INFLUENCE OF HOUSING DENSITY AND GRAZING ON BLOOD COMPONENTS AND EXPRESSION OF HEAT SHOCK PROTEIN IN BEEF CATTLE

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Abstract – This study investigated the role of heat shock protein (HSP) 27 as a stress marker in beef cattle. In the first experiment, 10 Japanese black steers were divided into two groups for a housing density stress experiment: a high-density (HD, n = 6) group and freedom (FR, n = 4) group. Blood samples were collected before and after high-density housing to measure IgG and N/L ratios. Skeletal muscles were obtained by biopsy to analyze HSP 27 gene expression at the time of the blood sample. Increases in IgG, N/L ratio, and HSP 27 gene expression were identified the HD group compared with the FR group ($p < 0.05$). In the second experiment, 8 Japanese Black steers were divided into two groups for a feeding experiment: a grazing (GR, n = 3) group and concentrate (CT, n = 5) group. Skeletal muscles were obtained before and after grazing. HSP 27 gene expression in the GR group was decreased in two muscles compared with that in the CT group at the end of grazing ($p < 0.05$). Expression of the HSP 27 gene in the GR group was lower after grazing than before grazing ($p < 0.05$). These results suggest that HSP 27 may be an index of the stress response and may reflect different stress responses according to the rearing system.

Key Words – Grazing, Heat shock protein (HSP) 27, Stress

• INTRODUCTION

Our previous study showed that decreases in expression of heat shock protein (HSP) 27 occur in grazed cattle by skeletal muscle proteome analysis [1]. HSP plays an important role in regulating protein folding and in coping with proteins affected by heat and other stresses [2]. HSP behaves as a molecular chaperone by binding to other cellular proteins, assisting intracellular transport, and folding into adequate secondary structures, thus preventing condensation of protein during stress [3, 4]. Grazed cows on native pasture are stressed more easily than those housed in group pens by exposure to high temperatures, high humidity, and high solar radiation in the hot season [5]. The objective of this study was to investigate the role of HSP 27 as a stress marker in beef cattle, especially grazing steers.

• MATERIALS AND METHODS

In the first experiment, 10 Japanese Black steers 8 months of age that had been bred at WeNARC were randomly selected and divided into two groups for a housing density stress experiment: a high-density (HD, n = 6) group and freedom (FR, n = 4) group. The HD and FR groups were housed in free stalls of six steers per 9.6 m^2 for 9 days and four steers with paddocks, respectively. They were fed concentrate according to the Japanese feeding standard for beef cattle (Italian ryegrass hay *ad libitum*) and had free access to water. Blood samples were collected before housing at high density; 3 and 9 days after housing at high density; and 21 days after the end of the experiment to measure immunoglobulin G (IgG) levels and neutrophil to lymphocyte (N/L) ratios. A portion of the blood samples were separated to obtain serum by centrifugation, and the serum samples were stored at -20°C until analysis.

Skeletal muscle tissues from the semitendinosus (ST) and longissimus lumborum (LL) muscles were obtained by biopsy at the time of the blood sample. These muscle samples were rapidly frozen in liquid nitrogen and stored at -80°C until RNA extraction.

In the second experiment, 8 Japanese Black steers 10 months of age that had been bred at WeNARC were randomly selected and divided into two groups: a grazing (GR, n = 3) group and concentrate (CT, n = 5) group. They were housed individually in a stall barn and fed concentrate *ad libitum* and Italian ryegrass hay at 1.5 kg/d from 10 to 27 months of age. After this control period, the three steers of the GR group were placed on an outdoor winter pasture until 31 months of age, while the five steers of the CT group were continued on the concentrate and grass hay diet in the stall barn. Skeletal muscle tissues from the ST and LL muscles were obtained by biopsy at 22 months of age and end of fattening. These muscle samples were stored as described above.

Serum samples were homogenized in SDS-PAGE buffer. Serum protein was separated by SDS-PAGE and transferred onto PVDF membranes by electroblotting. The blotting membranes were blocked in blocking buffer for 1 h, then incubated with a primary antibody specific for the target protein for 1 h at room temperature. Anti-bovine IgG rabbit polyclonal antibody was the primary antibody used for immunoblotting. The membrane was washed with washing buffer and further incubated with HRP-conjugated anti-rabbit IgG secondary antibodies for 1 h at room temperature. The HRP activity was detected using an ECL plus detection kit, and films were then scanned. The optical densities of proteins were analyzed using software (Diversity Database ver. 1.1). Whole blood was analyzed for the differential white blood cell counts by an outside laboratory (Japan Clinical Laboratories, Kyoto, Japan).

Total RNA was extracted from muscle tissues using TRIZOL reagent according to the manufacturer's protocol. The first-strand cDNA was synthesized from 3 μg of total RNA using SuperScript II RNase H^- reverse transcriptase with oligo(dT) primer. After reverse transcription, the gene expression of HSP 27 was performed by real-time PCR using an ABI 7500 detection system. The first-strand cDNA was diluted with deionized water and amplified using SYBR Green PCR Master Mix with gene-specific primers by real-time PCR. The housekeeping gene GAPDH was used as a normalizing control. The primers were designed using Primer Express.

Gene expression and blood component data were represented as means. The relationships between the groups were analyzed using one-way ANOVA and a post-hoc Fisher test. A p value of <0.05 was considered statistically significant.

- RESULTS AND DISCUSSION

Psychological stress induces an increase in serum IgG with high stress perception [6]. In this study, the approximate molecular weight of IgG was detected at 25 kDa by western blot analysis. The IgG level 9 days after housing at high density was significantly greater in the HD group than in the FR group ($p < 0.05$) (Fig. 1). It is well established that weaning stress of calves causes an increase in the N/L ratio with a reduction in lymphocytes [7]. Furthermore, transported cattle show a stress response with higher N/L ratios [8]. In the present study, the N/L ratio was calculated from the percentages of the neutrophil and lymphocyte compositions in the blood. The N/L ratio of the HD group was significantly higher than that of the FR group 3 days after housing at high density ($p < 0.05$) (Fig. 2). Increases in IgG and the N/L ratio of the HD group indicate that a stress response occurred in the HD group compared with the FR group.

Figure 1. Blood IgG amounts during experimental period. *$p < 0.05$

Figure 2. Blood N/L ratio during experimental period. *$p < 0.05$

To evaluate whether expression of HSP 27 is a stress response index in beef cattle, we analyzed HSP 27 gene expression in skeletal muscle by real-time PCR. Transport-stressed pigs showed a higher level of HSP 70 in the heart and kidney than that in control pigs [9]. In the present study, the expression of HSP 27 mRNA in the HD group significantly increased in the LL and ST muscles 3 and 9 days after housing at high density, respectively, compared with before housing at high density ($p < 0.05$) (Fig. 3). This result suggests that HSP 27 may be used as an index of the stress response.

Figure 3. HSP 27 mRNA abundance during high-density housing. Values with different letters are significantly different ($p < 0.05$).

To investigate whether expression of HSP 27 reflects differences in rearing systems, we analyzed HSP 27 gene expression in skeletal muscle when steers were outdoor grazed and indoor concentrate-fed. At the end of grazing, HSP 27 gene expression in the GR group was significantly decreased in the LL and ST muscles compared with that in the CT group ($p < 0.05$) (Fig. 4). Furthermore, expression of the HSP 27 gene in the ST muscle of the GR group was significantly lower after grazing than before grazing. These results suggest that grazing of steers may result in a lower stress response compared with housing in a stall barn and fed concentrate.

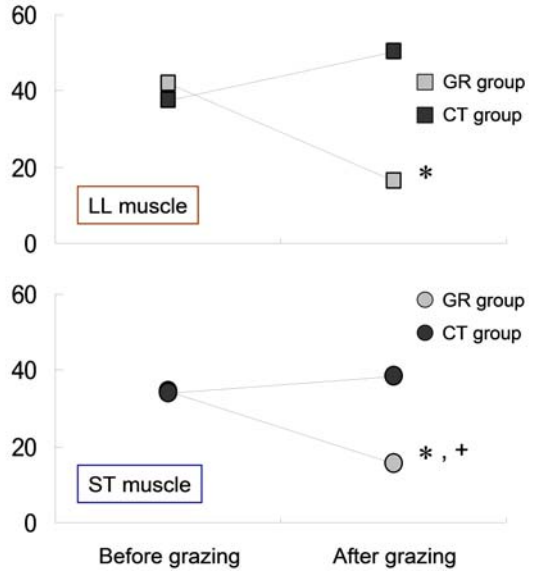

Figure 4. HSP 27 mRNA abundance before and after grazing. An asterisk indicates that the means differ between the two groups ($p < 0.05$). A cross indicates that the means differ between before and after grazing ($p < 0.05$).

- CONCLUSION

Our data confirm that increases in IgG, the N/L ratio, and HSP 27 gene expression reflect high-density housing stress and suggest that HSP 27 may be used as an index of the stress response. In addition, the present study suggests that the expression of HSP 27 may reflect different stress responses according to the rearing system.

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REFERENCES

1. Shibata M., Matsumoto K., Oe M., Kameyama O.M., Ojima K., Nakajima I., Muroya S. & Chikuni K. (2009). Differential expression of the skeletal muscle proteome in grazed cattle. Journal of Animal Science 87: 2700-2708.
2. Gething M. J. & Sambrook J. (1992). Protein folding in cell. Nature 355: 33-35.
3. Chirico W. J., Waters M. G. & Blobel G. (1988). 70K heat shock related preotin stimulate protein translocation into microsomes. Nature 332: 805-810.
4. Hartl F. U. (1996). Molecular chaperones in cellular protein folding. Nature 381: 571-580.
5. Kiyoshi K., Yamashita C., Yayota M., Ohtani S., & Ohashi H. (2010). Nutritional status and physiological stress responses in Japanese Black Cow grazing on a native pasture. Res. Bill. Aichi Agric. Res. Ctr. 42: 65-72. (In Japanese)
6. Maes M., Hendriks D., Gastel A., Demedts P., Wauters A., Neels H., Janca A. & Scharpe S. (1997). Effects of psychological stress on serum immunoglobulin, complement and acute phase protein concentrations in normal volunteers. Psychoneuroendcrinology 22: 397-409.
7. Kim M. H., Yang J. Y., Upadhaya S. D., Lee H.J., Yuu C. H. & Ha J. K. (2011). The stress of weaning influences serum levels of acute-phase proteins, iron-binding proteins, inflammatory cytokines, cortisol, and leukocyte subsets in Holstein calves. Journal of Veterinary Science 12: 151-157.
8. Chacon G., Garcia B. S., Villarroel M. & Maria G. A. (2005). Effect of transport stress on physiological responses of male bovines. Dtsch Tierarztl Wochenschr 112: 465-469.
9. Yu H., Bao E. D., Zhao R. Q. & Lv Q. X. (2007). Effect of transportation stress on heat shock protein 70 concentration and mRNA expression in heart and kidney tissues and serum enzyme activities and hormone concentrations of pigs. American Journal of Veterinary Research 68: 1145-1150.