

## Influence of Housing Density and Grazing on Heat Shock Protein 27 Expression in Skeletal Muscle of Beef Cattle

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### Abstract

Heat shock proteins (HSPs) are used as a stress biomarker in several studies as well as other stress biomarker, but the influence of different rearing environment on HSPs expression with other stress biomarker in cattle is unclear. To clear this point, two experiments were conducted to investigate the influence of rearing environment on HSP 27 in steer muscle. In experiment 1, 10 Japanese black steers were divided into 2 groups for a housing density stress experiment: a high-density (HD) group and freedom (FR) group. IgG levels and the neutrophil-to-lymphocyte (N/L) ratio in blood was analyzed as a stress marker. IgG levels and N/L ratios in the HD group were higher than those in the FR group. HSP 27 expression in the *semitendinosus* (ST) muscle of the HD group was higher than that of the FR group. These results suggest that HSP 27 expression may reflect the influence of the stress response with IgG levels and N/L ratios. In experiment 2, 8 Japanese Black steers were divided into 2 groups for a feeding experiment: a grazing (GR) group and concentrate-fed (CT) group. Blood IgG levels in the GR group after grazing were lower than those in the CT group. Expressions of the HSP 27 gene and its protein in the GR group were decreased in the *longissimus lumborum* and ST muscles compared with those in the CT group at the end of grazing. HSP 27 gene expression in the ST muscle of the GR group was lower after grazing than before grazing, but there was no significant difference in its expression in the CT group before and after grazing. The present study suggests that HSP 27 probably reflects not only differences in the rearing systems, but also the influence of the stress reaction.

**Keywords:** Beef cattle; Grazing; Heat shock protein 27; Housing density; IgG; Stress

### Introduction

Several stressors have influences on growth, reproduction, and meat quality in livestock production. Evaluation of various physiological and psychological stressors, such as housing density [1], transport [2], weaning [3], heat [4], examination [5], and job strain [6], have been studied in several animals using biomarkers. Cows grazed on native grassland 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 [7]. Our previous study shows that decrease in expression of heat shock protein (HSP) 27 occurred in grazed cattle by skeletal muscle proteome analysis [8]. HSPs play an important role in regulating protein folding and coping with proteins affected by heat and other stresses [9]. HSPs behave as molecular chaperones by binding to other cellular proteins, assisting intracellular transport, and folding into adequate secondary structures, thus preventing condensation of protein during stress [10,11]. HSPs have a homeostatic function in living tissues, stabilizing unfolded proteins, assisting with refolding of denatured proteins and preventing protein aggregation [12]. Tissues and cells up-regulate expression of HSP in respond to stress. Feasson et al. [13] indicates that an increase in the HSP 27 protein in skeletal muscle was observed after eccentric exercise stress compared with that before the stress. Another study reports that transport-stressed pigs show a higher level of HSP 70 in the heart and kidney than do control pigs [14]. Thus, although HSPs are used as a stress biomarker in several studies as well as other stress biomarker, the influence of different rearing environment on HSPs expression with other stress biomarker in cattle is unclear.

The aim of the present study was to investigate the expression of well-known stress biomarkers and HSP 27 in beef cattle under various feeding conditions. Whether expression of HSP 27 reflects differences in the rearing system of cattle is unknown. We hypothesized that expression of HSP 27 alters by the difference in the feeding environment of cattle, and moreover, its expression would be employed as a stress biomarker of cattle. To verify this hypothesis, we investigated whether HSP 27 expression occurs with changes in the expression

of known stress biomarkers under stress conditions by high-density housing. Furthermore, to examine changes in the expression of HSP 27 in different rearing conditions, we measured HSP 27 expression in outdoor-grazed and indoor concentrate-fed steers.

### Materials and Methods

#### Animal management

Management of steers and all procedures were performed according to the Animal Experimental Guidelines of the NARO Western Region Agricultural Research Center (NARO/WARC), Japan.

#### Experiment design and sample collection

In experiment 1, 10 Japanese Black steers of 8 months of age that had been bred at NARO/WARC were randomly selected and divided into 2 groups for a housing density stress experiment: a high-density (HD, 261 ± 12.3 kg, n = 6) group as the stress test group and a freedom (FR, 252 ± 6.26 kg, n = 4) group. Figure 1 shows details of the experimental design and schedule. All steers were housed in a free barn and fed a commercial concentrate diet (flaked corn, grain sorghum, corn gluten feed, wheat bran, and soybean meal; 17% CP and 70% TDN, Nishi-nihon Kumiai Shiryou, Hyogo, Japan) at 3.0 to 3.6 kg/head/day according to the Japanese feeding standard for beef cattle and grass hay (7.2% CP and 59% TDN on a dry matter basis) *ad libitum*, and all had free access to

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water. Blood samples were collected from both groups at the start of the experiment (-12 days), at 3 and 9 days after high-density housing, and at the end of the experimental period (30 days) (Figure 1) 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 *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles were obtained by biopsy at the time of the blood sample [15]. These muscle samples were rapidly frozen in liquid nitrogen and stored at -80°C until RNA extraction.

In experiment 2, 8 Japanese Black steers of 22 months of age that had been bred at NARO/WARC were randomly selected and divided into 2 groups: a grazing (GR, 654 ± 24.3 kg, n = 3) group and concentrate-fed (CT, 620 ± 22.8 kg, n = 5) group. They were housed individually in a tie stall barn and fed concentrate diet (flaked corn, flaked barley, wheat bran, and soybean meal; 11% CP and 73% TDN) *ad libitum* and grass hay (7.2% CP and 59% TDN on a dry matter basis) at 1.5 kg/day from 10 to 26 months of age. After this control period, the 3 steers of the GR group were placed on an outdoor pasture until 30 months of age, while the 5 steers of the CT group were continued on the concentrate and grass hay diet in the tie stall barn. Skeletal muscle tissues from the LL and ST muscles of both groups were obtained by biopsy at 22 months of age and at the end of fattening. These muscle samples were stored as described above. Blood samples were collected from both groups at the time of the muscle biopsy sample to measure IgG levels. The grass mass and chemical compositions of grass during the grazing period were

analyzed by an outside laboratory (Table 1) (Agricultural Chemistry Research Institute, Hokkaido, Japan).

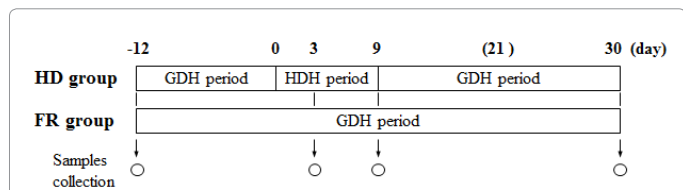
### Blood sample analyses

Serum samples were homogenized in SDS-PAGE buffer (pH 6.8) containing 50 mM Tris-HCl, 5% SDS, 10% (v/v) glycerol, and 5% (v/v) β-mercaptoethanol. Serum protein was separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (PVDF; GE Healthcare, Fairfield, CT) by electroblotting. After blotting, the membranes were stained (Ponceau S; Sigma, St. Louis, MO) to verify equal loadings. The blotting membranes were blocked in Tris-buffered saline with Tween-20 (TBS-T) (pH 7.6) comprising 20 mM Tris-HCl, 137 mM NaCl, and 0.1% (v/v) Tween-20 with 2% BSA (Sigma) for 1 h, then incubated with a primary antibody specific for target protein for 1 h at room temperature. The following primary antibody was used for immunoblotting: 1:10,000 dilution of rabbit polyclonal antibody to bovine IgG (701-401-002; Rockland Inc., Gilbertsville, PA). The membrane was washed with TBS-T and further incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG, 1:5,000 dilution (GE Healthcare) secondary antibody for 1 h at room temperature. The HRP activity was detected using an enhanced chemiluminescence (ECL) plus detection kit (GE Healthcare), and films were then scanned. The optical densities of proteins were analyzed using software (Diversity Database Ver.1.1; pdi, Huntington Station, NY). Whole blood was analyzed for the differential white blood cell counts by an outside laboratory (Japan Clinical Laboratories, Kyoto, Japan).

### Muscle sample analyses

Total RNA was extracted from muscle tissues using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The first-strand cDNA was synthesized from 3 μg of total RNA using SuperScript II RNase H<sup>-</sup> reverse transcriptase (Invitrogen) with oligo(dT) primer (Amersham Pharmacia Biotech, Piscataway, NJ). After reverse transcription, the gene expression of HSP 27 was performed by real-time PCR using an ABI 7500 detection system (Applied Biosystems, Foster City, CA). The first-strand cDNA was diluted with deionized water and amplified using SYBR Green PCR Master Mix (Applied Biosystems) with gene-specific primers by real-time PCR (Table 2). Every pair of oligonucleotide primers was designed to amplify a region including at least 1 intron. The real-time PCR reaction was carried out initially for 2 min at 50°C, then for 10 min at 95°C, then 50 cycles for 15 s at 95°C, and then for 1 min at 60°C. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a normalizing control. The primers were designed using Primer Express (Applied Biosystems).

Pulverized muscle tissue was homogenized in SDS-PAGE buffer (pH 6.8) as described above, then centrifuged at 15,000 × g for 10 min at 4°C. The supernatant fraction of each sample was taken, and the protein concentration was determined using a protein assay kit (RC DC; Bio-Rad Laboratories, Hercules, CA). Total protein was separated by SDS-PAGE and transferred onto PVDF membranes (GE Healthcare) by electroblotting. After blotting, the membranes were stained with



**Figure 1:** Experiment 1: Design and schedule for the housing density stress study in Japanese Black steers. The experimental period of the present study comprised high-density housing (HDH) and general-density housing (GDH) periods. Steers were housed in a free barn with narrow space (9.6 m<sup>2</sup>; 3.7-m width × 2.6-m length) in the HDH period and wide space (22.4 m<sup>2</sup>; 5.9-m width × 3.8-m length) with a paddock (30.6 m<sup>2</sup>) in the GDH periods. Ten steers were divided into 2 groups for a housing density stress experiment: a high-density (HD) group and freedom (FR) group. Six steers in the HD group were switched to HDH (0 day of the HDH period; 1.6 m<sup>2</sup>/head) after the first GDH period. Four steers in the FR group were housed in a wide space during the experimental period. Samples from both groups were collected at the start of the experimental period (-12 days), at 3 and 9 days of the HDH period, and at the end of the experimental period (30 days).

Period <sup>1</sup>	Grass mass (kg of DM / ha)	TDN (% of DM)	CP (% of DM)	ADF (% of DM)	NDF (% of DM)
Initial	1,746	71.2	14.2	20.0	36.4
Mid	2,524	69.7	17.1	21.6	42.4
End	3,902	72.2	7.8	18.0	36.4

<sup>1</sup>Initial, Mid, and End refer to samples taken at the beginning of grazing, at the middle of grazing, and at the end of grazing, respectively, during the experimental period.

**Table 1:** Grass mass and chemical compositions (% of DM) of grass during grazing.

Gene <sup>2</sup>	GenBank Accession No.	Forward primer (5' to 3')	Reverse primer (5' to 3')	Product size, bp
HSP 27	NM001025569	TCCCTGGACGTC AACCACTT	GGTGACGGGAATGGTGATCT	261
GAPDH	U85042	TGACCCCTTCATTGACCTCA	ACCCAGTGGACTCCACTACAT	201

<sup>1</sup>All sequence data were from bovine sequences.

<sup>2</sup>HSP 27, heat shock protein, 27 kDa; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

**Table 2:** Sequences of real-time PCR primers used in this study<sup>1</sup>.

Ponceau S (Sigma) to verify equal loadings. HSP 27 expression of the blotted membrane was detected using a 1:5,000 dilution of mouse monoclonal HSP 27 antibody (clone 8A7, Rockland Inc.). The following procedures were then carried out according to the method described above.

### Statistical analysis

Gene expression and blood component data were represented as means. The relationships between the groups were analyzed using one-way analysis of variance (ANOVA) and a post-hoc Fisher test. The relationships between results before and after the treatment were analyzed using one-way ANOVA and a post-hoc Fisher test. A *P* value of <0.05 was considered statistically significant.

## Results

### Experiment 1

To investigate whether expression of HSP 27 varies with stress, we designed a steer stress model using different housing densities and measured IgG levels and N/L ratios, which are well-known stress biomarkers, and HSP 27 expression. Six steers in the HD group were housed in a free barn with narrow space (1.6 m<sup>2</sup>/head) for 9 days of the high-density period as the stress test group. In contrast, 4 steers of the FR group were housed in a wide space (5.6 m<sup>2</sup>/head) throughout the experimental period. Body weight was not significantly different at the end of the experimental period (30 days) between the HD and FR groups (281 ± 10.8 kg and 281 ± 5.12 kg, respectively). Table 3 and Table 4 show the IgG levels and N/L ratios in the blood of the HD and FR groups during the experimental period. The approximate molecular weight of IgG was detected at 25 kDa by western blot analysis. The IgG levels of the HD group were significantly higher than those of the FR group at 9 days of the high-density period (*P*<0.05). The IgG levels of the HD

	HD group <sup>1</sup>	FR group <sup>2</sup>	Inter-group differences
Experimental days <sup>3</sup>			
-12 days	6.65 ± 0.89 <sup>a</sup>	7.50 ± 1.87	n.s.
3 days	10.3 ± 0.90 <sup>bc</sup>	7.38 ± 1.96	n.s.
9 days	12.6 ± 1.06 <sup>*,b</sup>	7.60 ± 1.63	* <i>P</i> < 0.05
30 days	8.43 ± 0.82 <sup>ac</sup>	6.96 ± 0.77	n.s.
Intra-group Differences	a VS. b VS. c <i>P</i> < 0.05	n.s.	

<sup>1</sup>Values are expressed as means ± SEM (n = 6).

<sup>2</sup>Values are expressed as means ± SEM (n = 4).

<sup>3</sup>Experimental days of both groups at the start of this experiment (-12 days), at 3 and 9 days after housing at high density, and at the end of the experimental period (30 days). Detailed information is provided in the legend of Figure 1.

**Table 3:** Blood IgG levels in steers of the high-density (HD) group and freedom (FR) group during the experimental period.

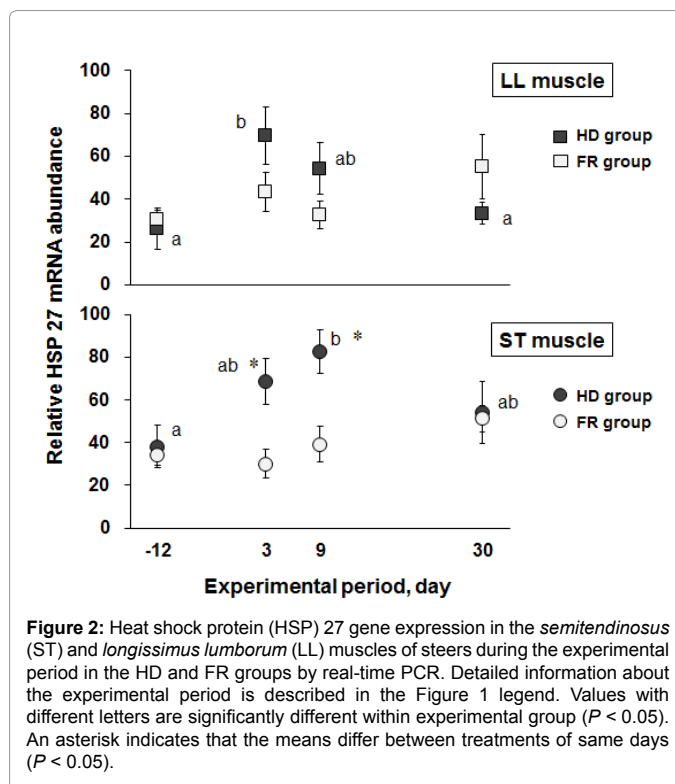
	HD group <sup>1</sup>	FR group <sup>2</sup>	Inter-group differences
Experimental days <sup>3</sup>			
-12 days	0.300 ± 0.04 <sup>a</sup>	0.290 ± 0.06 <sup>a</sup>	n.s.
3 days	0.487 ± 0.04 <sup>*,b</sup>	0.344 ± 0.04 <sup>ac</sup>	* <i>P</i> < 0.05
9 days	0.548 ± 0.07 <sup>b</sup>	0.798 ± 0.11 <sup>b</sup>	n.s.
30 days	0.559 ± 0.05 <sup>b</sup>	0.545 ± 0.05 <sup>c</sup>	n.s.
Intra-group Differences	a VS. b <i>P</i> < 0.05	a VS. b VS. c <i>P</i> < 0.05	

<sup>1</sup>Values are expressed as means ± SEM (n = 6).

<sup>2</sup>Values are expressed as means ± SEM (n = 4).

<sup>3</sup>Experimental days of both groups at the start of this experiment (-12 days), at 3 and 9 days after housing at high density, and at the end of the experimental period (30 days). Detailed information is provided in the legend of Figure 1.

**Table 4:** Blood neutrophil-to-lymphocyte (N/L) ratio in steers of the high-density (HD) group and freedom (FR) group during the experimental period.



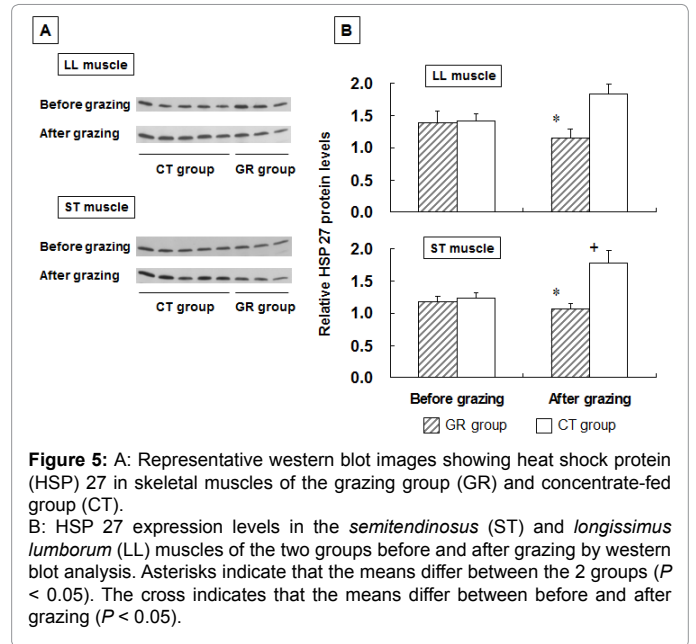
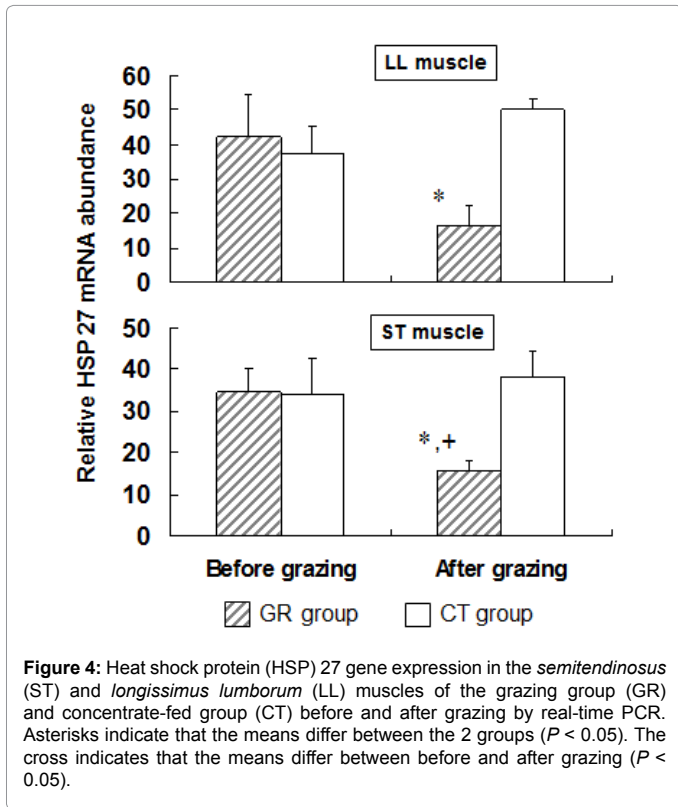
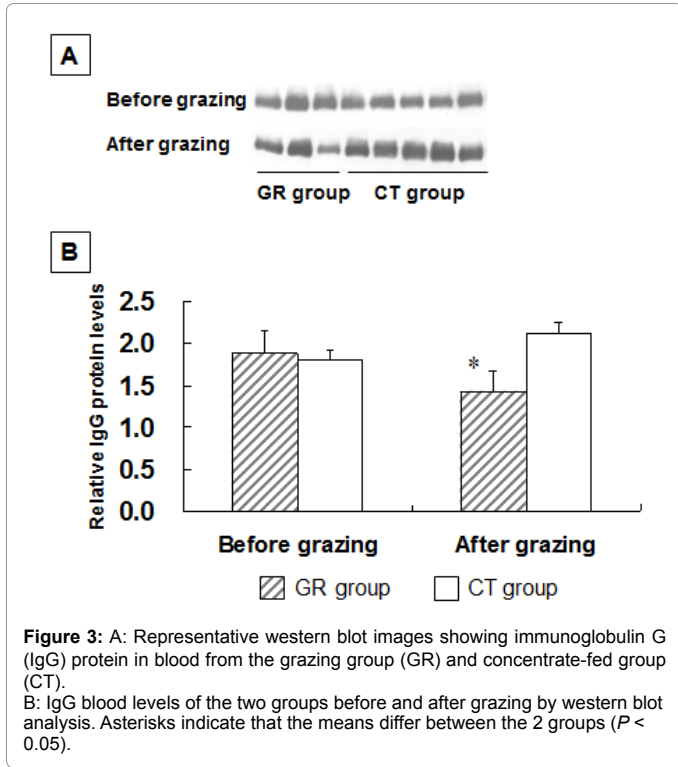
**Figure 2:** Heat shock protein (HSP) 27 gene expression in the *semitendinosus* (ST) and *longissimus lumborum* (LL) muscles of steers during the experimental period in the HD and FR groups by real-time PCR. Detailed information about the experimental period is described in the Figure 1 legend. Values with different letters are significantly different within experimental group (*P* < 0.05). An asterisk indicates that the means differ between treatments of same days (*P* < 0.05).

group were significantly higher during the high-density period (3 and 9 days) than at the start (-12 days) and end (30 days) of the experimental period (*P*<0.05), but there were no significant differences in the FR group during the experimental period. The N/L ratio was calculated from the neutrophil and lymphocyte compositions in the blood. The N/L ratios at 3 days of the high-density period were significantly higher in the HD group than in the FR group (*P*<0.05). The N/L ratios of the HD group were significantly higher at 3, 9, and 30 days than at -12 days (*P*<0.05). The N/L ratio of the FR group was the highest at 9 days during the experimental period. Figure 2 shows the expression of the HSP 27 gene in the skeletal muscles during the experimental period. The HSP 27 gene expression in the LL muscle of the HD group was significantly greater at 3 days of the high-density period than before the high-density period (*P*<0.05), and thereafter returned to base level. Expression of the HSP 27 gene in the ST muscle at 9 days of the high-density period was significantly greater than that before the high-density period (*P*<0.05), and thereafter tended to return to base level. At 3 and 9 days of the high-density period, HSP 27 expression was significantly increased in the ST muscle of the HD group compared with that of the FR group (*P*<0.05). Expression of HSP 27 occurred in synchrony with increases in IgG levels and N/L ratios under the stress condition.

### Experiment 2

To examine the influence of grazing on HSP 27 expression, we measured HSP 27 expression in outdoor-grazed and indoor concentrate-fed steers. Steers were outdoor-grazed for 3 months during the latter fattening period. Body weight at the end of the fattening period was not significantly different between the GR and CT groups (697 ± 12.9 kg and 707 ± 25.8 kg, respectively). Figure 3A, 3B shows that the blood IgG levels in the GR group after grazing were significantly lower than those in the CT group by western blot analysis (*P*<0.05). This result suggests that a stress response occurred in the CT group compared with the GR group. Figure 4 shows the HSP 27 gene expression in the skeletal

muscles of the GR and CT groups before and after grazing. Expression of the HSP 27 gene in the GR group was significantly decreased by 67% and 59% in the LL and ST muscles, respectively, compared with that in the CT group after grazing ( $P < 0.05$ ). Expression of the HSP 27 gene in the ST muscle of the GR group was significantly lower after grazing



than before grazing ( $P < 0.05$ ), but there was no significant difference in its expression in the CT group during the experimental period. The approximate molecular weight of HSP 27 was 27 kDa as detected by western blot analysis (Figure 5A). The HSP 27 protein expression in the GR group after grazing was significantly decreased by 37.5% and 40.3% in the LL and ST muscles, respectively, compared with that in the CT group ( $P < 0.05$ ) (Figure 5B). Expression of the HSP 27 protein in the ST muscle of the CT group was significantly higher after grazing than before grazing ( $P < 0.05$ ). In skeletal muscle, expression of HSP 27 protein corresponded to that of HSP 27 mRNA. A difference in the stress response was recognized between the GR and CT groups based on the change in IgG and HSP 27 expressions.

## Discussion

The aim of the present study was to investigate IgG levels and N/L ratios, which are well-known stress biomarkers, and expression of HSP 27 in beef cattle under various feeding conditions. We investigated whether HSP 27 expression occurs with changes in IgG levels and N/L ratios under stress conditions. Furthermore, to examine changes in the expression of HSP 27 in different rearing conditions, we measured HSP 27 expression in outdoor-grazed and indoor concentrate-fed steers.

Serum biomarkers associated with stress have been detected in pigs housed at high density using biochemical and proteome analysis [1]. To create a steer stress model, we designed high- and low-density housing systems and measured various stress indices. Immunoglobulins have been utilized as stress markers in several studies. Psychological stress by academic examination induces an increase in serum IgA, IgG, and IgM in students with high stress perceptions [16]. IgA, IgG, and IgM titers in saliva during exams are higher in students with symptoms of psychological stress [5]. In one study, the serum IgG concentration in the high job strain group was higher than that in the low job strain group, but there was no difference in IgA and IgM between the 2 groups [6]. These studies indicated that Ig has been detected in several stress condition as stress marker. In the present study, the IgG levels at 9 days of the high-density period were higher in the HD group than in the FR group. Furthermore, increase in the IgG levels in the HD group was shown during the high-density period compared with the levels at the

start and end of the experimental period. These results suggest that a stress response occurred in the HD group.

Furthermore, the N/L ratio expresses the severity of afflictions such as surgical stress [17]. It is well established that weaning stress of calves causes an increase in the N/L ratio with a reduction in lymphocytes [3]. In addition, transported cattle show a stress response with higher N/L ratios [2]. The N/L ratio of grazed cows on native grassland is higher than that of cows housed in group pens in mid-summer [7]. The N/L ratio has been used as a stress marker in many studies and was employed the present study as a stress marker. The N/L ratio of the HD group was higher than that of the FR group at 3 days of the high-density period. In addition, the N/L ratios in the HD group were the lowest before the high-density housing period. Increases in IgG levels and N/L ratios of the HD group indicate that a stress response occurred in the HD group compared with the FR group.

We examined whether changes in the expression of HSP 27 occur in the high-density conditions, because changes in the IgG levels and N/L ratios were confirmed in the steers housed in high-density conditions. HSPs have been utilized in several studies as stress markers. For instance, increased HSP 70 in some species, such as fish, amphibians, arthropods, and plants, is a biomarker for detection of heat stress [18]. High ambient temperature induces an additional accumulation of HSP 27 mRNA and protein in leukocytes compared with the exercise-induced expression at low temperatures [4]. In addition, an increase in the HSP 27 protein level in skeletal muscle was observed at 1 and 14 days after eccentric exercise stress compared with that before the stress [13]. Transport-stressed pigs show a higher level of HSP 70 in the heart and kidney than do control pigs [14]. An increase in HSP 90 in the white blood cells of lactating beef cows occurred with long-term caloric stress induced by feeding a low-energy diet [19]. Previous reports have indicated that expression of HSPs depends not only on the ambient temperature, but also on several other stress factors such as exercise, transport, and low-calorie diets. In the present study, the expression of HSP 27 mRNA in the HD group increased in the LL and ST muscles of the high-density period compared with that before the high-density period. Moreover, HSP 27 expression was increased in the ST muscle of the HD group compared with that of the FR group. Results in the experiment 1 indicate that expression of HSP 27 occurred in synchrony with increases in IgG levels and N/L ratios under stress conditions in steers. These results suggest that HSP 27 expression may also reflect the influence of the stress response.

Our previous study indicated that decreases in expression of HSP 27 occurred in grazed cattle compared with indoor concentrate-fed cattle in a stall barn by skeletal muscle proteome analysis [8]. In the experiment 1, we confirmed that HSP 27 expression takes place in synchrony with increases in stress biomarkers under stress conditions in steers. To verify whether expression of HSP 27 as a stress biomarker reflects differences in rearing systems, we analyzed blood IgG levels and HSP 27 expression in skeletal muscle when steers were outdoor-grazed and indoor concentrate-fed. At the end of grazing, expression of the HSP 27 gene and its protein in the GR group was decreased in the LL and ST muscles compared with that in the CT group. Furthermore, HSP 27 gene expression in the ST muscle of the GR group was lower after grazing than before grazing. Results in the experiment 2 indicate that expression of HSP 27 reflected a difference between outdoor grazing and indoor concentrate-feeding. Furthermore, decreases in the IgG levels of the GR group after grazing indicate that the stress response was suppressed in the GR group compared with the CT group. These results suggest that HSP 27 may be employed to detect the stress response under the differences in rearing environment as a stress biomarker.

In terms of the effect of HSP on meat quality, Bernard et al. [20] reported that low expression of HSP 40 in muscle was associated with elevated beef tenderness. Although the molecular classes of HSP in the present study are different from the previous report [20], decreased HSP 27 expression might also affect beef tenderness. Further studies are needed to clarify the correlation between HSP 27 and beef quality, including tenderness, taste or nutrients.

The present study confirmed that expression of both HSP 27 protein and mRNA levels were present in skeletal muscle. Apart from skeletal muscle, HSP 27 protein expression will be also identified in the dressed carcass and retail meat. This procedure may be useful for retail beef to determine whether beef cattle encountered a stressful environment. Moreover, to aid stress evaluation in the beef cattle production field by HSP 27, further studies should determine whether HSP 27 can be measured in other samples, such as blood, saliva and urine.

## Conclusion

In the present study, we confirmed that HSP 27 gene expression may reflect the influence of the stress response because its expression took place in synchrony with increases in the IgG level and N/L ratio under a high-density housing stress condition in steers (experiment 1). In addition, we revealed that decreases in both HSP 27 expression and IgG levels occur during outdoor grazing compared with indoor concentrate-feeding in steers (experiment 2). These results indicate that outdoor grazing would be less stressful than indoor concentrate-feeding because increases in both HSP 27 expression and IgG levels occurred under the stress condition of experiment 1 in the present study. The present study suggests that HSP 27 probably reflects not only the difference in the rearing system, but also the influence of the stress reaction.

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