NOTE
Internal Medicine

Serum iron concentration as a marker of inflammation in young cows that underwent dehorning operation

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ABSTRACT. This study aimed to assess the usefulness of serum iron (Fe) concentration as a marker of inflammation caused by the dehorning operation. Five young Holstein cows aged 205.0 ± 10.7 days and weighing 207.2 ± 24.1 kg underwent the dehorning operation. Blood samples were withdrawn before dehorning (pre) and at time periods of t=0.5, 2, 4, 6, 8, 12, 24, and 48 hr. The serum amyloid A (SAA) concentration was significantly high at t=48 hr ($P<0.01$). The serum Fe concentration significantly decreased, reaching 90.0 ± 36.4 μg/dl at t=24 hr ($P<0.001$). Therefore, serum Fe concentration showed significant and negative correlation with SAA concentration (r^2=0.500, $P<0.01$). In conclusion, serum Fe concentration is a useful marker of inflammation in young cows that have undergone the dehorning operation.

KEY WORDS: calf, dehorning, Inflammation, iron, serum amyloid A

Inflammation is a complex response to cell or tissue injury. Acute inflammation causes a non-specific systemic reaction as an acute phase response. The host's response to inflammation, the "acute phase response", is a highly conserved series of physiological reactions, including marked changes in plasma protein concentrations. The systemic reaction to proinflammatory cytokines is the production and secretion of acute phase proteins (APPs) by the liver [6]. Testing for APPs allows early detection of inflammation because APPs are released early during the inflammatory response. The common APPs measured in cattle are haptoglobin (Hp), serum amyloid A (SAA), lipopolysaccharide binding protein, alpha (1)-acid glycoprotein, and transferrin. [8, 10, 21].

Iron (Fe) plays important roles in many enzymatic activities and is an essential trace element for the host and pathogen [17]. Neumann [16] reported that serum Fe levels decreased in 90% of cats and 60% of dogs with inflammatory diseases. This reduction has been attributed to decreased intestinal absorption and reductions in the release of Fe by the reticulo-endothelial cells [1, 4, 5]. Previous studies have also demonstrated that serum Fe concentration decreased in acute traumatic reticuloperitonitis and mastitis in cows [2, 9]. Erskine *et al.* [9] demonstrated that serum Fe concentration decreased in 20 hr after experimental induction of infection with bovine *E. coli*. In addition, serum Fe levels decreased within 24 hr postoperatively in horses with osteochondritic lesions, laryngeal neuropathy, and ovarian tumors [11]. Serum Fe concentration in cows not only reflects inflammatory responses but may also be a sensitive inflammatory marker.

An evaluation of the relationship between inflammation and the status of serum Fe concentration may be useful for the evaluation of host response to inflammation during surgery because measurement of the serum Fe concentration is cheap and easily performed. However, comparative studies have not yet been conducted on Fe concentration in serum collected from cattle who underwent surgery. Therefore, the aim of the present study was to measure changes in the serum Fe concentration in cows that underwent the dehorning operation, and assess the usefulness of serum Fe concentration as a marker of inflammation.

All procedures were performed in accordance with the Good for the Care and Use of Laboratory Animals of the School of Veterinary Medicine at Rakuno Gakuen University and the National Research Council [13]. Three male and two female Holstein cows, aged 205.0 ± 10.7 days and weighing 207.2 ± 24.1 kg, were enrolled in this study. These cows were patients at the animal medical teaching hospital in Rakuno Gakuen University, and were scheduled to undergo the dehorning operation. These young cows were clinically normal, as assessed by vital signs, attributes, and appetite. In addition, no abnormal findings were observed in temperature, heart rate, respiratory rate, ruminal movement, and fecal production. The time at which 1 mg/kg of xylazine (Bayer, Osaka, Japan) was intravenously administered was defined as t=0 min. After 2 min of Xylazine administration, the cows were injected with 5 ml of 2% lidocaine hydrochloride (Xylocaine Injection 2%, Aspen Japan, Tokyo, Japan) on both sides as a

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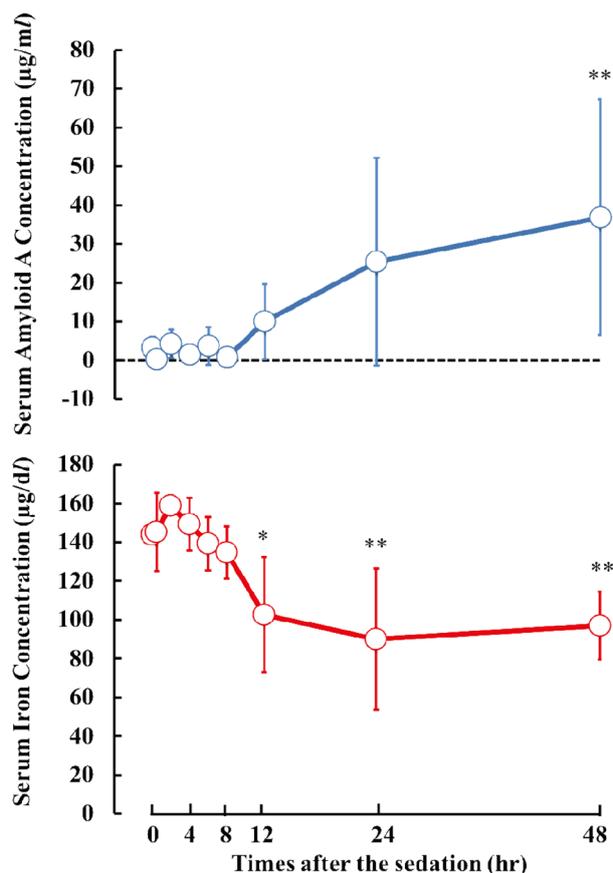
Fig. 1. Sequential changes in serum amyloid A (SAA, upper) and iron (Fe, lower) concentrations in cows that underwent dehorning. *; vs. initial $P<0.05$, **; vs. initial $P<0.01$ by the Dunnet-test.
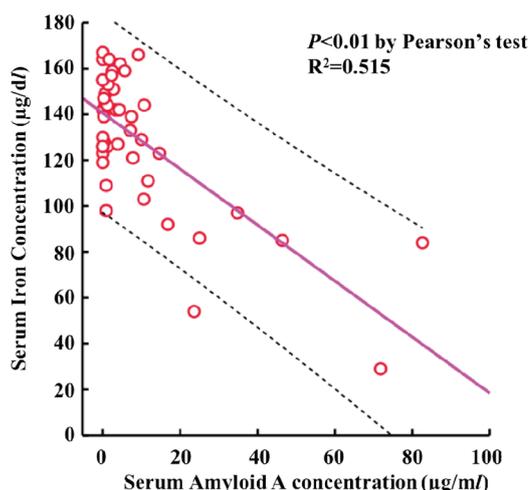
Fig. 2. Relationship between serum amyloid A (SAA) and iron (Fe) concentration in cows that underwent dehorning. The central line represents the regression line; the dashed lines represent the 95% confidence interval.

local anesthetic block of the cornual branch of the zygomaticotemporal (lachrymal) nerve. Dehorning and hemostasis at the horn base were carried out using the Barnes dehorner and electric dehorner, respectively. Because all surgical procedures were completed within 15 min, 50 μg/kg of atipamezole (Antisedan, Nihon Zenyaku Kogyo Co., Ltd., Fukushima, Japan) was intravenously administered at t=15 min as an antagonist of the sedative. All cows stood up immediately after the atipamezole injection.

Blood samples (10 ml each) were withdrawn from the contra lateral jugular vein before sedation (pre) and at t=0.5, 2, 4, 6, 8, 12, 24 and 48 hr, and the serum samples were stored in separate tubes. The serum was harvested after centrifugation at 3,000 rpm for 15 min at room temperature and was stored at −80°C until analysis. The SAA was measured with an automated latex agglutination turbidimetric immunoassay (LAT: SAA-1, Eiken Chemical Co., Tokyo, Japan) using an automated clinical chemical analyzer (Hitachi 7170S, Hitachi Ltd., Tokyo, Japan) [7]. The serum Fe concentration was measured by 2-Nitroso-5-[N-n-propyl-N-(3-sulfopropyl) amino] phenol (Nitroso-PSAP) methods using a commercial kit (N-assay L Fe-H Nittobo, Nitto Boseki, Co., Ltd., Tokyo, Japan). Duplicate measurements were performed, and the mean concentrations of SAA and iron were used for further statistical analyses.

Statistical analyses were performed using a commercial software package (IBM SPSS Statistics, v.21, IBM Co, Somers, NY, U.S.A.). Data were reported as the mean± standard deviation (SD). The mean values of each dependent variable were compared using the one-way analysis of variance (ANOVA), and was followed by the use of a post hoc test that depended on multiple comparisons with the initial value (Dunnett test). Pearson's rank correlation test was also used to evaluate the correlation between SAA and serum Fe concentration. P values lower than 0.05 were considered significant.

All the test cows underwent dehorning surgery uneventfully, and did not develop complications after that. Figure 1 shows the sequential changes in SAA and Fe concentrations of the cows that underwent dehorning. The initial value of SAA was 3.2 ± 2.9 μg/ml. The SAA value after dehorning reached 36.9 ± 30.3 μg/ml at t=48 hr. This value was significantly higher than the initial value ($P<0.01$). The initial value of serum Fe concentration was 143.8 ± 4.1 μg/dl. The Fe concentration in serum significantly decreased, and reached 90.0 ± 36.4 μg/dl at t=24 hr ($P<0.001$). Significantly low levels of serum Fe in cows that underwent the dehorning operation was maintained at t=12 ($P<0.05$), 24 ($P<0.01$), and 48 hr ($P<0.01$), when compared to the initial value. As described in Fig. 2, serum Fe concentration was significantly and negatively correlated with SAA concentration (r^2=0.500, $P<0.01$).

Significant increase in SAA and significant decrease in serum Fe concentrations due to inflammation associated with the dehorning operation were confirmed in this study. In cattle, the most sensitive acute phase proteins are SAA and Hp, showing a substantial rise in response to acute inflammation [19]. In the present study, the SAA level was increased in cows with non-infectious inflammation, as reported previously [2]. However, measurement of SAA concentration is too expensive to be performed routinely in veterinary diagnostic laboratories.

Hepcidin is essential for systemic Fe homeostasis. Hepcidin is secreted predominantly from hepatocytes in response to anemia, hypoxia, or inflammation [18], and its major functions are to sequester Fe [23]. Interleukin-6 (IL-6) was identified as an important upstream mediator of hepcidin induction by inflammation [14]. IL-6 infusion in humans induces hepcidin excretion and a decrease in serum iron [15]. Therefore, the decrease in serum Fe concentration in this study might have been due to increased hepcidin secretion caused by inflammation. The novelty of our study lies in the fact that no previous study has observed a sequential change in serum Fe concentration in cows with non-infectious inflammation. Suojala *et al*. [20] showed that variation in SAA concentration was slower than that in Hp in cows with mastitis. In this study, changes in SAA concentration were slower than those of serum Fe concentrations in cows that underwent the dehorning operation. Serum Fe concentration decreases rapidly in response to inflammation, and this can be explained as the host's defense mechanism [3]. A low-Fe environment, which is a consequence of Fe sequestration in the body for control of bacterial proliferation, is essential for the bacteriostatic system to operate in the body [22]. Our results suggest that variation of serum Fe concentration is a superior marker of inflammation compared to variation of SAA concentration, as a marker of inflammation in young cows that underwent the dehorning operation.

Some factors such as hypoproteinemia, anemia, hypoxia, renal disease, and intestinal disease, unrelated to inflammation, may also alter serum Fe concentration [12, 18]. However, initial serum Fe concentration in all cows were normal based on the reference value [2] in this study. In addition, all cows that underwent the dehorning operation were clinically normal based on physical examination. Therefore, inflammation due to the dehorning operation is the only possible factor affecting the variation of serum Fe concentration in this study.

In conclusion, our results demonstrated that serum Fe concentration is a useful marker of inflammation in young cows that have undergone the dehorning operation. Measurement of serum Fe concentration is cheap and easily performed, and hence can be used for clinical cases by the veterinary practitioners. Further studies need to investigate the relationship between inflammation and hepcidin expression in cows.

CONFLICT OF INTERESTS. The authors have no conflict of interests directly relevant to the content of this article.

REFERENCES

1. Andriopoulos, B. Jr., Corradini, E., Xia, Y., Faasse, S. A., Chen, S., Grgurevic, L., Knutson, M. D., Pietrangelo, A., Vukicevic, S., Lin, H. Y. and Babitt, J. L. 2009. BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat. Genet.* **41**: 482–487. [Medline] [CrossRef]
2. Baydar, E. and Dabak, M. 2014. Serum iron as an indicator of acute inflammation in cattle. *J. Dairy Sci.* **97**: 222–228. [Medline] [CrossRef]
3. Borges, A. S., Divers, T. J., Stokol, T. and Mohammed, O. H. 2007. Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses. *J. Vet. Intern. Med.* **21**: 489–494. [Medline] [CrossRef]
4. Cherayil, B. J. 2011. The role of iron in the immune response to bacterial infection. *Immunol. Res.* **50**: 1–9. [Medline] [CrossRef]
5. Cherayil, B. J., Ellenbogen, S. and Shanmugam, N. N. 2011. Iron and intestinal immunity. *Curr. Opin. Gastroenterol.* **27**: 523–528. [Medline] [CrossRef]
6. Constante, M., Jiang, W., Wang, D., Raymond, V. A., Bilodeau, M. and Santos, M. M. 2006. Distinct requirements for Hfe in basal and induced hepcidin levels in iron overload and inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **291**: G229–G237. [Medline] [CrossRef]
7. Christensen, M., Jacobsen, S., Ichiyanagi, T. and Kjelgaard-Hansen, M. 2012. Evaluation of an automated assay based on monoclonal anti-human serum amyloid A (SAA) antibodies for measurement of canine, feline, and equine SAA. *Vet. J.* **194**: 332–337. [Medline] [CrossRef]
8. Eckersall, P. D. and Bell, R. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Vet. J.* **185**: 23–27. [Medline] [CrossRef]
9. Erskine, R. J. and Bartlett, P. C. 1993. Serum concentrations of copper, iron, and zinc during Escherichia coli-induced mastitis. *J. Dairy Sci.* **76**: 408–413. [Medline] [CrossRef]
10. Idoate, I., Vander Ley, B., Schultz, L. and Heller, M. 2015. Acute phase proteins in naturally occurring respiratory disease of feedlot cattle. *Vet. Immunol. Immunopathol.* **163**: 221–226. [Medline] [CrossRef]
11. Jacobsen, S., Nielsen, J. V., Kjelgaard-Hansen, M., Toelboell, T., Fjeldborg, J., Halling-Thomsen, M., Martinussen, T. and Thoefner, M. B. 2009. Acute phase response to surgery of varying intensity in horses: a preliminary study. *Vet. Surg.* **38**: 762–769. [Medline] [CrossRef]
12. Jones, M. L. and Allison, R. W. 2007. Evaluation of the ruminant complete blood cell count. *Vet. Clin. North Am. Food Anim. Pract.* **23**: 377–402, v. [Medline] [CrossRef]
13. National Research Council. 1996. Guide for the Care and Use of Laboratory Animals. pp. 1–70. National Academy Press, Washington, D.C.
14. Nemeth, E., Valore, E. V., Territo, M., Schiller, G., Lichtenstein, A. and Ganz, T. 2003. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood* **101**: 2461–2463. [Medline] [CrossRef]
15. Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K. and Ganz, T. 2004. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J. Clin. Invest.* **113**: 1271–1276. [Medline] [CrossRef]
16. Neumann, S. 2003. Serum iron level as an indicator for inflammation in dogs and cats. *Comp. Clin. Pathol.* **12**: 90–94. [CrossRef]
17. Ong, S. T., Ho, J. Z., Ho, B. and Ding, J. L. 2006. Iron-withholding strategy in innate immunity. *Immunobiology* **211**: 295–314. [Medline] [CrossRef]
18. Przybylowski, P., Malyszko, J. S., Macdougall, I. C. and Malyszko, J. 2013. Iron metabolism, hepcidin, and anemia in orthotopic heart transplantation recipients treated with mammalian target of rapamycin. *Transplant. Proc.* **45**: 387–390. [Medline] [CrossRef]
19. Pyörälä, S. 2003. Indicators of inflammation in the diagnosis of mastitis. *Vet. Res.* **34**: 565–578. [Medline] [CrossRef]
20. Suojala, L., Orro, T., Järvinen, H., Saatsi, J. and Pyörälä, S. 2008. Acute phase response in two consecutive experimentally induced *E. coli* intramammary infections in dairy cows. *Acta Vet. Scand.* **50**: 18. [Medline] [CrossRef]
21. Vels, L., Røntved, C. M., Bjerring, M. and Ingvartsen, K. L. 2009. Cytokine and acute phase protein gene expression in repeated liver biopsies of dairy cows with a lipopolysaccharide-induced mastitis. *J. Dairy Sci.* **92**: 922–934. [Medline] [CrossRef]
22. Ward, C. G., Bullen, J. J. and Rogers, H. J. 1996. Iron and infection: new developments and their implications. *J. Trauma* **41**: 356–364. [Medline] [CrossRef]
23. Zhang, A. S. 2010. Control of systemic iron homeostasis by the hemojuvelin-hepcidin axis. *Adv. Nutr.* **1**: 38–45. [Medline] [CrossRef]