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Short communication

Upregulated heat shock protein beta-1 associated with caloric restriction and high feed efficiency in *longissimus dorsi* muscle of steer

U.S. Jung[a], M.J. Kim[a], T. Wang[b], J.S. Lee[a], S.W. Jeon[a], N.C. Jo[c], W.S. Kim[c], M. Baik[d], H.G. Lee[a,*]

[a] Department of Animal Science and Technology, Konkuk University, Seoul 05029, Republic of Korea
[b] Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Jilin Agricultural University, Jilin 130118, China
[c] Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Republic of Korea
[d] Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 02792, Republic of Korea

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ABSTRACT

The objective of this study was to identify myogenic proteins associated with caloric restriction and feed efficiency in bovine *longissimus dorsi* muscle. Thirty-one Korean native steers were allocated to 100% *ad libitum* (n = 16) or 80% of *ad libitum* (n = 15) groups. Regardless of nutritional level, a subset of these animals were assigned to groups with high or low feed efficiency (n = 5) at a later time point based on feed efficiency. A total of 7 differentially expressed proteins were found between groups with different nutrition levels while a total of 12 differentially expressed proteins were found between groups with different feed efficiencies. Interestingly, heat shock protein beta-1 (HSPB1) was a differentially expressed protein that showed up in both results (nutrition level and feed efficiency). It was up-regulated in both the 80% *ad libitum* group and the high feed efficiency group. In *in vitro* study, mRNA expression level of HSPB1 was increased ($P < 0.05$) after myogenic differentiation. Results of this study suggest that HSPB1 might be a myogenic protein involved in response to caloric restriction and feed efficiency in *longissimus dorsi* muscle of Korean native steer.

1. Introduction

Caloric restriction (CR) and feed efficiency (FE) could affect muscle development. It has been reported that steers exposed to low nutrition diet have enlarged muscle fibers compared to steers exposed to moderate-nutrition diet (Long et al., 2010). Restricted feeding can lead to the production of leaner carcasses (Murphy and Loerch, 1994). In general, CR has profound effect on myogenic activity of muscle stem cells such as satellite cell by altering their gene expression profile and enhancing mitochondrial energy production in mice (Cerletti et al., 2012). FE is an economically important factor in beef production. Generally, FE (g gain/kg feed) is regarded as the inverse of feed conversion ratio (FCR) or residual feed intake (RFI). Several FE studies have investigated candidate genes associated with carcass characteristics and meat quality in bovine (Baker et al., 2006; Al-Husseini et al., 2014). Lancaster et al. (2009) have reported that gains in *longissimus dorsi* muscle (LM) area are negatively correlated with FCR in growing bulls. In addition, muscle development and cytoskeletal architecture are modulated by gene expression which varies according to FE (Bottje and Kong, 2013). In this regard, we considered that CR and FE might affect muscle metabolism in LM of cattle. However, proteins involved in CR and FE during muscle development in bovine have not been identified. Therefore, it is necessary to identify a physiological marker associated with these two factors (CR and FE) in LM. Moreover, two-dimensional gel electrophoresis (2-DE) and spontaneously immortalized bovine embryonic fibroblasts (BEFS) could be used to identify genes involved in CR and FE. These genes might be used in feed development or animal selection for breeding, consequently increasing the productivity of beef cattle. Taken together, the objectives of this study were: 1) to use 2-DE to discover differentially expressed proteins common in bovine LM according to CR and FE; 2) to predict the roles of identified genes during myogenesis using BEFS.

2. Materials and methods

2.1. Animals, diets, experimental design, and sample collection

All experimental procedures involving animals were performed according to the Animal Experimental Guidelines. They were approved by the Animal Research Ethics Committee of Chungnam National University. A total of 31 Korean native steers (292.0 ± 6.95 kg) at 10 months of age were used for 4 months for this study. It has been

* Corresponding author.
E-mail address: hglee66@konkuk.ac.kr (H.G. Lee).

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Table 1
Nutrient composition (g/kg DM or as stated) of experimental diets.

Item[a]	14 months of age
DM (g/kg)	631
Crude protein	95
Ether extract	24
Neutral detergent fiber	585
Acid detergent fiber	413
Acid detergent lignin	61
NDICP	30
ADICP	17
Ash	82
Net energy for gain (KJ/kg)	2.64

[a] NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein.

reported that intramuscular fat is mainly developed after 14 months of age (Cianzio et al., 1985). Therefore, experimental period of 4 months was used to investigate the effect of CR and FE on muscle development stage. Thirty-one Korean native steers were randomly distributed to 16 pens (2 animals/pen). Of these 31 steers, 16 were provided normal feed *ad libitum* while the remaining 15 were assigned to the CR group and fed *ad libitum* of 80% of normal feed intake consumed on a previous day. One steer was removed due to mechanical accident. During the 4-month feeding trial, chemical composition of the experimental diet of the normal group was 2.64 KJ/kg net energy for gain. Experimental diets were calculated to meet the requirement of the National Research Council (NRC, 2001) (Table 1). Individual daily feed intake was measured using an automated feeding machine (TMR FEEDER; Dawoon, Incheon, Korea). Body weight was recorded monthly before morning feeding. FE (g gain/kg intake) was calculated for the total experimental period (Table 2). Animals were divided into low FE (LF: n = 5) and high FE (HF: n = 5) groups. Those with median values were excluded. Difference in FE of the two groups was 20 or more.

For proteomic analysis, each LM sample was obtained at age of 14 months (normal group: n = 7, CR group: n = 5, LF group: n = 5, and HF group: n = 5) using a spring-loaded biopsy instrument (Biotech Nitra, Republic of Slovakia). Whole blood sample (10 ml) was taken *via* jugular veins after morning meal and added into a tube containing EDTA (Becton and Dickson, New Jersey, USA).

2.2. Protein extraction and two-dimensional gel electrophoresis

Proteomic analysis was performed for pooled samples (100 μg) containing equal quantities of protein from LM sample of each group to identify differentially expressed proteins. Weinkauf et al. (2006) have reported that sample pooling is efficient in 2-DE as it reduces non-specific expression background. Differentially expressed spots with at least a 2-fold change in intensity were subjected to ESI-Q-TOF/MS analysis. Details of 2-DE and ESI-Q-TOF/MS analysis have been previously described (Jin et al., 2012).

2.3. Blood variables

Whole blood (1 ml) was subjected to complete blood cell count analysis using HM2 (VetScan HM2 Hematology System, Abaxis, USA). Plasma albumin, blood urea nitrogen, glucose, total cholesterol, triglyceride, total protein, and γ-glutamyl transpeptidase levels were measured using Toshiba Accute Biochemical Analyzer-TBA-40FR (Toshiba Medical Instruments, Otawara-shi, Tochigi-ken, Japan).

2.4. Cell culture

MyoD-overexpressing BEFS cells (BEFS-MyoD) undergoing differentiation into myogenic lineages were used in this study. Details for cell culture have been described previously (Yin et al., 2010),

2.5. Total RNA extraction and real-time PCR analysis

Details of RNA isolation, cDNA synthesis, and real-time PCR procedure have been described previously (Zhang et al., 2014). Primers were designed using National Center for Biotechnology Information Primer-BLAST (Table 3). Relative fold-changes were determined using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All data were normalized against β-actin as housekeeping gene.

2.6. Statistical analysis

Data (6 observations for body weight, 147 observations for daily feed intake, and 1 observation for blood composition) were presented as mean with standard error of mean (SEM). They were analyzed with independent-sample *t*-test. Real-time PCR data from BEFS cell lines were presented as mean ± SD. They were analyzed using Tukey's test. Statistical analysis was performed using SPSS software package (SPSS Inc., Chicago, IL, USA). *P*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Growth performance

Body weight of steers in the restricted group with 80% of *ad libitum* feed intake was 8.8% lower at 14 months of age compared to that of steers in the normal group with 100% *ad libitum* intake. In addition, feed intake, average daily gain, and FE at 9–14 months of age were significantly different. Although steers in the HF group consumed 15.2% less feed on average than steers in the LF group, average daily gain of steers in the HF group was greater ($P = 0.08$) than that in the LF group. Therefore, HF steers utilized nutrients more efficiently than LF steers (56.8 g/kg *vs* 31.4 g/kg for HF *vs.* LF groups, $P < 0.05$) (Table 2).

Table 2
Effects of caloric restriction and feed efficiency on growth performance.

		Nutritional level[a]		Feed efficiency[b]		SEM[c]		*P*-values[d]	
Trait	Age	Normal	Restricted	HF	LF	N	FE	N	FE
Initial BW, kg	10	294.4	289.3	287.6	307.2	9.80	19.05	ns	ns
Final BW, kg	14	370.9	338.1	359.4	356.4	12.29	28.00	*	ns
Daily feed intake (kg/d)	10–14	12.7	11.4	10.8	12.8	0.49	1.22	**	ns
Average daily gain (g/d)	10–14	642.9	410.1	603.4	413.4	45.44	78.19	**	*
Feed efficiency (g gain/kg feed)	10–14	51.0	36.6	56.8	31.4	4.39	5.70	**	**

[a] Steers were fed *ad libitum* (normal group, n = 16) or 80% of *ad libitum* (restricted group, n = 15).
[b] Steers were assigned to groups with high (HF, n = 5) and low feed efficiency (LF, n = 5) regardless of nutritional level.
[c] N, nutritional level; FE, feed efficiency.
[d] Probability values for the effect of nutritional level (N) and feed efficiency (FE); (* $P < 0.05$, ** $P < 0.01$, and ns = non-significant).