PhD
Email: novi.mayasari@unpad.ac.id
Tel: +62-81214609646

INTRODUCTION

During transition period, immune status in dairy cows were suppressed and need to be increased. It is known that dairy cows are characterized with immune suppression during transition period, which is related with severe negative energy balance (EB), and high rate of infection diseases and metabolic disorders (8). Innate immunity is the first line defense against infection (1), and natural antibodies (NAb) are a part of humoral innate immunity before get any antigenic stimulation (2). CD5+ B-1 cells produce natural antibodies in healthy individuals and NAb mainly consist of immunoglobulin M (IgM), IgG and IgA (3,4). In previous research, NAb binding keyhole limpet hemocyanin (KLH) were higher in cows with an improved EB in early lactation (7). Transition period is the crucial time for dairy cows especially in the first two weeks after calving. In early lactation, cows experienced negative EB, which is related to immunosuppression (9). Negative EB was not only related to NAb but also was associated with enhanced level of inflammatory biomarkers (10) and metabolic disorders (11) in dairy cows during early lactation.

In early lactation, increased disease rates are commonly reported among high-yielding dairy cows and characterized by the occurrence of an inflammatory response indicated by acute phase protein (APR) (12). Inflammation evokes white blood cells (WBC) to release of tumor necrosis factor-alpha (TNF-α) and (interleukin-1 and -6) (IL 1 or 6). As a consequence, TNF-α and IL-1 or 6 triggered the release of acute phase response (13). During the response of acute phase protein, positive acute phase reactants (+AP) including haptoglobin and ceruloplasmin were increased in plasma and negative

acute phase reactants (-AP) including cholesterol and albumin were reduced in plasma (13,14).

Cows with no dry period had better energy and metabolic status (15), however these cows had higher ceruloplasmin and oxidative stress compared with cows with a 60-d dry period (10). In an earlier study, cows with no dry period had a higher plasma NAb (IgG) binding liposaccharide (LPS), and higher NAb (IgG and IgM) binding KLH and LPS in milk compared with cows with a short or conventional dry period (16). The relationship between inflammatory biomarkers and NAb titer in plasma during the first two weeks after calving in cows with no dry period are less known. The objective of this study was to determine the association between plasma NAb and inflammatory biomarkers (haptoglobin, ceruloplasmin, albumin and cholesterol) in the first two weeks after calving in cows with no dry period.

MATERIALS AND METHODS

Animals and Experimental Design

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Wageningen University. The registration number of the experimental protocol was 2010026. The experimental design was described in our earlier study (11). In present study, we investigated data of inflammatory biomarkers and NAb titers from cows with no dry period from earlier study (10). Holstein-Friesian dairy (n=55) were selected from the Dairy Campus research herd. Cows were housed in a freestall with slatted floor and cubicles. Cows were milked twice daily (0500 and 1630 h).

Rations

Ration composition was described earlier (11). Prepartum, cows with no dry period received a lactating cow ration supporting 25 kg of milk yield per day. Forage composition during prepartum and postpartum-treatments consisted of grass silage, corn silage, wheat straw, and a protein source with different ratio. Rations were isocaloric. Concentrate and forage were supplied separately and provided adlibitum.

Blood Sampling

Blood samples were taken from all cows (n=55) in the morning from the tail vein at week 1 and 2 postpartum. Blood samples collected in evacuated tubes containing litium-heparin and immediately put on ice. All blood samples were centrifuged at 3,000 × g for 15 min at 4˚C., frozen, and stored as plasma at -20˚C until use.

Laboratory Analysis

Natural antibody titers binding Megathura crenulata-derived KLH (Sigma, H7017 Sigma Aldrich Co., St Louis, MO) in plasma of cows were measured by an indirect enzyme-linked immunosorbent assay (ELISA) technique as outlined in previous study (16). In brief, titers of natural antibodies in plasma of IgG and IgM isotype were detected using 1:20,000. IgG and IgM were detected using diluted sheep anti-bovine IgG-heavy chain conjugated to horseradish peroxidase (Cat. No. E10-118P, Bethyl Laboratories) and rabbit anti-bovine IgM-whole molecule conjugated to horseradish peroxidase (Cat. No. A10-100P, Bethyl Laboratories), respectively. During ELISA, both IgG and IgM used four-step serial dilutions and started with the ratio 1:40. After washing, a substrate containing tetra methyl benzidine (TMB from Sigma) and 0.05% distillated water was added. The plates were incubated for 10-15 minutes at room temperature and the reaction was stopped by adding 1.25 M sulfuric acid. To measure the extinctions of the titers of IgG and IgM, a Multiskan reader with a wavelength of 450 nm was used.

Inflammatory biomarkers were measured using a clinical auto-analyzer (ILAB 650, Instrumentation Laboratory, Lexington, MA, USA). In current study, the level of total cholesterol, albumin, haptoglobin and ceruloplasmin was determined with the method described and were standardized for each assay (21).

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS, version 9.4). To assess associations of NAb (IgG and IgM) titers binding KLH with inflammatory biomarkers (haptoglobin, ceruloplasmin, albumin and cholesterol), the titers from week one and two were added as a linear covariate to developed statistical logistic regression model. The regression coefficient (β) from the statistical model and the p-value corresponding to the β are displayed.

RESULTS

Inflammatory biomarkers and natural antibodies

In the current study, cows with no dry period were investigated for the associations between inflammatory biomarkers and natural antibodies in plasma in the first two weeks postpartum. Earlier study found that cows with no dry period in present study compared with a short or a conventional dry period had higher cholesterol, higher ceruloplasmin and tended to have higher haptoglobin levels in plasma in early lactation (10). The increase of haptoglobin levels in plasma was earlier related with high production of liver macrophages (known as Kupffer cells) during inflammation (22-24). Previous studies showed a positive relationship between plasma ceruloplasmin levels and clinical health problems in cows in early lactation (25-27).

Cows in the current study not only had higher inflammatory biomarkers (haptoglobin and ceruloplasmin) but they also showed increased NAb binding KLH in early lactation (16). Our earlier study showed that the higher NAb binding KLH was associated

with mammary health in early lactation. The levels of inflammatory biomarkers and plasma antibodies binding KLH for cows with no dry period in the first two weeks after calving are shown in Fig 1, 2 and 3.

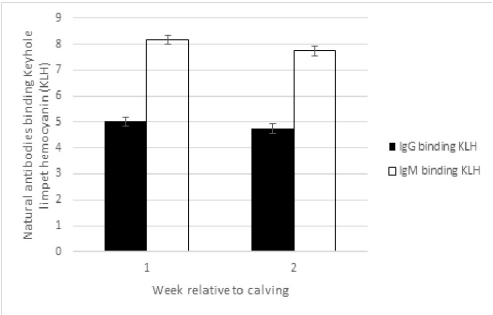

Fig. 1 : Natural antibodies for isotype IgG and IgM binding keyhole limpet hemocyanin in plasma of cows with no dry period in the first two weeks after calving . Values represent means ± SEM.

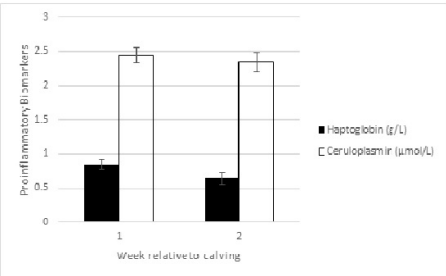

Fig. 2 : Proinflammatory biomarkers for haptoglobin and ceruloplasmin in plasma of cows with no dry period in the first two weeks after calving. Values represent means ± SEM.

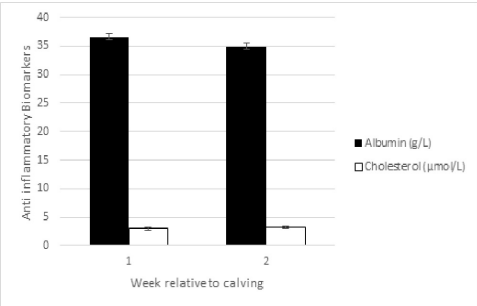

Fig. 3 : Antiinflammatory biomarkers for albumin and cholesterol in plasma of cows with no dry period in the first two weeks after calving. Values represent means ± SEM.

The association between inflammatory biomarkers and natural antibodies

The association between inflammatory biomarkers and NAb binding KLH in cows with no dry period in the first two weeks postpartum are shown in Table I. In the current study, we found a positive relationship between NAb IgG and haptoglobin levels in plasma (β = 0.97, *P* = 0.03). The increased plasma NAb IgG binding KLH levels were accompanied by an increased plasma haptoglobin levels. In addition, our study indicated that the increased plasma NAb IgG binding KLH levels tended to be related with increased ceruloplasmin levels (β = 0.45, *P* = 0.11), at least in the first two weeks after calving. The positive association between IgG and (+) acute protein response could be related with a severe inflammatory condition (14) of cows with no dry period in early lactation. In the current study, we did not find any association between IgM binding KLH with inflammatory biomarkers.

Table I : Regression coefficient (β) and *P*-value of plasma natural antibodies (NAb) binding keyhole limpet hemocyanin (KLH) related to haptoglobin, ceruloplasmin, albumin and cholesterol in dairy cows with 0-d dry period in the first two weeks after calving

Variable	IgG binding KLH		IgM binding KLH	
	β	*P*-value	β	*P*-value
Haptoglobin (g/L)	0.97	0.03	0.4	0.17
Ceruloplasmin (µmol/L)	0.45	0.11	-0.06	0.74
Albumin (g/L)	-0.01	0.82	-0.03	0.46
Cholesterol (µmol/L)	0.18	0.35	0.03	0.79

DISCUSSION

In the current experiment, cows with no dry period had improved EB and lower daily milk yield with similar dry matter intake (15). In addition, cows with no dry period had higher NAb titers (16) and inflammation status. It was suggested that the higher NAb titers for cows in plasma with no dry period are related with the improved EB (16). In our earlier study, specific plasma NAb were associated with high somatic cell count (SCC) and clinical mastitis. Our earlier study showed that "increasing plasma NAb titer for IgM binding KLH in the week before the occurrence of high SCC were associated with a decreased odd of high SCC occurrence. Moreover, increasing titers of IgM binding KLH or LPS in plasma in the three weeks before the incidence of the disease was associated with decreased odds of CM occurrence" (10 p. 8). It was suggested that NAb levels in plasma or in milk, may be an additional health biomarker to select for mastitis resistance in dairy cows (28,29).

In the current study, high SCC in cows with no dry period was not associated with inflammatory biomarkers (10). There were several causes for inflammation during transition period such as differentiation of mammary gland cell and high oxidative stress (30). A previous study showed that cows with subclinical mastitis (31) and clinical mastitis (32) had increased haptoglobin levels in plasma. Haptoglobin is an acute phase protein synthesized in the liver in response to inflammation (33) and it can be measured in serum. Haptoglobin binds to haemoglobin and so inhibits bacterial proliferation by reducing the availability of iron. Haptoglobin measurement has been of particular interest for detecting

inflammation in cattle and dairy cows due to its virtual absence in the serum of healthy animals (34). Moreover, haptoglobin is more commonly available as a routine analysis compared with many other acute phase protein. Ceruloplasmin is plasma α-2 glycoprotein and one of the important positive acute phase protein. Ceruloplasmin plays an important role for immune system which help to transport copper in the blood by the enzymes lysyl oxidase and Cu-Zn superoxide dismutase. Moreover, ceruloplasmin involved in iron metabolism (ferroxidase) (35). As we know, copper improves immune function by acting on the levels of various enzymes mediating the antioxidant system and protects cells against oxidative damage. Low level of ceruloplasmin in plasma decreased phagocytosis and antimicrobial therefore increase inflammatory conditions (36).

In the previous study, some clinical health problems like fever, metritis, mastitis, retained placenta) were related with high levels of ceruloplasmin and a tendency for high haptoglobin levels in plasma (10). A previous study reported that clinical health problems has been associated with consequences of prolonged inflammations before calving (37). It seems that the increases of inflammatory biomarkers and NAb in plasma were correlated not only due to specific diseases or health problem but may be due to several causes of inflammation conditions in early lactation.

CONCLUSION

In conclusion, a positive correlation between haptoglobin and natural antibodies IgG binding keyhole limpet hemocyanin were found in the first weeks after calving in cows with no dry period. The association between components within immune responses showed complex cause-effect of defensive effect in the body. An inflammatory status and antibody responses attributed to negative EB should be disentangled in various subclinical and clinical health problems related with inflammation may partly explain the changes in inflammatory biomarkers in early lactation.

ACKNOWLEDGEMENT

The authors thank the Dutch Dairy Board (PZ, Zoetermeer, the Netherlands), the Product Board Animal Feed (PDV, Zoetermeer, the Netherlands), and CRV (Arnhem, the Netherlands), for financing the experiment. The authors thank G. de Vries Reilingh and Annarita Ferrari for their technical support during the laboratory analysis.

REFERENCES

1. Matter MS, Ochsenbein AF. Natural antibodies target virus–antibody complexes to organized lymphoid tissue. Autoimmun Rev. 2008;7(6):480-6. URL : http://ac.els-cdn.com/S1568997208000426/1-s2.0-S1568997208000426-main.pdf?_tid=2f0474fa-3fac-11e3-ac58-00000aab0f01&acdnat=1382949630_978145fa9eaf7ace6ffba1f28852791b
2. Ochsenbein AF, Fehr T, Lutz C, Suter M, Brombacher F, Hengartner H, et al. Control of early viral and bacterial distribution and disease by natural antibodies. Science. 1999;286(5447):2156-9. URL : http://www.sciencemag.org/content/286/5447/2156.full.pdf
3. Kohler H, Bayry J, Nicoletti A, Kaveri SV. Natural autoantibodies as tools to predict the outcome of immune response? Scandinavian journal of immunology. 2003;58(3):285-9.
4. Avrameas S, Ternynck T, Tsonis IA, Lymberi P. Naturally occurring B-cell autoreactivity: A critical overview. Journal of Autoimmunity. 2007;29(4):213-8. URL : http://onlinelibrary.wiley.com/store/10.1046/j.1365-3083.2003.01314.x/asset/j.1365-3083.2003.01314.x.pdf?v=1&t=i38r9ksv&s=f0ee0c86009ae3a6efa5301ae2239859027 5d544
5. Ochsenbein AF, Zinkernagel RM. Natural antibodies and complement link innate and acquired immunity. Immunol Today. 2000;21(12):624-30. URL : http://www.scopus.com/inward/record.url?eid=2-s2.0-0034508025&partnerID=40&md5=b4fa17c0bd806d9ac1e0c9a71696f7c0
6. Zinkernagel RM. Immunological memory≠ protective immunity. Cell Mol Life Sci. 2012;69(10):1635-40. URL :http://download.springer.com/static/pdf/639/art%253A10.1007%252Fs00018-012-0972-y.pdf?auth66=1393755567_b3f5f42a47536ca66b992d8e438c2098&ext=.pdf
7. Van Knegsel ATM, de Vries Reilingh G, Meulenberg S, van den Brand H, Dijkstra J, Kemp B, et al. Natural antibodies related to energy balance in early lactation dairy cows. Journal of Dairy Science. 2007;90(12):5490-8. URL : http://www.sciencedirect.com/science/article/pii/S0022030207720222
8. Friggens NC, Newbold JR. Towards a biological basis for predicting nutrient partitioning: the dairy cow as an example. animal. 2007;1(01):87-97. URL : https://www.cambridge.org/core/journals/animal/article/towards-a-biological-basis-for-predicting-nutrient-partitioning-the-dairy-cow-as-an-example/774876F0D2E6242D69C16EDDFD378BA1
9. Mallard BA, Dekkers JC, Ireland MJ, Leslie KE, Sharif S, Vankampen CL, et al. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. Journal of Dairy Science. 1998;81(2):585-95. URL : http://www.sciencedirect.com/science/article/pii/S0022030298756127
10. Mayasari N, Chen J, Ferrari A, Bruckmaier RM, Kemp B, Parmentier HK, et al. Effects of dry period

length and dietary energy source on inflammatory biomarkers and oxidative stress in dairy cows. Journal of Dairy Science. 2017;100(6):4961-75. URL : https://www.sciencedirect.com/science/article/pii/S0022030217302722
11. Van Knegsel ATM, Hammon HM, Bernabucci U, Bertoni G, Bruckmaier RM, Goselink RMA, et al. Metabolic adaptation during early lactation: key to cow health, longevity and a sustainable dairy production chain. CAB Rev. 2014;9:15. URL : https://www.researchgate.net/publication/281736646_Metabolic_adaptation_during_early_lactation_Key_to_cow_health_longevity_and_a_sustainable_dairy_production_chain
12. Trevisi E, Amadori M, Cogrossi S, Razzuoli E, Bertoni G. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Research in Veterinary Science. 2012;93(2):695-704. URL : http://dx.doi.org/10.1016/j.rvsc.2011.11.008
13. Petersen HH, Nielsen JP, Heegaard PMH. Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary Research. 2004;35(2):163-87. URL : https://www.vetres.org/articles/vetres/pdf/2004/02/V4202.pdf
14. Bionaz M, Trevisi E, Calamari L, Librandi F, Ferrari A, Bertoni G. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. Journal of Dairy Science. 2007;90(4):1740-50. URL : https://www.sciencedirect.com/science/article/pii/S0022030207716600
15. Van Knegsel ATM, Remmelink GJ, Jorjong S, Fievez V, Kemp B. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. Journal of Dairy Science. 2014;97(3):1499-512. URL : https://www.sciencedirect.com/science/article/pii/S0022030214000423
16. Mayasari N, Rijks W, de Vries Reilingh G, Remmelink GJ, Ducro B, Kemp B, et al. The effects of dry period length and dietary energy source on natural antibody titers and mammary health in dairy cows. Preventive Veterinary Medicine. 2016;127:1-9. URL : https://www.sciencedirect.com/science/article/pii/S0167587716300733
17. Van Es AJH. Feed evaluation for dairy cows. Livest Prod Sci. 1975;4:95-175.
18. Tamminga S, Van Straalen WM, Subnel APJ, Meijer RGM, Steg A, Wever CJG, et al. The Dutch protein evaluation system: The DVE/OEB system. Livest Prod Sci. 1994;40:139-55.
19. Skinner JG, Brown RA, Roberts L. Bovine haptoglobin response in clinically defined field conditions. Vet Rec. 1991;128(7):147-9.
20. Owen JA, Better FC, Hoban J. A simple method for the determination of serum haptoglobins. J Clin Pathol. 1960;13(2):163-4.
21. Sunderman FW, Nomoto S. Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. Clinical chemistry. 1970;16(11):903-10. URL : http://clinchem.aaccjnls.org/content/clinchem/16/11/903.full.pdf
22. Ramadori G, Christ B, editors. Cytokines and the hepatic acute-phase response. Seminars in liver disease; 1999: © 1999 by Thieme Medical Publishers, Inc.
23. Trebicka J, Krag A, Gansweid S, Appenrodt B, Schiedermaier P, Sauerbruch T, et al. Endotoxin and tumor necrosis factor-receptor levels in portal and hepatic vein of patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt. European journal of gastroenterology & hepatology. 2011;23(12):1218-25.
24. Ametaj BN, Bradford BJ, Bobe G, Nafikov RA, Lu Y, Young JW, et al. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can J Anim Sci. 2005;85(2):165-75. URL : http://dx.doi.org/10.4141/A04-043
25. Conner JG, Eckersall PD, Wiseman A, Aitchison TC, Douglas TA. Bovine acute phase response following turpentine injection. Research in veterinary science. 1988;44(1):82-8.
26. Sheldon IM, Noakes DE, Rycroft A, Dobson H. Acute phase protein responses to uterine bacterial contamination in cattle after calving. Vet Rec. 2001;148(6):172-5.
27. Chassagne M, Barnouin J, Chacornac JP. Biological predictors for early clinical mastitis occurrence in Holstein cows under field conditions in France. Preventive Veterinary Medicine. 1998;35(1):29-38. URL : http://www.sciencedirect.com/science/article/pii/S0167587797000925
28. Ploegaert TCW, Wijga S, Tijhaar E, Van der Poel JJ, Lam TJGM, Savelkoul HFJ, et al. Genetic variation of natural antibodies in milk of Dutch Holstein-Friesian cows. J Dairy Sci. 2010;93(11):5467-73. URL : https://www.sciencedirect.com/science/article/pii/S0022030210005886
29. Thompson-Crispi KA, Miglior F, Mallard BA. Genetic parameters for natural antibodies and associations with specific antibody and mastitis in Canadian Holsteins. Journal of Dairy Science. 2013;96(6):3965-72. URL : https://www.sciencedirect.com/science/article/pii/S0022030213002774
30. Trevisi E, Zecconi A, Bertoni G, Piccinini R. Blood and milk immune and inflammatory profiles in periparturient dairy cows showing a different liver activity index. Journal of dairy research. 2010;77(03):310-7.
31. Safi S, Khoshvaghti A, Jafarzadeh SR, Bolourchi M, Nowrouzian I. Acute phase proteins in the diagnosis of bovine subclinical mastitis. Veterinary Clinical Pathology. 2009;38(4):471-6. URL : https://onlinelibrary.wiley.com/store/10.1111/j.1939-165X.2009.00156.x/asset/j.1939-165X.200

9.00156.x.pdf?v=1&t=io2mpa4h&s=0376338936 3193fdba6cc36f716daed8e68b5268
32. Pyörälä S. Indicators of inflammation in the diagnosis of mastitis. Veterinary research. 2003;34(5):565-78. URL : http://www.vetres.org/articles/vetres/pdf/2003/05/V3507.pdf
33. Crawford ML, Quigley Iii JD, Martin KR. Immunoglobulin Concentrations in Serum in Response to Injectable Immunoglobulin in Neonatal Dairy Calves. Journal of Dairy Science. 1995;78(7):1567-72. URL : https://www.sciencedirect.com/science/article/pii/S0022030295767790
34. Eckersall PD, Young FJ, McComb C, Hogarth CJ, Safi S, Weber A, et al. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet Rec. 2001;148(2):35-41.
35. Floris G, Medda R, Padiglia A, Musci G. The physiopathological significance of ceruloplasmin: a possible therapeutic approach. Biochemical pharmacology. 2000;60(12):1735-41. URL : http://www.sciencedirect.com/science/article/pii/S0006295200003993
36. Cerone S, Sansinanea A, Streitenberger S, Garcia M, Auza N. Cytochrome c oxidase, Cu, Zn-superoxide dismutase, and ceruloplasmin activities in copper-deficient bovines. Biol Trace Elem Res. 2000;73(3):269-78. URL : https://link.springer.com/article/10.1385%2FBTER%3A73%3A3%3A269
37. Trevisi E, Amadori M, Archetti I, Lacetera N, Bertoni G. Inflammatory response and acute phase proteins in the transition period of high-yielding dairy cows. F Veas (Ed), Acute Phase Proteins (2nd), InTech, Rijeka, Croatia (2011),. 2011:355–80.