# EFFECTS OF DIETARY FLAX AND VITAMIN E ON BEEF INTRAMUSCULAR FAT CONCENTRATION OF BIOHYDROGENATION INTERMEDIATES

Manuel Juárez[1,*], Michael E. R. Dugan[1], Jennifer L. Aalhus[1], Noelia Aldai[1], John A. Basarab[2], Vern S. Baron[1], Tim A. McAllister[3]

[1]Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C & E Trail, Lacombe, AB, Canada T4L 1W1

[2]Alberta Agriculture and Rural Development, Research Centre, 6000 C & E Trail, Lacombe, AB, Canada T4L 1W1

[3]Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403-1st Ave. S. Lethbridge, AB, Canada T1J 4B1

[*]Corresponding author (phone: +1-403-782-8118; fax: +1-403-782-6120; e-mail: manuel.juarez@agr.gc.ca)

*Abstract*—**Dietary flax has been previously reported to increase total omega-3 (n-3) and polyunsaturated (PUFA) fatty acids in beef. In order to prevent the detrimental effects of increased PUFA on beef quality, feedlot diets can be supplemented with antioxidants, such as vitamin E. However, the combined effects of flax and vitamin E on concentrations of biohydrogenation intermediates in beef intramuscular fat have not been investigated. Eighty feedlot steers were fed four barley-based diets: control (no flax; 451 IU dl-α-tocopheryl acetate/head/day), high vitamin E (1051 IU dl-α-tocopheryl acetate/head/day), 10% ground flax or 10% ground flax with high vitamin E. Besides the expected effects on PUFA indices, feeding flax also resulted in modifications to levels of 18:3n-3 biohydrogenation intermediates, yielding increased *t*11- and *t*13/14-18:1 and total atypical 18:2 isomers and reductions in *t*10-18:1. Vitamin E supplementation provided some protection for 18:3n-3 and its biohydrogenation intermediates and an interaction with dietary flax and resulted in greater increases in total n-3, but also total *trans* 18:1 and atypical 18:2 isomers. Changes in beef fatty acid composition resultant from feeding flax and vitamin E in a barley based diet were at variance with profiles typically seen when finishing on either pasture or other high grain diets. Given the physiological effects of many of the biohydrogenation intermediates are unknown, the human health consequences of such modifications to the intramuscular fatty acid profile of beef warrant further investigation.**

*Index Terms*—**CLA, linoleic, linolenic, α-tocopherol, *trans***

## I. INTRODUCTION

Omega-3 (n-3) fatty acids are currently the only class of fatty acids with regulatory label claim status for meat and meat products in Canada (≥300 mg n-3 per serving; CFIA, 2003). Current market penetration of animal based n-3 products are in the monogastric and dairy sectors, generally resulting from feeding flax to monogastrics (Haak, Raes, Van Dyck, & De Smet, 2008) or bypass lipid products to dairy cattle (Castañeda-Gutiérrez, De Veth, Lock, Dwyer, Murphy, & Bauman, 2007). Several authors have studied the effects of including dietary flax on beef quality and fatty acid composition (Bartoň, Marounek, Kudrna, Bureš, & Zahrádková, 2007; Drouillard, Seyfert, Good, Loe, Depenbusch, & Daubert., 2004; Raes, Haak, Balcaen, Claeys, Demeyer, & De Smet, 2004; Scollan, Choi, Kurt, Fisher, Enser, & Wood, 2001). However, the impact of dietary flax on the accumulation of biohydrogenation intermediates in beef, such as *trans*-18:1 and 18:2 atypical (non conjugated) isomers, as reported in dairy studies (Kay, Roche, Kolver, Thomson, & Baumgard, 2005; Pottier, Focant, Debier, De Buysser, Goffe, Mignolet, et al., 2006), has not been fully explored.

The increase in n-3, together with other polyunsaturated fatty acids (PUFA), may lead to problems in beef colour and lipid stability. Vitamin E has been shown to have a positive impact on colour and lipid stability of fresh and frozen beef because of its location in biological membranes and fat (Liu, Lanari, & Schaefer, 1995). Moreover, dietary vitamin E could influence ruminal pathways of PUFA biohydrogenation pathways, possibly affecting microbial communities in the rumen (Hughes & Tove, 1980; Pottier, et al., 2006), leading to modifications in the final fatty acid profile observed in beef carcasses.

Thus, a study was undertaken to elucidate the effect of dietary flaxseed and vitamin E on the concentration of biohydrogenation intermediates in intramuscular fat from beef cattle fed a typical western Canadian finisher diet high in ground barley.

## II. MATERIALS AND METHODS

*A*. Animal management

Eighty feedlot steers were housed in 8 feedlot pens (2 pens per dietary treatment, 10 animals per pen, n=20 animals per dietary treatment) and fed *ad libitum* diets consisting of 83.5 % steam-rolled barley, 8 % grass hay, 4.4 % feedlot supplement, 4 % molasses and 0.1 % fortified trace mineralized salt over a 90 day period (DM basis). The animals were fed four different diets: Control (no flaxseed and 451 IU dl-α-tocopheryl acetate/head/day), vitamin E (no flaxseed and 1051 dl-α-tocopheryl IU/head/day), Flax (10% ground flaxseed substituted for steam-rolled barley and 451 IU dl-α-tocopheryl acetate/head day) and vitamin E/Flax (10% ground flaxseed and 1051 IU dl-α-tocopheryl acetate/head/day).

Animals were slaughtered over 5 dates (4 animals per dietary treatment per kill day) at a targeted ultrasound backfat of 8-9 mm. Animals were raised and slaughtered in accordance with the principals and guidelines established by the Canadian Council on Animal Care (CCAC, 1993). Following overnight chill at 2ºC, at approximately 24 h *post-mortem*, the left *longissimus thoracis* (LT; rib-eye) was collected and one steak from the posterior end (12$^{th}$ rib) was removed and frozen (-80ºC) for subsequent fatty acid determination.

*B*. Lipid analysis

Intramuscular lipids were extracted from the meat samples with 2:1 chloroform:methanol and extracts along with adipose tissue were methylated using 0.5 N sodium methoxide, to prevent isomerization and artefact formation from conjugated linoleic acids (CLA), and using 5% methanolic HCl to permit analysis of N-acyl lipids and dimethyl acetals. Fatty acid methyl esters were analyzed using the GC and Ag+-HPLC equipment and methods outlined by Cruz-Hernandez, Deng, Zhou, Hill, Yurawecz, Delmonte, et al. (2004). The *trans*-18:1 isomers were analyzed using two complementary GC temperature programs (Kramer, Hernandez, Cruz-Hernandez, Kraft, & Dugan, 2008). For the identification of fatty acids by GC, the reference standard #461 from Nu-Check Prep Inc. (Elysian, MN) was used. Branch-chain fatty acids were identified using a GC reference standard BC-Mix1 (Applied Science, State College, PA). CLA isomers #UC-59M (Nu-Chek Prep Inc. Elysian, MN) was used since it contains all four positional CLA isomers, and additional CLA isomers were obtained from Matreya Inc. (Pleasant Gap, PA).

*C*. Statistical Analysis

Statistical analyses were conducted using the MIXED procedure of SAS (SAS, 2003). The model included the fixed effects of diet (flax/no flax) and vitamin E (normal/enhanced), the diet x vitamin E interaction and the random kill and pen (only when significant) effects. Treatment means were determined using the LSMEANS option and separated using F-test protected LSD ($P \leq 0.05$).

## III. RESULTS AND DISCUSSION

Feeding flaxseed increased total PUFA and n-3 fatty acids (Table 1) in intramuscular fat ($P < 0.001$). High levels of dietary vitamin E led to increases in total n-3 ($P = 0.026$), either compared to the control or to the flaxseed supplemented diet. On the other hand, total saturated (SFA; $P < 0.001$), n-6 ($P = 0.046$) and the n-6/n-3 ratio ($P < 0.001$) decreased when flaxseed was included in the diet. As reported by several authors when feeding flaxseed to beef cattle (Bartoň, Marounek, Kudrna, Bureš, & Zahrádková, 2007; Scollan, Choi, Kurt, Fisher, Enser, & Wood, 2001), these effects were mainly attributed to reductions ($P < 0.001$) in 16:0 and 20:4n-6 and increases ($P < 0.001$) in 18:3n-3 and 20:5n:3 (data not shown).

A flax by vitamin E interaction was found for total atypical 18:2 isomers ($P = 0.027$), with the highest level of atypical 18:2 isomers in intramuscular fat being found when vitamin E was included in the flaxseed containing diet (Table 1). The flax by vitamin E interaction for total atypical 18:2 isomers was mostly related to an interaction in *t*11,*c*15-18:2 levels ($P < 0.001$). However, it should be noted that flaxseed by itself significantly increased ($P < 0.001$) levels of several other atypical 18:2 isomers (*c*9,*t*13-/*t*8,*c*12-, *t*8,*c*13- and *c*9,*t*12-).

Adding either flaxseed or vitamin E had no effect ($P > 0.05$) on the level of total CLA (Table 1). According to the literature, the effect of dietary flaxseed on CLA is not clear. Some authors agree with the lack of an effect of flaxseed on CLA (Raes, Haak, Balcaen, Claeys, Demeyer, & De Smet, 2004), while others report an increase (Bartoň, Marounek, Kudrna, Bureš, & Zahrádková, 2007) or a trend for an increase (Mach, Devant, Díaz, Font-Furnols, Oliver, García, et al., 2006) in *c*9,*t*11-CLA. In the present experiment, however, feeding flaxseed or vitamin E had no effect on the major CLA isomer (*c*9,*t*11-CLA; $P > 0.05$). Flaxseed, vitamin E and flaxseed by vitamin E interactions were found to cause minor affects on CLA isomers, but these forms were present at very low (< 0.05%) concentrations.

Changes in monounsaturated (MUFA) were complex. Feeding flaxseed increased total MUFA ($P = 0.011$), but these changes were not consistent for individual MUFA. The main 18:1 isomer, *c*9-18:1, increased when vitamin E was added to the control diet and decreased when it was added to the flaxseed containing diet ($P < 0.001$). The *cis*-18:1 isomers which are intermediates of PUFA, specifically 18:3n-3, hydrogenation (*c*12-, *c*13-, *c*14- and *c*16-18:1) were increased with flaxseed feeding ($P < 0.05$). In addition, a flax by vitamin E interaction showed that feeding flaxseed in combination with additional vitamin E resulted in a further increase in the level of *c*15-18:1 ($P = 0.046$).

The effects of feeding flaxseed and vitamin E on *trans*-18:1 isomers was as complex as the *cis*-18:1 isomers. Total

*trans*-18:1 increased when feeding flaxseed (Table 1), and a flax by vitamin E interaction indicated adding vitamin E to the control diet decreased total *trans*-18:1 but increased it when included in the flaxseed containing diet ($P < 0.001$). This interaction was also found for *t*6-*t*8-, *t*9 and *t*10-18:1 ($P < 0.05$). However, an opposing flax by vitamin E interaction was found for *t*11- and *t*16-18:1 ($P < 0.05$). The increased level of total *trans*-18:1 when feeding flaxseed was mainly attributed to large increases ($P < 0.001$) in *t*12-, *t*13/14-, *t*15- and *t*16-18:1, and this was to the point where *t*13/14-18:1 was actually more concentrated than *t*10- or *t*11-18:1. This is unusual, as *t*10- or *t*11-18:1 are typically the most abundant isomers when feeding concentrate or forage based diets respectively (Aldai, Dugan, Rolland, & Kramer, 2009).

Table 1. Beef intramuscular fatty acid profile (% total fatty acids) from four different dietary treatments

| | **Diet** | | | | **SEM** | ***P* value** | | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **VitE** | **Flax** | **Flax E** | | **VitE** | **Flax** | **VitE*Flax** |
| **Total FA (mg.g$^{-1}$ meat)** | 35.2 | 37.4 | 43.1 | 39.0 | 4.978 | 0.762 | 0.126 | 0.315 |
| SFA | 43.4 | 42.2 | 39.9 | 39.8 | 0.789 | 0.219 | <.001 | 0.251 |
| MUFA | 48.6 | 49.3 | 50.6 | 49.6 | 0.460 | 0.690 | 0.011 | 0.071 |
| PUFA | 6.10 | 6.60 | 7.71 | 8.76 | 0.601 | 0.063 | <.001 | 0.503 |
| n-3 | 0.95 | 1.04 | 2.05 | 2.41 | 0.142 | 0.026 | <.001 | 0.176 |
| n-6 | 3.88 | 4.33 | 3.31 | 3.71 | 0.420 | 0.154 | 0.046 | 0.939 |
| n-6/n-3 | 4.11 | 4.19 | 1.61 | 1.54 | 0.110 | 0.923 | <.001 | 0.281 |
| **Σ*Trans* 18:1** | 3.62$^{c}$ | 2.85$^{d}$ | 4.24$^{b}$ | 4.76$^{a}$ | 0.178 | 0.475 | <.001 | <.001 |
| *t*6-*t*8- | 0.26$^{a}$ | 0.19$^{b}$ | 0.19$^{b}$ | 0.24$^{a}$ | 0.014 | 0.255 | 0.472 | <.001 |
| *t*9- | 0.33$^{a}$ | 0.25$^{b}$ | 0.25$^{b}$ | 0.28$^{b}$ | 0.014 | 0.095 | 0.093 | <.001 |
| *t*10- | 1.74$^{a}$ | 1.00$^{b}$ | 0.77$^{b}$ | 1.04$^{b}$ | 0.121 | 0.061 | <.001 | <.001 |
| *t*11- | 0.64$^{c}$ | 0.71$^{bc}$ | 0.78$^{a}$ | 0.72$^{ab}$ | 0.036 | 0.911 | 0.004 | 0.011 |
| *t*12- | 0.11 | 0.13 | 0.39 | 0.43 | 0.012 | 0.018 | <.001 | 0.417 |
| *t*13-/*t*14- | 0.27 | 0.28 | 0.99 | 1.14 | 0.046 | 0.069 | <.001 | 0.137 |
| *t*15- | 0.21 | 0.21 | 0.54 | 0.62 | 0.040 | 0.201 | <.001 | 0.263 |
| *t*16- | 0.06$^{c}$ | 0.08$^{c}$ | 0.32$^{a}$ | 0.29$^{b}$ | 0.011 | 0.270 | <.001 | 0.007 |
| ***c*9-18:1** | 35.8$^{bc}$ | 37.5$^{a}$ | 36.7$^{ab}$ | 34.8$^{c}$ | 0.544 | 0.827 | 0.070 | <.001 |
| *c*11- | 1.55 | 1.62 | 1.56 | 1.53 | 0.066 | 0.682 | 0.320 | 0.229 |
| *c*12- | 0.12 | 0.14 | 0.21 | 0.24 | 0.017 | 0.022 | <.001 | 0.699 |
| *c*13- | 0.47 | 0.47 | 0.55 | 0.52 | 0.025 | 0.592 | 0.004 | 0.504 |
| *c*14- | 0.04 | 0.04 | 0.12 | 0.13 | 0.007 | 0.040 | <.001 | 0.147 |
| *c*15- | 0.31$^{c}$ | 0.24$^{c}$ | 1.10$^{b}$ | 1.45$^{a}$ | 0.121 | 0.162 | <.001 | 0.046 |
| *c*16- | 0.04 | 0.04 | 0.08 | 0.10 | 0.007 | 0.062 | <.001 | 0.313 |
| *c*17- | 0.09$^{ab}$ | 0.08$^{b}$ | 0.09$^{ab}$ | 0.10$^{a}$ | 0.006 | 0.834 | 0.084 | 0.046 |
| **Σ18:2 atypical isomers** | 0.68$^{c}$ | 0.64$^{c}$ | 1.75$^{b}$ | 2.00$^{a}$ | 0.082 | 0.092 | <.001 | 0.027 |
| *c*9,*t*13-/*t*8,*c*12- | 0.16 | 0.17 | 0.58 | 0.62 | 0.021 | 0.267 | <.001 | 0.513 |
| *t*8,*c*13- | 0.11 | 0.11 | 0.43 | 0.48 | 0.024 | 0.139 | <.001 | 0.145 |
| *c*9,*t*12- | 0.06 | 0.06 | 0.15 | 0.17 | 0.006 | 0.043 | <.001 | 0.154 |
| *t*9,*c*12- | 0.03$^{c}$ | 0.03$^{c}$ | 0.05$^{b}$ | 0.07$^{a}$ | 0.006 | 0.058 | <.001 | 0.005 |
| *t*11,*c*15- | 0.20$^{c}$ | 0.14$^{c}$ | 0.42$^{b}$ | 0.55$^{a}$ | 0.033 | 0.152 | <.001 | <.001 |
| *c*9,*c*15- | 0.08$^{ab}$ | 0.09$^{a}$ | 0.08$^{ab}$ | 0.07$^{b}$ | 0.006 | 0.943 | 0.184 | 0.020 |
| **ΣCLA** | 0.59 | 0.58 | 0.60 | 0.64 | 0.037 | 0.604 | 0.407 | 0.579 |
| *c*9,*t*11- | 0.33 | 0.36 | 0.34 | 0.34 | 0.023 | 0.509 | 0.971 | 0.436 |
| *t*7,*c*9- | 0.10$^{a}$ | 0.07$^{b}$ | 0.07$^{b}$ | 0.08$^{b}$ | 0.007 | 0.174 | 0.109 | 0.005 |

VitE: vitamin E; FA: fatty acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: conjugated linoleic acid; $^{a,b,c,d}$: means with different superscripts in the same row are statistically different ($P < 0.05$)

The accumulation of 18:3n-3 biohydrogenation intermediates in intramuscular fat when feeding flaxseed and vitamin E were at variance with what is typically seen when feeding beef cattle either concentrate or forage based diets. Forage feeding typically yields increased levels of *t*11- versus *t*10-18:1 and, after *c*9,*t*11-CLA, the second most abundant CLA isomer is frequently *t*11, *c*13-CLA for forage fed and *t*7, *c*9-CLA for concentrate fed beef cattle (Aldai, Dugan, Rolland, & Kramer, 2009). When feeding forage or grain based diets, the levels of atypical 18:2 isomers are low. Feeding a relatively high level of 18:3n-3 from flaxseed resulted in unusual accumulations of 18:3n-3 partial hydrogenation intermediates with a marked shift away from CLA towards atypical 18:2 isomers, and relatively large shifts toward *c*15-18:1 and *t*13/14-18:1accumulation. Feeding an increased level of vitamin E in the diet resulted in higher levels of 18:3n-3 when flaxseed was added to the diet, which suggests that vitamin E can provide some protection against 18:3n-3 hydrogenation. In addition, when either vitamin E or flaxseed was added to the control diet, they reduced the accumulation of *t*10-18:1. Although a reduction in *t*10-18:1 could be viewed as positive, since it is associated with increased cardiovascular disease risk in animal models (Mensink, Zock, Kester, & Katan, 2003), the increase in levels of levels of *t*9,*c*12- and *c*9,*t*12-18:2 could be viewed as a negative from a cardiovascular perspective (Baylin, Kabagambe, Ascherio, Spiegelman, & Campos, 2003; Lemaitre, King, Mozaffarian, Sootodehnia, & Siscovick, 2006). Furthermore, the health effects of many of the other PUFA partial hydrogenation intermediates identified have yet to be

investigated.

## IV. CONCLUSION

Besides the increase in total n-3 and PUFA, the inclusion of flaxseed and vitamin E in feedlot diets resulted in an intramuscular fatty acid profile different to those reported for forage or grain based diets, especially in the concentration of 18:3 biohydrogenation intermediates. While the decrease in *t*10-18:1 and the increase in *t*11-18:1 could be considered positive for human health, the health consequences of the large increases in other biohydrogenation intermediates, such as 18:2 atypical isomers identified here for the first time, remains unknown.


## ACKNOWLEDGEMENT

Dr. M. Juárez acknowledges the receipt of a NSERC fellowship funded through the AAFC ABIP-FOBI program. Dr. N. Aldai acknowledges the receipt of a research contract from the 7th European Community Program (Marie Curie International Outgoing Fellowship).


## REFERENCES


Aldai, N., Dugan, M. E. R., Rolland, D. C., & Kramer, J. K. G. (2009). Survey of the fatty acid composition of Canadian beef: Backfat and *longissimus lumborum* muscle. *Canadian Journal of Animal Science, 89*(3), 315-329.

Bartoň, L., Marounek, M., Kudrna, V., Bureš, D., & Zahrádková, R. (2007). Growth performance and fatty acid profiles of intramuscular and subcutaneous fat from Limousin and Charolais heifers fed extruded linseed. *Meat Science, 76*(3), 517-523.

Baylin, A., Kabagambe, E. K., Ascherio, A., Spiegelman, D., & Campos, H. (2003). High 18:2 *trans*-fatty acids in adipose tissue are associated with increased risk of nonfatal acute myocardial infarction in Costa Rican adults. *Journal of Nutrition, 133*(4), 1186-1191.

Castañeda-Gutiérrez, E., De Veth, M. J., Lock, A. L., Dwyer, D. A., Murphy, K. D., & Bauman, D. E. (2007). Effect of supplementation with calcium salts of fish oil on n-3 fatty acids in milk fat. *Journal of Dairy Science, 90*(9), 4149-4156.

CCAC. (1993). Canadian Council on Animal Care. In E. B. Olfert, B. M. Cross, & A. A. McWilliam (Eds.). *A guide to the care and use of experimental animals, . Vol. 1, (2nd ed.). Ottawa, ON: CCAC*.

CFIA. (2003). Canadian Food Inspection Agency. Guide to Food Labeling and Advertising. In).

Cruz-Hernandez, C., Deng, Z., Zhou, J., Hill, A. R., Yurawecz, M. P., Delmonte, P., Mossoba, M. M., Dugan, M. E. R., & Kramer, J. K. G. (2004). Methods for analysis of conjugated linoleic acids and *trans*-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. *Journal of AOAC International, 87*(2), 545-562.

Drouillard, J. S., Seyfert, M. A., Good, E. J., Loe, E. R., Depenbusch, B., & Daubert., R. (2004). Flaxseed for finishing beef cattle: Effects on animal performance, carcass quality, and meat composition. In *60th Flax Inst., Fargo, ND. Flax Inst., Dep. Plant Sci., Fargo, ND*, (pp. 108-117).

Haak, L., Raes, K., Van Dyck, S., & De Smet, S. (2008). Effect of dietary rosemary and α-tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. *Meat Science, 78*(3), 239-247.

Hughes, P. E., & Tove, S. B. (1980). Identification of deoxy-α-tocopherolquinol as another endogenous electron donor for biohydrogenation. *Journal of Biological Chemistry, 255*(24), 11802-11806.

Kay, J. K., Roche, J. R., Kolver, E. S., Thomson, N. A., & Baumgard, L. H. (2005). A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. *Journal of Dairy Research, 72*(3), 322-332.

Kramer, J. K. G., Hernandez, M., Cruz-Hernandez, C., Kraft, J., & Dugan, M. E. R. (2008). Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using ag-ion SPE fractionation. *Lipids, 43*(3), 259-273.

Lemaitre, R. N., King, I. B., Mozaffarian, D., Sootodehnia, N., & Siscovick, D. S. (2006). *Trans*-fatty acids and sudden cardiac death. *Atherosclerosis Supplements, 7*(2), 13-15.

Liu, Q., Lanari, M. C., & Schaefer, D. M. (1995). A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of animal science, 73*(10), 3131-3140.

Mach, N., Devant, M., Díaz, I., Font-Furnols, M., Oliver, M. A., García, J. A., & Bach, A. (2006). Increasing the amount of n-3 fatty acid in meat from young Holstein bulls through nutrition. *Journal of Animal Science, 84*(11), 3039-3048.

Mensink, R. P., Zock, P. L., Kester, A. D. M., & Katan, M. B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. *American Journal of Clinical Nutrition, 77*(5), 1146-1155.

Pottier, J., Focant, M., Debier, C., De Buysser, G., Goffe, C., Mignolet, E., Froidmont, E., & Larondelle, Y. (2006). Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. *Journal of Dairy Science, 89*(2), 685-692.

Raes, K., Haak, L., Balcaen, A., Claeys, E., Demeyer, D., & De Smet, S. (2004). Effect of linseed feeding at similar linoleic acid levels on the fatty acid composition of double-muscled Belgian Blue young bulls. *Meat Science, 66*(2), 307-315.

SAS. (2003). SAS® user's guide: Statistics. SAS for Windows, version 9.1. *SAS Institute, Inc., Cary, NC*.

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., & Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *British Journal of Nutrition, 85*(1), 115-124.