INTRODUCTION

A recent study has reported that Cr-Met in the form of chelate could improve carcass characteristics including marbling score in Korean native steers (Sung et al., 2015). Another follow up study in Holstein steers during raising and late fattening period showed that 4 months is an optimum period for feeding Cr-Met chelate to improve daily gain and carcass characteristics of Holstein steers during the fattening period; however, fatty acids profile of beef was not measured (Song et al., 2013). Therefore, the apparent effects of Cr-Met chelate supplementation on blood metabolites and profile of fatty acids of beef in Holstein steers during late fattening period has not yet been investigated.

Chromium (Cr) is one of the essential micronutrients for ruminants and is considered to be a metabolic modifier. The organic source of Cr is a promising form due to higher bioavailability than inorganic sources (NRC, 1997). Cr supplementation has been reported to show an influence on some blood metabolites such as glucose (Wang et al., 2007) and could improve carcass quality such as intermuscular fat and percentage of muscle (Boleman et al., 1995) in pigs. Furthermore, Cr is also known to be a key constituent of glucose tolerance factor (regulates blood glucose level) and maintains glucose homeostasis (Sung et al., 2015). In addition, supplemental Cr was also found to play an important role in serum cholesterol homeostasis (Ohh et al., 2004). Cr supplementation decreased the level of total cholesterol, low density lipoprotein (LDL) cholesterol, and
triglyceride in the blood, but increased the level of high density lipoprotein (HDL) cholesterol (Anderson, 1995; Sung et al., 2015). Schwarz and Merts (1959) and Bunting et al. (1994) reported that Cr supplementation can alter glucose metabolism in rats and in calves. Positive responses were reported in pigs by Page et al. (1993), Lindemann et al. (1995), Boleman et al. (1995), and Mooney and Cromwell (1995, 1997) in swine for carcass leanness. Moreover, according to Ohh et al. (2004), the positive effect of Cr supplementation can be associated with its obvious influence on the systematic division of energy between adipose and lean tissue. Reports of lessened fat over the 10th rib and decreased yield grades have been reported in lambs supplemented with Cr tripicolinate (Kitchalong et al., 1995). However, others reported no responses in carcass leanness to supplemental Cr (Harris et al., 1995; Ward et al., 1995). Likewise, Cr research has been conducted in dairy (Al-Saiady et al., 2004) and beef cattle (Swanson et al., 2000; Pollard et al., 2002; Stahlhut et al., 2006). Treatments were control with no Cr-Met supplementation and the different level of Cr-Met supplementation in diet with or without yeast resulted in improving carcass quality including marbling score and glucose tolerance rate. The aim of the present study was to evaluate the effects of Cr-Met chelate on blood metabolites and fatty acids profile of beef from Holstein beef steers.

MATERIALS AND METHODS

Treatments, feeding and experimental procedure

To assess the effect of Cr-Met on performance and beef quality, fifteen Holstein steers were randomly assigned into two dietary treatment groups; Non Cr-Met feeding (NCM, 7 head), Cr-Met feeding over a 4 months (4CM, 8 head). Average body weights of groups were 483±25.7 kg, 486±27.5 kg for NCM and 4CM, respectively at the beginning of the experiment. The feeding amount of Cr-Met (Innobio Co., Ltd., Shiheung, Korea) to animals was limited to 400 ppb/cow/d. The duration for the study was 4 months. The rate of forage to concentrate was 20:80 and forages included alfalfa cube and perennial ryegrass hay according to NRC requirements of beef cattle (2000). Concentrate, alfalfa cubes and Perennial ryegrass hay were fed twice daily, in the morning at 0900 am and at 0500 pm in the afternoon to achieve controlled feeding of fixed ratio of F:C as 20:80. The intake of concentrate was 8±0.4 kg (dry matter [DM]) and the forage intake was 2 kg (1±0.2 kg of alfalfa cube +1±0.2 kg of Perennial ryegrass hay, DM) for both groups. The composition of the experimental diets is presented in Table 1.

Feed, blood and beef quality analysis

Common nutrients were analyzed according to AOAC (1990); neutral detergent fiber and the level of acid detergent fiber were analyzed using the method of Goering and Van Soest (1991). A total digestible nutrient (Table 1) was calculated using the regression equation Ward (1981). Steers had ad libitum access to water. Blood samples from each steer at the beginning and the end of the experiment was collected from the jugular vein at 1300 h by using a vacutainer (no additive: BD, Franklin Lakes, NJ, USA) for serum separation. The serum samples were collected at the beginning, on a monthly basis and at the end of the experiment by centrifuging it at 2,500×g for 15 minutes and the samples were stored at −20°C for further processing. The serum samples were later analyzed for total protein, albumen, alkaline phosphatase (ALP), calcium, creatine, triglyceride, cholesterol, glucose, LDL, and HDL, using kits abiding with the manufacturer’s protocol (Modular analytic E170, Roche, Germany). Upon completion of the field experiment, all steers were slaughtered in order to measure the fatty acids composition of beef from loin side. Samples from each steer were frozen at −20°C for 12 hours, and were thawed prior to analysis. According to the method of lipid extraction (Folch et al., 1957), 6 g of sample and chloroform/methanol (2:1) solution were homogenized in a 25 mL homogenizer (Diax 6000, Heidelberg, Germany) at 1,100×g for 30 seconds. Next 6 mL of 0.9% KCl solution was added to the homogenate, followed by centrifugation at 2,500×g (GS-6R Centrifuge, Beckman, Ramsey, MN, USA) for 10 minutes. The fluid was filtered through filter paper and lipid was concentrated using a nitrogen gas concentrator (MGS-2200, Eyelaa Tokyo Rikakikai Co., Ltd, Tokyo, Japan) following the method of AOAC (1990). Each of the fatty acid methyl ester standards (Sigma-Aldrich Co., Saint Louis, MO, USA) was qualitatively compared with the analytic conditions used for Gas Chromatography (Agilent 6890N, Agilent Technologies, Santa Clara, CA, USA). For this, a sample of beef and tissue was kept for the split ratio of 1:10. The oven injector

| Table 1. Chemical composition of experimental feed |
|-----------------------------------------------|
|             | DM | CP | CF | EE | Ash | NDF | ADF | NFE | TDN |
| Concentrate | 89.0 | 19.0 | 6.0 | 2.2 | 7.5 | 19.4 | 10.6 | 62.2 | 72.1 |
| Alfalfa cube | 89.7 | 14.4 | 20.4 | 1.5 | 12.1 | 46.6 | 38.2 | 51.6 | 56.2 |
| Perennial ryegrass hay | 92.6 | 6.7 | 29.8 | 2.5 | 5.3 | 62.3 | 35.7 | 55.7 | 56.8 |

DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFE, nitrogen free extract; TDN, total digestible nutrient.
was heated with 220°C. A carrier of gas 1mL/min was heated with 150°C for one min. A column HP-Innowax (30 m length×0.32 id×0.25 μm thicknesses) maintained for a detector temperature of 275°C. The oven maintained the temperature of 200°C to 250°C at 3°C/min and 250°C for 5 min.

**Statistical analysis**

All the data are reported as the sample mean±the standard deviation. Pairwise comparisons between means of different groups were performed using a t-test. The difference between two subsets of data is considered statistically significant if the t-test gives a significance level p (p value) less than 0.05.

**RESULTS AND DISCUSSIONS**

**Blood metabolites**

In assessing the effect of Cr-Met supplementation on normal body functions (renal function, clinical enzymology etc.) we observed that Cr-Met supplementation did not affect (p>0.05) albumen, ALP, calcium and creatine in blood (Table 2). Al-Saiady et al. (2004) also did not find any change in the albumin level in Holstein cows supplemented with chelated Cr. Similar results for creatine levels in pigs was observed by Lindemann et al. (2008). Kaneko et al. (1989) reported that an increase in ALP activity may occur in response to bone or liver damage. However, in the current study no increase (p>0.05) in ALP activity was observed. Furthermore, no effect on urea nitrogen and total protein was observed in any of the other trials (Ohh and Lee, 2005; Sung et al., 2015). Similar results were reported by Kitchalong et al. (1995) in lambs and Bunting et al. (1994) in steers with Cr-picolinate.

**Table 2. Effect of Cr-Met on blood levels of Holstein steers during late fattening period**

|                     | NCM (n=7) | 4CM (n=8) | p value |
|---------------------|-----------|-----------|---------|
| Albumin (g/dL)      | 2.9±0.7   | 3.3±0.2   | 0.45    |
| Alkaline phosphatase (U/L) | 262.3±137.6 | 196.5±24.6 | 0.27    |
| Urea-nitrogen (mg/dL) | 9.0±4.9   | 10.2±2.6  | 0.58    |
| Calcium (mg/dL)     | 9.6±0.6   | 9.7±0.7   | 0.73    |
| Creatine (mg/dL)    | 1.2±0.1   | 1.3±0.2   | 0.81    |
| Glucose (mg/dL)     | 93.3±4.0  | 87.1±5.0  | 0.37    |
| Total protein (g/dL)| 8.1±1.7   | 6.9±0.6   | 0.29    |
| Triglyceride (mg/dL)| 24.0±6.0  | 26.7±5.5  | 0.78    |
| Cholesterol (mg/dL) | 164.0±7.5 | 141.5±12.1 | 0.43    |
| HDL (mg/dL)         | 72.3±3.5a | 101.3±8.3a| 0.01    |
| LDL (mg/dL)         | 51.0±3.5a | 35.1±7.5a | 0.04    |

NCM, non Cr-Met feeding; CM, Cr-Met feeding; HDL, high density lipoprotein; LDL, low density lipoprotein; ±, standard deviation.

*Means with different superscript in same row differs significantly (p<0.05).

However, Dominguez-vara (2009) showed inconsistent results in urea nitrogen levels with the addition of Cr-yeast. In this study, supplementation with Cr-Met chelate did not affect (p>0.05) serum glucose levels in Holstein steers.

**Fatty acid composition of beef**

In the current study, no significant differences (p>0.05) in composition of fatty acids (caprate, laurate, myristate, pentadecanoate, palmitate, palmitoleate, margarate, cis-11 heptadecenoate, stearate, oleate, trans-vaccenate, linoleate, cis-11 eicosenate, docosa hexaenoic acid, and docosa pentadecenoic acid) were observed. Whereas, gamma linolenate (C18:3n6, p<0.05) and arachidonic acid (C20:4n6, p = 0.07) were higher in the 4CM group than the
Conversely, supplemental Cr had no effect on plasma NEFA concentration lessened plasma NEFA concentration. Hayirli et al. (2001) found that Cr picolinate had lower plasma NEFA concentration vs. control. Kitchalong et al. (1995) and Matthews et al. (2001) reported that swine supplemented with Cr shortly after calving (days 97 and 155), especially young cows (Stahlhut et al., 2006). Matthews et al. (2001) reported that swine supplemented with Cr picolinate had lower plasma NEFA concentration vs. control. Similarly, studies on sheep (Kitchalong et al., 1995) and dairy cattle (Hayirli et al., 2001) found that Cr supplementation lessened plasma NEFA concentration. Conversely, supplemental Cr had no effect on plasma NEFA concentration of NEFA measured in blood has been shown to reflect fat mobilized from body fat reserves. Plasma NEFA concentrations were lower in beef cows receiving supplemental Cr shortly after calving (days 97 and 155), especially young cows (Stahlhut et al., 2006). Matthews et al. (2001) reported that swine supplemented with Cr picolinate had lower plasma NEFA concentration vs. control. Similarly, studies on sheep (Kitchalong et al., 1995) and dairy cattle (Hayirli et al., 2001) found that Cr supplementation lessened plasma NEFA concentration. Conversely, supplemental Cr had no effect on plasma NEFA concentration.

### Table 3. Effect of Cr-Met supplementation on fatty acid profile of beef (loin side) from Holstein steers during late fattening period

| Fatty acids (%) | NCM (n = 7) | 4CM (n = 8) | p value |
|-----------------|-------------|-------------|---------|
| C10:0 (caprate) | 0.06±0.01   | 0.05±0.02   | 0.57    |
| C12:0 (laurate) | 0.09±0.01   | 0.08±0.01   | 0.73    |
| C14:0 (myristate)| 3.26±0.13   | 3.11±0.66   | 0.69    |
| C15:0 (pentacontaneoate) | 0.27±0.03 | 0.24±0.02 | 0.49    |
| C16:0 (palmitate) | 29.01±0.62 | 28.72±2.75 | 0.47    |
| C16:1n7 (palmitoleate) | 4.74±0.95 | 4.58±0.77 | 0.83    |
| C17:0 (margarate) | 0.71±0.11   | 0.69±0.10   | 0.75    |
| C17:1n6 (cis-11-heptadecanoate) | 0.67±0.15 | 0.65±0.04 | 0.57    |
| C18:0 (stearate) | 11.31±0.86  | 12.29±1.24  | 0.43    |
| C18:1n9 (oleate) | 47.00±0.29  | 46.49±2.42  | 0.39    |
| C18:1n7 (trans-vaccenate) | 1.91±0.10 | 1.76±0.36 | 0.37    |
| C18:2n6 (linoleate) | 0.24±0.04   | 0.24±0.03   | 0.81    |
| C18:3n6 (gamma-Linoleate) | 0.07±0.06a  | 0.23±0.03a  | 0.04    |
| C20:1n9 (cis-11-Eicosenoate) | 0.26±0.10  | 0.29±0.04   | 0.68    |
| C20:4n6 (arachidonate) | 0.32±0.07  | 0.48±0.10   | 0.07    |
| C22:4n6 (DHA) | 0.04±0.07   | 0.05±0.09   | 0.73    |
| C22:5n3 (DPA) | 0.04±0.08   | 0.05±0.08   | 0.39    |
| Total           | 100.00      | 100.00      |         |

NCM, non Cr-Met feeding; CM, Cr-Met feeding; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ±, standard deviation.

*p<0.05.

### Table 4. Effect of Cr-Met on fatty acid ratio of Holstein steers (loin side) during late fattening period

| Fatty acids (%) | NCM (n = 7) | 4CM (n = 8) | p value |
|-----------------|-------------|-------------|---------|
| SFA             | 44.72±1.35  | 44.55±2.10  | 0.47    |
| UFA             | 55.28±1.35  | 54.45±2.10  | 0.59    |
| Total           | 100.00      | 100.00      |         |
| MUFA            | 54.58±1.34  | 53.71±2.06  | 0.73    |
| PUFA            | 0.70±0.08a  | 0.87±0.07a  | 0.04    |
| UFA/SFA         | 1.24±0.07   | 1.15±0.10   | 0.31    |
| MUFA/SFA        | 1.22±0.07   | 1.19±0.10   | 0.83    |
| PUFA/SFA        | 0.02±0.01   | 0.02±0.01   | 0.83    |

NCM, non Cr-Met feeding; CM, Cr-Met feeding; SFU, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ±, standard deviation.

*p<0.05.

**Means with different superscript in same row differs significantly (p<0.05).**
influences the fatty acid composition of beef (Smith et al., 2009). Zea et al. (2007) reported that saturated fatty acids (SFA) were higher in animals fed with concentrates, while animals fed with silage had higher levels of PUFA and a higher PUFA/SFA ratio. Furthermore, Smith et al. (2009) reported that grain feeding arouses the activity of adipose tissue stearoyl-CoA desaturase in marbling adipose tissue and lowers ruminal isomerization/hydrogenation of dietary PUFA, resulting in a noticeable increase in monounsaturated fatty acid (MUFA) in beef over time. The current study found no differences (p>0.05) in the values of SFA, unsaturated fatty acid (UFA), MUFA, UFA/SFA, MUFA/SFA, and PUFA/SFA.

CONCLUSIONS

It can be concluded that feeding Cr-Met supplementation in 400 ppb/d to Holstein steers for 4 months during late fattening period can improve some blood metabolites and beef quality regarding fatty acid composition by increasing PUFA and gamma-linoleate compositions of beef. However, further research is warranted to validate the present results.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

Authors are thankful to Austin Thelen (Department of Communication, Oregon State University, USA) and Lindsay M. Loehden (Linfield College, USA) for their help in editing the manuscript.

REFERENCES

Al-Saiady, M. Y., M. A. Al-Shaikh, S. Y. Al-Mufarrej, T. A. Al-Showeimi, H. H. Mogawer, and A. Dirrar. 2004. Effect of chelated chromium supplementation on lactation performance and blood parameters of Holstein cows under heat stress. Anim. Feed Sci. Technol. 117:223-233.
Anderson, R. A. 1995. Chromium, glucose tolerance, and lipid metabolism. J. Adv. Med. 8:37.
AOAC. 1990. Official Methods of Analysis. 15th edition. Association of Official Analytical Chemists, Washington, DC, USA.
Boleman, S. L., S. J. Boleman, T. D. Bidner, L. L. Southern, T. L. Ward, J. E. Pontif, and M. M. Pike. 1995. Effect of chromium picolinate on growth, body composition, and tissue accretion in pigs. J. Anim. Sci. 73:2033-2042.
Bunting, L. D., J. M. Fernandez, Jr. D. L. Thompson, and L. L. Southern. 1994. Influence of chromium picolinate on glucose usage and metabolic criteria in growing Holstein calves. J. Anim. Sci. 72:1591-1599.
Chang, H., D. N. Mowat, and B. A. Mallard. 1995. Supplemental chromium and niacin for stressed feeder calves. Can. J. Anim. Sci. 75:351-358.
Chang, X. and D. N. Mowat. 1992. Supplemental chromium for stressed and growing feeder calves. J. Anim. Sci. 70:559-565.
Domínguez-Vara, I. A., S. S. González-Muñoz, J. M. Pino-Rodriguez, J. L. Bórguez-Gastelum, R. Bárcena-Gama, G. Mendoza-Martínez, L. E. Zapata, and L. L. Landois-Palencia. 2009. Effects of feeding selenium-yeast and chromium-yeast to finishing lambs on growth, carcass characteristics, and blood hormones and metabolites. Anim. Feed Sci. Technol. 152:42-49.
Folch, J., M. Lee, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.
Goering, H. K. and P. J. Van Soest. 1991. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook. No.379. ARS, USDA, Washington, DC, USA.
Harris, J. E., S. D. Crow, and M. D. Newcomb. 1995. Effect of chromium picolinate on growth performance and carcass characteristics on pigs fed adequate and low protein diets. J. Anim. Sci. 73 (Suppl. 1):194 (abstract).
Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. J. Dairy Sci. 84:1218-1230.
Kaneko, J. J. 1989. Reference values for blood gas and electrolyte determinations. In: Clinical Biochemistry of Domestic Animals. 4th ed. (Ed. J. J. Kaneko). Academic Press, San Diego, CA, USA. 564 p.
Kegley, E. B., J. W. Spears, and T. T. Brown. 1997. Effect of shipping and chromium supplementation on performance, immune response, and disease resistance of steers. J. Anim. Sci. 75:1956-1964.
Kitchaleng, L., J. M. Fernandez, L. D. Bunting, L. L. Southern, and T. D. Bidner. 1995. Influence of chromium tripicolinate on glucose metabolism and nutrient partitioning in growing lambs. J. Anim. Sci. 73:2694-2705.
Lindemann, M. D., G. L. Cromwell, H. J. Monegue, and K. W. Pursur. 2008. Effect of chromium source on tissue concentration of chromium in pigs. J. Anim. Sci. 86:2971-2978.
Lindemann, M. D., C. M. Wood, A. F. Harper, E. T. Kornegay, and R. A. Anderson. 1995. Dietary chromium picolinate additions improve gain:feed and carcass characteristics in growing-finishing pigs and increase litter size in reproducing sows. J. Anim. Sci. 73:457-465.
Matthews, J. O., L. L. Southern, J. M. Fernandez, E. J. Pontif, T. D. Bidner, and R. L. Odgaard. 2001. Effect of chromium picolinate and chromium propionate on glucose and insulin kinetics of growing barrows and on growth and carcass traits of growing-finishing barrows. J. Anim. Sci. 79:2172-2178.
Mertz, W. 1993. Chromium in human nutrition: A review. J. Nutr. 123:626-633.
Mooney, K. W. and G. L. Cromwell. 1995. Effects of dietary chromium picolinate supplementation on growth, carcass
characteristics, and accretion rates of carcass tissues in growing-finishing swine. J. Anim. Sci. 73:3351-3357.

Mooney, K. W. and G. L. Cromwell. 1997. Efficacy of chromium picolinate and chromium chloride as potential carcass modifiers in swine. J. Anim. Sci. 75:2661-2671.

NRC. 1997. The role of chromium in animal nutrition. National Academy of Sciences. National Academy Press, Washington, DC, USA.

Nutrient Requirements of Beef Cattle. 2000. 7th rev. ed. National Academy Press, Washington, DC, USA.

Ohh, S. J., C. H. Kim, J. S. Shin, K. I. Sung, and H. S. Kim. 2004. Effects of different forms of Chromium supplements on serum glucose, insulin and lipids in rats. J. Feed Sci. Nutr. 9:342-345.

Ohh, S. J. and J. Y. Lee. 2005. Dietary chromium-methionine chelate supplementation and animal performance. Asian Australas. J. Anim. Sci. 18:898-907.

Page, T. G., L. L. Southern, T. L. Ward, and D. L. Thompson Jr. 1993. Effect of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. J. Anim. Sci. 71:656-662.

Pollard, G. V., C. R. Richardson, and T. P. Karnezos. 2002. Effects of supplemental organic chromium on growth, feed efficiency and carcass characteristics of feedlot steers. Anim. Feed Sci. Technol. 98:121-128.

Roginski, E. E. and W. Mertz. 1969. Effect of chromium supplementation on glucose and amino acid metabolism in rats fed a low protein diet. J. Nutr. 97:525-30.

Schwarz, K. and W. Mertz. 1959. Chromium (III) and the glucose tolerance factor. Arch. Biochem. Biophys. 85:292 (Letters to the Editors).

Smith, S. B., C. A. Gill, D. K. Lunt, and M. A. Brooks. 2009. Regulation of fat and fatty acid composition in beef cattle. Asian Australas. J. Anim. Sci. 22:1225-1233.

Song, S. Y., J. Ghassemi Nejad, S. J. Ohh, B. H. Lee, H. S. Kim, and K. I. Sung. 2013. Effects of chromium-methionine chelate feeding for different duration on growth and carcass characteristics of Holstein steers in the late fattening stage. Ann. Anim. Resour. Sci. 24:38-43.

Stahlhut, H. S., C. S. Whisnant, K. E. Lloyd, E. J. Baird, L. R. Legleiter, S. L. Hansen, and J. W. Spears. 2006. Effect of chromium supplementation and copper status on glucose and lipid metabolism in Angus and Simmental beef cows. Anim. Feed Sci. Technol. 128:263-265.

Sung, K. I., J. Ghassemi Nejad, S. M. Hong, S. J. Ohh, B. H. Lee, J. L. Peng, D. H. Ji, and B. W. Kim. 2015. Effects of forage level and chromium-methionine chelate supplementation on performance, carcass characteristics and blood metabolites in Korean native (Hanwoo) steers. J. Anim. Sci. Technol. 57:14-20.

Swanson, K. C., D. L. Harmon, K. A. Jacques, B. T. Larson, C. J. Richards, D. W. Bohnert, and S. J. Paton. 2000. Efficacy of chromium-yeast supplementation for growing beef steers. Anim. Feed Sci. Technol. 86:95-105.

Wang, M. Q., Z. R. Xu, L. Y. Zha, and M. D. Lindemann. 2007. Effects of chromium nanocomposite supplementation on blood metabolites, endocrine parameters and immune traits in finishing pigs. Anim. Feed Sci. Technol. 139:69-80.

Ward, T. L., L. L. Southern, and R. A. Anderson. 1995. Effect of dietary chromium source on growth, carcass characteristics, and plasma metabolite and hormone concentrations in growing-finishing swine. J. Anim. Sci. 73 (Suppl.):189 (abstract).

Wardh, M. F. 1981. Models for Estimating Energy and Protein Utilization for Feeds. PhD Thesis, Utah State University, Logan, UT, USA.

Zea, S. J., M. D. Diaz, and J. A. C. Santaolalla. 2007. Sex and beef production system on meat and fat quality. Archivos de Zootecnia 56:817-828.