Bos indicus cattle possess greater basal concentrations of HSP27, alpha B-crystallin, and HSP70 in skeletal muscle *in vivo* compared to *Bos taurus* cattle

C. R. Mullins,[*] H. N. Zerby,[*] L. A. Fitzpatrick,† and A. J. Parker†

*Department of Animal Sciences, The Ohio State University, Columbus, Ohio, 43210

†School of Veterinary and Biomedical Sciences, James Cook University, Townsville, QLD, Australia, 4811

[1]Acknowledgements: The authors would like to acknowledge the assistance of Martin Holzwart and the James Cook University Fletcherview Research Station. Research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH, USA.

[2]Corresponding author: zerby.8@osu.edu

ABSTRACT: The objectives of the present study were to evaluate the basal concentrations of heat shock proteins (HSP) between *Bos indicus* and *Bos taurus* cattle and determine if HSP basal concentrations change as an animal matures. A total of 40 cattle were used in a 2 × 2 factorial design to evaluate the effects of genotype and age (heifers and mature cows) on basal concentrations of Heat Shock Protein 27 (HSP27), alpha B-crystallin (Cryab), and Heat Shock Protein 70 (HSP70). A muscle sample was collected from the longissimus thoracis (LT) and concentrations of HSP were quantified using ELISA. There were no significant differences in HSP concentration for the interaction between age and genotype or for age alone. *Bos indicus* cattle had greater ($P < 0.05$) basal concentrations of HSP27, Cryab, and HSP70 in the LT than *Bos taurus* cattle. The results of this study suggest that HSP could partially explain differences in tenderness of meat between *Bos indicus* and *Bos taurus* cattle.

Key words: beef tenderness, *Bos indicus*, *Bos taurus*, heat shock protein, meat quality

INTRODUCTION

Ensuring consistent beef quality with regard to palatability, and especially tenderness, will contribute to more profitable marketing opportunities for all members of the beef supply chain. Protein expression early *post-mortem* in some instances, can be used as a reliable biomarker to accurately predict ultimate meat quality characteristics (Picard et al., 2012). A class of chaperone proteins found ubiquitously in skeletal muscle, known as heat shock proteins (HSP), have come under investigation for their potential role in meat tenderness (de Jong et al., 1998). Studies investigating the concentration of the HSP in meat from either *Bos taurus* or *Bos indicus*-type cattle have generally shown HSP concentrations to be decreased in beef that is more tender. In general, as the percentage of *Bos indicus* genotype increases in cattle, the resulting meat possesses decreased tenderness when compared with meat from *Bos taurus* type cattle (O'Connor et al., 1997; Highfill et al., 2012). Previous studies have investigated the relative up- and down-regulation of HSP in meat within similar genotypes and among various meat quality attributes (Lomiwes et al., 2014); however, studies examining the basal *in vivo* HSP concentrations between *Bos indicus* and *Bos taurus* type cattle are lacking. Additionally, as beef from older cattle is known to be less tender, it stands to be seen if *in vivo* HSP concentration changes as an animal ages. We hypothesized that tropically-adapted *Bos indicus* cattle have a greater basal concentration of HSP27, alpha B-crystallin (Cryab), and HSP70 in skeletal muscle *in vivo* than *Bos taurus* cattle and that older animals have a greater basal concentration of the HSP. This study was conducted to determine if skeletal muscle HSP concentration differs between *Bos indicus* and *Bos taurus* cattle and between younger and older cattle. Should a difference be noted between genotypes, future studies can seek to correlate tenderness with HSP concentration.

67 ## MATERIALS AND METHODS

68 ### *Animals and Sample Collection*

69 All methods and procedures involving animals were reviewed and approved by the James Cook
70 University Animal Ethics Committee and were in compliance with the Queensland Animal Care
71 and Protection Act. Forty non-lactating and non-pregnant female cattle were randomly selected
72 from two separate populations: *Bos taurus,* dairy-type animals (Friesian or Friesian × Jersey; n =
73 20) were sourced from James Cook University's School of Veterinary and Biomedical Sciences,
74 James Cook University, Townsville, Queensland, Australia (19.32, 78° S, 146.75, 83° E); and
75 *Bos indicus*, beef-type animals (Brahman; n = 20), were sourced from James Cook University's
76 Fletcherview Research Station, Charters Towers, Queensland, Australia (20.046, 03°'S, 146.158,
77 02°'E), 140 km southwest of Townsville. Ten animals were randomly selected from appropriate
78 age groups within each herd to establish two distinct age categories (heifers, 18 months-of-age;
79 mature cows, > 7 years-of-age) resulting in four experimental groups of 10 animals each. The
80 individual animal served as the experimental unit. At the time of sampling the *Bos taurus* heifers
81 and mature cows and *Bos indicus* heifers were maintained at one site in Townsville and were fed
82 Rhodes grass (*Chloris gayana*) hay *ad libitum.* The *Bos indicus* mature cows were maintained at
83 the Fletcherview research station grazing a mixed diet of predominately Rhodes grass and buffel
84 grass (*Cenchus ciliaris*) supplemented with Rhodes grass hay during the dry season. All the
85 cattle were part of the university's veterinary science teaching herd and as such were extensively
86 handled, docile and of good temperament. The *Bos taurus* heifers were transported from dairy
87 farms in north Queensland to Townsville one month before sampling. All other cattle received
88 the same management.
89

Animals were sequentially separated from their respective group and individually restrained in a crush with a head bail. The site between the 12th and 13th ribs on the animal's left side over the longissimus thoracis (LT) was clipped and aseptically prepped with an iodine solution and an alcohol solution. Ilium Lignocaine 20 Injection (Troy Laboratories, Glendenning, NSW, Australia) was injected intramuscularly until sensation at the surgical site was lost. Muscle biopsy samples (approximately 1 g) were collected from the LT between the 12th and 13th ribs by a veterinarian using a scalpel to dissect through the hide and fascia. Samples were cleaned of visible blood and fat, placed in 2-mL cryotubes, snap-frozen in liquid nitrogen, and then stored at -80°C until further analysis. The surgical site was sutured and the animal monitored over the next 7 days for signs of infection or illness which was promptly treated, if present. A live weight and rectal temperature was recorded for each animal after the tissue sample was collected.

Sampling days were distributed over a period of 4 consecutive weeks. The ambient temperature and relative humidity were retrieved from national weather service for each day and time muscle samples were collected, and temperature-humidity index (THI) was calculated according to Thom (1959).

Quantitative measurements of heat shock proteins

Longissimus thoracis samples were prepared for HSP quantification by thawing them at room temperature (22°C) for 15 minutes. Any remaining visible blood, connective tissue, or fat was removed, and samples were homogenized in 10 volumes of 1X PBS solution. Homogenized samples were then subjected to two freeze-thaw cycles, centrifuged at 1,500 x *g* for 15 minutes at

112 4°C, and the supernatant was collected for immediate analysis. The supernatant served as the
113 sample source for each of the HSP ELISA assays.
114
115 Samples were analyzed with complete bovine ELISA immunoassay kits [MyBioSource, San
116 Diego, CA, www.mybiosource.com; Cat. No MBS008260 (Cryab); Cat. No MBS011935
117 (HSP27); Cat. No MBS019766 (HSP70)] following the manufacturer's directions, briefly
118 described as follows. All reagents and standards were slowly thawed to 22°C for 30 minutes.
119 Then 50 µL of sample diluent was added to 2 wells, 50 µL of each of the six different standards
120 supplied in the kit were loaded into wells in duplicate to generate the standard curve, and 50 µL
121 of supernatant from each sample were loaded in duplicate in the remaining wells. HRP-conjugate
122 reagent (100 µL) was then added to each well, and the plate was covered with an adhesive strip
123 and incubated at 37°C for 60 minutes. The plate was manually washed by decanting the liquid in
124 the wells and using a squirt bottle to fill each well with Wash Solution (1X) prepared by mixing
125 20 mL of Wash Solution (20X) in 380 mL of distilled water. After repeating the wash step 4
126 times, the plate was further dried by inverting onto an absorbent cloth. Then 50 µL of
127 Chromagen Solution A was added to each well immediately followed by 50 µL of Chromagen
128 Solution B. The plate was immediately covered with aluminum foil to protect it against light and
129 incubated at 37°C for 15 minutes. After incubation, 50 µL of Stop Solution was added to each
130 well. Then the plate was placed in a microplate reader (Varioskan Flash Microplate Reader,
131 Thermo Fisher Scientific, Waltham, MA), which was set at 450 nm to provide an optical density
132 reading for each muscle tissue sample. Optical density readings for both the standards and the
133 muscle tissue samples were adjusted by subtracting the absorbance of the control from each
134 standard and sample reading, and then duplicate readings were averaged. A standard curve was

generated from the standard readings and used to calculate the heat shock protein concentration in each muscle tissue sample.

Data Analysis

Data were analyzed as a 2 × 2 factorial structure (Genotype × Age) using SPSS statistical package 22.0 (IBM Corp, 2013). The following statistical model was used to estimate the main effects of genotype (*Bos indicus* or *Bos taurus*) and age (heifers, 18 months-of-age; or mature cows, >7 years-of-age) on HSP concentration: $Y_{ijk} = \mu + G_i + A_j + (G \times A)_{ij} + e_{ijk}$, where Y_{ijk} is the dependent variable, μ is the overall mean of the population, G_i is the mean effect of the genotype, A_j is the mean effect of the age, e_{ijk} is the unexplained residual element assumed to be independent and normally distributed. Means were separated and considered significantly different when $P < 0.05$.

RESULTS AND DISCUSSION

Animal temperature

Core rectal body temperature of every animal at the time of sampling was measured to ensure no animals were afflicted by acute heat stress which is known to up-regulate the heat shock proteins. The ambient temperature, humidity, and animal core body temperature that corresponded with respective sampling days are presented in **Table 1**. All animals were within the normal range for cattle body temperature. The lower mean body temperature in the *Bos indicus* mature cows is notable and was statistically lower ($P < 0.05$) than the other groups. Body temperature was tested as a covariate in the model, however, it was not significant when included in the model and was subsequently removed from the final model. Gaughan et al. (2013) reported that body

158 temperature did not affect plasma concentration of HSP70 in feedlot steers, and that there was no
159 relationship between temperature-humidity index (THI) and HSP70 concentration when average
160 THI ranged from 68.3°C to 75.5°C on the sampling days.
161
162 ***Heat shock protein concentration and genotype***
163 There were no significant interactions between genotype and age for concentrations of HSP27,
164 Cryab, or HSP70 in the LT, thus, only main effects are presented and discussed throughout the
165 remainder of the manuscript. Among the 3 HSP measured in this study, the mean basal
166 concentration across age and genotype was greatest for HSP27 followed by HSP70 and then
167 Cryab (4.66, 2.17, 0.76 ng/g wet tissue, respectively). The LT of *Bos indicus* cattle had a greater
168 ($P < 0.05$) concentration of basal HSP27, Cryab, and HSP70 when compared to the LT of *Bos*
169 *taurus* cattle (**Table 2**). The absolute difference in mean concentration (ng/g wet tissue) of HSP
170 between the LT from *Bos indicus* and *Bos taurus* was numerically larger for HSP27 (1.64),
171 followed by HSP70 (0.51), and then Cryab (0.38). However, it should be noted that if the
172 differences between mean HSP concentrations were ranked on relative difference rather than
173 absolute difference, the rank or order would change to Cryab, followed by HSP27 and then
174 HSP70 with *Bos indicus* LT having 67%, 43%, and 27% greater concentrations, respectively,
175 than *Bos taurus* LT.
176
177 To our knowledge, no previous studies have directly compared *in vivo* HSP concentrations in
178 skeletal muscle between *Bos indicus* and *Bos taurus* cattle. The results of this study support our
179 hypothesis that *Bos indicus* cattle have greater basal concentrations of HSP27, Cryab, and
180 HSP70 *in vivo*. It is plausible that the greater concentrations of HSP in the *Bos indicus* cattle

enhance their ability to thrive in tropical environments when compared to *Bos taurus* cattle. However, the elevated levels of HSP may also serve as a strong inhibitor of *post-mortem* tenderization and partially explain the tendency for *Bos indicus* cattle to produce less tender beef.

Small heat shock proteins are expressed at low levels in muscle until an inciting event, such as heat stress in recently harvested animals, dramatically increases their synthesis (Lindquist and Craig, 1988; Sugiyama et al., 2000). Heat Shock Protein 27 and Cryab are regarded as small heat shock proteins. They work in myofibers to protect myofibrillar proteins from degradation and denaturation (Paulsen et al., 2007). Furthermore, it has been reported that in the presence of increasing ATP levels concomitant with post-stress cell recovery, small HSP release the damaged proteins to HSP70, a member of a class of larger heat shock proteins which utilize the energy of ATP to renature the proteins (Wang and Spector, 2001). These 3 proteins—HSP27, Cryab, and HSP70—work in concert, binding and renaturing denatured proteins. The irreversible decline in ATP levels *post-mortem*, however, limits the ability of the small HSP to release their substrates to HSP70 (Wang and Spector, 2001).

The process of tenderization in meat is largely attributed to the proteolytic activities of the calpain and cathepsin enzyme systems, which degrade select myofibrillar and cytoskeletal proteins and are down-regulated by calpastatin and cystatin, respectively (Herrera-Mendez et al., 2006; Ouali et al., 2006). Though it is widely accepted that the increased toughness associated with *Bos indicus* beef is in part due to elevated levels of calpastatin and its interactions with calpain-1 and calpain-2, several studies have continued to suggest that other proteases, proteins, and mechanisms are also involved in significantly improving *post-mortem* tenderness (Huff-

Lonergan et al., 2010; Kemp et al., 2010; Kemp and Parr, 2012). Bernard et al. (2007) reported a down-regulation of both HSP27 and Cryab in more tender meat from Charolais bull calves and Kim et al. (2008) reported a down-regulation of HSP27 in more tender meat from male Korean native cattle (*Bos taurus*). Additionally, Carvalho et al. (2014) conducted a study using *Bos indicus* (Nellore) cattle to compare protein expression between LT steaks that were sorted into tough and tender categories, and the steaks in the tender category had a lesser concentration (16.6%) of HSP70 compared to steaks in the tough category.

Heat shock protein concentration and animal age

It is well established that meat from older animals is considerably less tender when compared with that from younger animals, thus a further objective of this study was to determine if basal muscle HSP concentration increases with advanced maturity. There were no significant differences in basal HSP concentrations between heifers (18 months-of-age) and mature cows (>7 years-of-age) for HSP27, Cryab, or HSP70 (**Table 3**). However, it should be noted that there was a trend ($P = 0.087$) for the HSP27 basal concentration of mature cows to be greater than that of heifers.

Similar to the results of this study, Locke (2000) reported that both young and aged rats possessed similar ($P > 0.05$) concentrations of HSP70 in skeletal muscle both at basal levels and following heat stress. In contrast to the results of this study, Gutierrez and Guerriero (1991) reported that bovine skeletal muscle from adult cattle contained a greater content of HSP70 when compared with muscle from 3-month old calves. The difference in the results of this study compared with that of Gutierrez and Guerriero (1991) may be related to the difference in the age

227 of the cattle in the "younger" treatment group, which was 3-months of age in their study
228 compared with 18 months-of-age in the current study. Given that the younger animals were in
229 different stages of their respective growth curves, they may have expressed differing
230 concentrations of HSP compared to their adult counterparts. It should also be noted that
231 Kristensen et al. (2004) demonstrated a more complex relationship with the expression of HSP72
232 and age in Holstein females where older heifers (305-560 days old) expressed a greater ($P <$
233 0.05) concentration of plasma HSP72 compared to calves, young heifers, cows in early lactation,
234 and cows in late lactation. This is in direct contrast to the findings of the current study where
235 heifers of approximately the same age as those used by Kristensen et al. (2004) did not show
236 greater concentrations of any measured HSP in comparison with the older cows. The differences
237 reported by Kristensen et al. (2004) and the results of this study may have been related to the
238 different HSP measured in the two studies (HSP72 versus HSP27, Cryab, and HSP70) and the
239 different source of the HSP (plasma versus skeletal muscle).
240
241 The results of this study suggest that *Bos indicus* cattle have a genetic predisposition, regardless
242 of age, to produce a greater basal concentration of HSP27, Cryab, and HSP70 in the LT *in vivo*
243 than *Bos taurus* cattle. Future studies are needed to investigate the relationship between
244 tenderness and *in vivo* muscle HSP concentration with both *Bos indicus* and *Bos taurus*
245 genotypes to determine if the higher HSP concentration of the *Bos indicus* animals, at least
246 partially, explains the decreased tenderness noted in those animals. Previous studies have
247 correlated HSP concentrations with tenderness within both *Bos indicus* and *Bos taurus*
248 genotypes, but no studies have examined if inherent differences in the *in vivo* expression of HSP
249 between these genotypes is associated with the resulting differences in meat tenderness.

Furthermore, future research can seek to determine if it is the absolute level or relative level of *in vivo* HSP concentration which best predicts resulting tenderness.

LITERATURE CITED

Bernard, C., I. Cassar-Malek, M. Le Cunff, H. Dubroeucq, G. Renand, and J. F. Hocquette. 2007. New indicators of beef sensory quality revealed by expression of specific genes. J. Agric. Food Chem. 55:5229-5237. doi:10.1021/jf063372l

Carvalho, M. E., G. Gasparin, M. D. Poleti, A. F. Rosa, J. C. C. Balieiro, C. A. Labate, R. T. Nassu, R. R. Tullio, L. C. A. Regitano, G. B. Mourao, and L. L. Coutinho. 2014. Heat shock and structural proteins associated with meat tenderness in nellore beef cattle, a *Bos indicus* breed. Meat Sci. 96:1318-1324. doi:10.1016/j.meatsci.2013.11.014

de Jong, W. W., G. -J. Caspers, and J. A. M. Leunissen. 1998. Genealogy of the α-crystallin small heat-shock protein superfamily. Int. J. Biol. Macromol. 22:151-162.

Gaughan, J. B., S. L. Bonner, I. Loxton, and T. L. Mader. 2013. Effects of chronic heat stress on plasma concentration of secreted heat shock protein 70 in growing feedlot cattle. J. Anim. Sci. 91:120-129. doi:10.2527/jas.2012-5294

Gutierrez, J. A., and V. Guerriero. 1991. Quantitation of Hsp70 in tissues using a competitive enzyme-linked immunosorbent assay. J. Immunol. Methods. 143:81-88.

Herrera-Mendez, C. H., S. Becila, A. Boudjellal, and A. Ouali. 2006. Meat ageing: Reconsideration of the current concept. Trends Food Sci Technol. 17:394-405. doi:10.1016/j.tifs.2006.01.011

Highfill, C. M., O. Esquivel-Font, M. E. Dikeman, and D. H. Kropf. 2012. Tenderness profiles of ten muscles from F1 *Bos indicus* x *Bos taurus* and *Bos taurus* cattle cooked as steaks and roasts. Meat Sci. 90:881-886. doi:10.1016/j.meatsci.2011.11.022

294 Huff-Lonergan, E., W. Zhang, and S. M. Lonergan. 2010. Biochemistry of *postmortem* muscle -
295 Lessons on mechanisms of meat tenderization. Meat Sci. 86:184-195.
296 doi:10.1016/j.meatsci.2010.05.004
297 Kemp, C. M., P. L. Sensky, R. G. Bardsley, P. J. Buttery, and T. Parr. 2010. Tenderness - An
298 enzymatic review. Meat Sci. 84:248-256. doi:10.1016/j.meatsci.2009.06.008
299 Kemp, C. M., and T. Parr. 2012. Advances in apoptotic mediated proteolysis in meat
300 tenderisation. Meat Sci. 92:252-259. doi:10.1016/j.meatsci.2012.03.013
301 Kim, N. K., S. Cho, S. H. Lee, H. R. Park, C. S. Lee, Y. M. Cho, Y. H. Choy, D. Yoon, S. K. Im,
302 and E. W. Park. 2008. Proteins in longissimus muscle of Korean native cattle and their
303 relationship to meat quality. Meat Sci. 80:1068-1073. doi:10.1016/j.meatsci.2008.04.027
304 Kristensen, T. N., P. Lovendahl, P. Berg, and V. Loeschcke. 2004. Hsp72 is present in plasma
305 from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and
306 age classes. Cell Stress Chaperones. 9:143-149. doi:10.1379/CSC-17.1
307 Lindquist, S., and E. A. Craig. 1988. The heat-shock proteins. Annu. Rev. Genet. 22:631-677.
308 Locke, M. 2000. Heat shock transcription factor activation and Hsp72 accumulation in aged
309 skeletal muscle. Cell Stress Chaperones. 5:45-51.
310 Lomiwes, D., M. M. Farouk, E. Wiklund, and O. A. Young. 2014. Small heat shock proteins and
311 their role in meat tenderness: A review. Meat Sci. 96:26-46.
312 doi:10.1016/j.meatsci.2013.06.008
313 O'Connor, S. F., J. D. Tatum, D. M. Wulf, R. D. Green, and G. C. Smith. 1997. Genetic effects
314 on beef tenderness in *Bos indicus* composite and *Bos taurus* cattle. J. Anim. Sci. 75:1822-
315 1830.

316 Ouali, A., C. H. Herrera-Mendez, G. Coulis, S. Becila, A. Boudjellal, L. Aubry, and M. A.
317 Sentandreu. 2006. Revisiting the conversion of muscle into meat and the underlying
318 mechanisms. Meat Sci. 74:44-58. doi:10.1016/j.meatsci.2006.05.010
319 Paulsen, G., K. Vissing, J. M. Kalhovde, I. Ugelstad, M. L. Bayer, F. Kadi, P. Schjerling, J.
320 Hallen, and T. Raastad. 2007. Maximal eccentric exercise induces a rapid accumulation of
321 small heat shock proteins on myofibrils and a delayed HSP70 response in humans. Am. J.
322 Physiol. Regul. Integr. Comp. Physiol. 293:R844-R853. doi:10.1152/ajpregu.00677.2006
323 Picard, B., F. Lefevre, and B. Lebret. 2012. Meat and fish flesh quality improvements with
324 proteomic applications. Animal Frontiers. 2:18-25. doi:10.2527/af.2012-0058
325 Sugiyama, Y., A. Suzuki, M. Kishikawa, R. Akutsu, T. Hirose, M. M. Waye, S. K. Tsui, S.
326 Yoshida, and S. Ohno. 2000. Muscle develops a specific form of small heat shock protein
327 complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. J. Biol.
328 Chem. 275:1095-1104. doi: 10.1074/jbc.275.2.1095
329 Thom, E. C. 1959. The discomfort index. Weatherwise. 12:57–59. doi:10.1080/00431672.1959
330 Wang, K., and A. Spector. 2001. ATP causes small heat shock proteins to release denatured
331 protein. Eur. J. Biochem. 268:6335-6345.
332

333 ***Tables and Figures***

334 **Table 1**

335 Descriptive data for treatment animals and environmental conditions at time of tissue collection.

Age[1]	Genotype	Weight, kg	THI[2]	Ambient Temperature, °C	Relative Humidity, %	Body Temperature, °C
		Mean (SD)				Mean (SD)
Heifer	*Bos indicus*	285.7 (15.3)	72.6	25.8	49	39.1[a] (0.4)
Mature Cow	*Bos indicus*	472.6 (37.3)	67.7	21.0	68	38.2[b] (0.7)
Heifer	*Bos taurus*	296.0 (30.3)	70.0	23.9	47	39.2[a] (0.3)
Mature Cow	*Bos taurus*	622.8 (49.8)	67.8	22.8	38	38.9[a] (0.2)

336 [a.b]Within a column, means without a common superscript differ ($P < 0.05$).

337 [1]Age: heifers, 18 months-of-age; mature cows, >7 years-old-age.

338 [2]Temperature-humidity index (THI) calculated from the formula: $THI = (0.8 \times T_A) + [(RH \times$

339 $0.01) \times (T_A - 14.4)] + 46.4$.

340

341 **Table 2**

342 Mean basal concentration of HSP27, alpha B-crystallin (Cryab), and HSP70 in the longissimus

343 thoracis by genotype.

			Concentration, ng/g wet tissue			
Protein	Genotype	*n*	Mean (SEM)	Max	Min	P-value
HSP27	*Bos indicus*	20	5.48 (0.61)	11.08	1.46	0.041
	Bos taurus	20	3.84 (0.50)	7.51	0.94	
Cryab	*Bos indicus*	20	0.95 (0.10)	1.86	0.28	0.009
	Bos taurus	20	0.57 (0.09)	1.32	0.06	
HSP70	*Bos indicus*	20	2.42 (0.17)	3.63	0.86	0.034
	Bos taurus	20	1.91 (0.15)	3.63	1.15	

344

345 **Table 3**

346 Mean basal concentration of HSP27, alpha B-crystallin (Cryab), and HSP70 in the longissimus

347 thoracis by age.

			Concentration, ng/g wet tissue			
Protein	Age[1]	*n*	Mean (SEM)	Max	Min	P-value
HSP27	Heifer	20	3.98 (0.51)	7.95	1.33	0.087
	Mature Cow	20	5.34 (0.61)	11.08	0.94	
Cryab	Heifer	20	0.77 (0.02)	1.86	0.12	0.866
	Mature Cow	20	0.75 (0.09)	1.44	0.06	
HSP70	Heifer	20	2.09 (0.17)	3.63	0.86	0.489
	Mature Cow	20	2.25 (0.17)	3.63	1.22	

348 [1]Age: heifers, 18 months-of-age; mature cows, >7 years-old-age.

349