ABSTRACT
Thirty-six Holstein bulls (initial body weight, 345 ± 61 kg) were randomly assigned to six dietary treatments with a 2\(\times\)3 factorial arrangement, with two levels of AH (alfalfa hay) (10 and 20% of AH) combining with three levels of FO (fish oil) (0, 1 and 2.1% of DM) to investigate the effects of AH proportion and FO supplementation on performance, carcase characteristics, and meat fatty acids profile. DMI (dry matter intake) (kg/day) was lower \((p < .01)\) for high (8.0) than for low (8.7) AH. Highest level of FO reduced DMI \((p < .01)\) regardless of AH level. Dietary inclusion of FO increased the concentration of VA \((p < .01)\), CLA \((p < .01)\) and n-3 \((p < .01)\) fatty acids which subsequently reduced n-6: n-3 \((p < .01)\). The results indicate that AH can be replaced by corn (zea mays) silage to mitigate the detrimental effect of supplemented fat on dry matter intake. Moreover, FO can be supplemented to feedlot diet to enrich ruminant products without deleterious effects on carcass characteristics.

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Fish oil; forage type; performance; fatty acids profile

Introduction
Eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are the main PUFAs present in FO which have beneficial effects on cardiovascular disease, coronary heart disease and blood pressure (Kris-Etherton et al. 2002). Dohme et al. (2003) reported a lower lipolysis and biohydrogenation (BH) of FO predominant fatty acids (EPA and DHA) and subsequently enhanced duodenal flow of EPA and DHA compared to soybean (glycine max) oil, indicating the possibility of an incorporation in ruminant products. Moreover, the PUFA, specially DHA in FO not only escape from BH, but also inhibit the last step of other dietary PUFA ruminal BH (reduction of vaccenic acid; VA to c18:0) through the inhibition of bacteria and/or enzymes responsible for this conversion (AbuGhazaleh and Jenkins 2004) leading to enhanced flow of VA and c9t11 CLA (common linoleic and linolenic acid BH intermediates) to the small intestine. These BH intermediates have been reported to have inhibitory effects on growth of cancer cell lines and also suppressive effects on chemically induced tumour development in animal models (Parodi 2003). Furthermore, dietary inclusion of FO in sucrose-fed rats have been shown to reduce the hypertrophy of visceral fat stores and retroperitoneal adipose tissues (Peyron-Caso et al. 2002).

Reduced DMI is a common consequence of FO supplementation in ruminants diet due to its odour, low palatability and high content of long-chain fatty acids with high degree of unsaturation (Wistuba et al. 2006; Shingfield et al. 2012). Type of forage has been reported to modulate responses of dietary fat supplementation. Onetti et al. (2004) reported a decline in DMI, milk fat yield and milk fat percentage when tallow was added to corn silage (CS)-based diet, but this deleterious effect was eliminated when 50% of corn silage was replaced by equal alfalfa hay (AH). On the other hand, Kowsar et al. (2008) reported that replacing AH with corn silage increased intake of DM (dry matter), NEL (net energy of lactation) and NDF (neutral detergent fibre). Moreover, AH has been shown to form a stable rumen environment and subsequently more complete rumen BH of unsaturated fatty acids. Onetti et al. (2002) reported increased concentration of C18:0 and reduced trans-10 C18:1 and total trans fatty acids in milk fat with increased proportion of alfalfa silage relative to corn silage. Hence, the first objective of current study was to prevent the likely deleterious effect of FO supplementation on DMI and...
consequently performance of the bull through using different proportion of AH and CS (corn silage) under different proportion of AH and CS (corn silage) and secondly, the effects of FO supplementation on, meat fatty acids profile, carcass characteristics and body fat deposition in Holstein bulls under different proportion of AH and CS.

Materials and methods
Animal, housing and diets
This experiment was conducted at the Natural Resources and Agricultural Research Farm of the Tehran University (Karaj, Iran). This experiment was conducted according to the guidelines and procedures of Iranian Ministry of Agriculture (experimental permission 858). Thirty-six Holstein bulls with initial body weight (BW) of 345 ± 61 kg and age of 11–13 months were blocked by weight and randomly assigned to six treatments following a 3×2 factorial arrangement. Bulls were housed in individual tie-stall pens with the dimensions of 1.20×1.70 m. A plastic mat was used as bedding in the floor. The experiment began after 2 weeks of adaptation to the experimental diets. Dietary treatments were two proportions of AH (10 and 20%, DM basis) combined with three concentrations of FO (0, 1 and 2.1%). All diets were balanced to meet the nutrient requirement of National Research Council (1996) and were isocaloric and isonitrogenous. The diet consisted of 30% of forage and 70% concentrate mix (DM basis). FO was added to concentrate and was prepared every 10 days to prevent fatty acids oxidation. The bulls were offered a total mixed ration (TMR) ad libitum and fed twice daily at 8:00 h and 17:00 h with free access to water and salt. Feed offered and refusals were recorded daily before the morning feeding to estimate DMI. Bulls were weighted monthly before morning feed. Feed ingredients and diets were sampled every 2 weeks. Ingredients and chemical composition of diet are shown in Tables 1 and 2.

Slaughtering and data collection
At the end of period (90 days), bulls were weighted at two consecutive days and slaughtered to measure carcass traits. Hot carcass weight (HCW) was recorded immediately after slaughter and cold carcass weight (CCW) was recorded 24 h after slaughter. Dressing percentage was calculated using HCW weight. Visceral fat, prirenal fat, longissimus muscle area and subcutaneous fat thickness were measured. Longissimus dorsi muscle sample was taken from the 12–13 rib site and frozen at −20°C until subsequent analyses. The length between the anterior edge of the first rib and the anterior end of the pubic symphysis was measured as carcass length. Digital planimeter (Delta-T Devices, Cambridge, UK) was used to determine longissimus dorsi muscle area.

Meat quality
The grounded sub-sample of longissimus dorsi muscle was used for determination of pH (24 h after slaughter) using a calibrated pH metre (Metrohm MG, Herisau, Switzerland). For assessing the meat colour, the 2.5 cm thick bloomed (1 h at 14°C) samples of longissimus dorsi muscle were prepared (48 h after slaughter) to be read by a

### Table 1. Ingredient and chemical composition of dietary treatments.

| FO (%) | 10% alfalfa hay (AH) | 20% alfalfa hay (AH) |
|--------|---------------------|---------------------|
|        | 0       | 1       | 2.1     | 0       | 1       | 2.1     |
| Alfalfa hay | 10   | 10   | 10   | 20   | 20   | 20   |
| Corn silage  | 20   | 20   | 20   | 10   | 10   | 10   |
| Barley grain | 41   | 41   | 41   | 41   | 41   | 41   |
| Wheat grain | 2   | 1   | 0.5  | 2   | 1   | 0.5  |
| Soybean meal | 2   | 2   | 2   | 2   | 2   | 2   |
| Canola meal | 12   | 13   | 14   | 10   | 11   | 11   |
| Beet pulp  | 5   | 5   | 5   | 5   | 5   | 5   |
| Wheat bran | 5   | 4   | 2   | 4   | 5   | 5   |
| Zeolite  | 1   | 1   | 1   | 1   | 1   | 1   |
| Calcium carbonate | 0.5   | 0.55  | 0.68 | 0.4  | 0.5  | 0.62 |
| Sodium bicarbonate | 0.7   | 0.7  | 0.7  | 0.7  | 0.7  | 0.7  |
| Vitamin and mineral premix\* | 0.7   | 0.7  | 0.7  | 0.7  | 0.7  | 0.7  |
| Salt | 0.2   | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Fish oil | 0   | 1    | 2.1  | 0   | 1    | 2.1  |

### Table 2. Fatty acids composition of fish oil.

| Fatty acids | Fish oil (%) |
|-------------|--------------|
| C14:0       | 6.20         |
| C16:0       | 22.50        |
| C16:1       | 7.90         |
| C18:0       | 4.80         |
| C18:1       | 25.80        |
| C18:2       | 3.60         |
| C18:3       | 1.30         |
| C20:4       | 0.70         |
| C20:5       | 8.30         |
| C22:6       | 17.60        |
Hunter Lab colourimeter (Hunter Lab, D25, optical sensor, Model DP-9000; VA, USA) according to the L* (lightness), a* (redness) and b* (yellowness) system [Commission Internationale de l’éclairage (CIE) 1986]. Hue angle and chroma (the measure of colour vividness) were calculated as $\tan^{-1} (b*/a*)$ and $(a^{*2}+b^{*2})^{1/2}$, respectively (Hunter and Harold 1987). The shear force of longissimus dorsi muscle samples were assessed (72 h after slaughtering) after cooking in plastic bags at 75°C for 60 min (Hoffman et al. 2003) and then samples were left at room temperature to be cold. The thickness of the samples was 1 cm$^2$ with the length of 3 cm. The cooked meat samples were used along the fibre axis to be sheared transversely by a Warner–Bratzler device, mounted in a texture analyser (HSKS-Hounsfield, Red hill, Surrey, UK) testing machine. The average of three replicates from each bull, of the maximum force needed to shear the samples perpendicularly to the fibres’ direction was recorded.

**Chemical composition of meat and hepatic**

The grounded samples of longissimus dorsi muscle and hepatic were used to determine chemical composition including DM, fat, protein and ash according to the Association of Official Analytical Chemists (AOAC) methods (AOAC 1990). Cholesterol content of longissimus dorsi muscle samples were measured by method of Janssen and Meijer (1995). Briefly, one g of ground samples was placed in 15 mL tubes and 3 mL of Folch et al. (1957) solvent (containing methanol-to-chloroform ratio of 1:2, respectively) was added to the tubes. Tubes were vortexed, 1 mL of water was added to the tubes and tubes were centrifuged at 2500g for 10 min to form separated phases. The lower phase was transferred to weighted micro tubes and dried under $N_2$ at room temperature. Micro tubes were weighted again to calculate lipid residue (mg) and six times of it’s weight, the equal portion (by volume in microlitre) of Triton-X100 and chloroform was added to tubes and then chloroform was added to tubes to reach the final volume of 500 $\mu$L. The tubes were vortexed and 50 $\mu$L of it was transferred to another tubes and the solvent was evaporated under $N_2$ and the residue was used to assay cholesterol content via enzymatic kite (Pars Azmoon Co., Tehran, Iran) through an automatic spectrophotometer (Clima Plus, RAL, Madrid, Spain).

**TBARS value**

Ground samples of longissimus dorsi muscle were used to determine oxidative stability 2 months after slaughtering. The peroxides extent of samples was assessed through measuring TBARS according to the method of Esterbauer and Cheeseman (1990) and TBARS were expressed as milligrams of malonaldehyde (MDA)/g of wet tissue.

**Fatty acids analysis**

Fatty acids profiles of longissimus dorsi muscle were measured by extracting lipid fraction according to the method of Folch et al. (1957). Samples were evaporated under $N_2$ and fatty acids were methylated with methanolic HCl by the method described by Ichihara and Fukubayashi (2010). The FA methyl esters were fractionated over a CP-SIL88 column (100 m $\times$ 0.25 mm i.d., film thickness 0.20-$\mu$m fused silica; Varian, Palo Alto, CA) in a gas chromatograph (Agilent Technologies GC, model 7890A, Co., Palo Alto, CA) using flame ionisation detection. Helium was used as the carrier gas at a constant flow rate of 1.7 mL/min. The oven temperature was programmed as follows: 175°C, held for 4 min; 175–250°C at 3°C/min; and then maintained for 20 min. The temperature of the injector port and detector was 250°C. Samples (1 $\mu$L) were injected with an auto-sampler. Output signals of individual fatty acids were identified from the retention times of known calibration standard and pentadecanoic acid (Sigma, St. Louis, MO) was used as an internal standard (Sigma) for quantification of fatty acids.

**Statistical analyses**

Data were analysed by MIXED procedure of SAS (SAS Institute Inc., 2002; Cary, NC) for $3 \times 2$ factorial arrangement. The mixed model included the fixed effects of AH, FO and their interaction and the random effects of animal. Least-square means were computed and tested for differences by the Tukey’s test. The effect of increasing levels of FO in the diet was examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. The experimental unit was the individual animal. Initial BW was included into the model as a covariate. For colour data, pH was used as a covariate. Differences
et al. (2002) and Ruppert et al. (2003) were successful in increasing the alfalfa silage: corn silage ratio, Onetti (2004) through replacing corn silage by alfalfa hay. By corn silage-based diet supplemented by tallow (2004) eliminated the decline in DMI of dairy cow-fed bulls. However, Onetti et al. (2008) who reported increased DMI when AH was partially replaced with corn silage. The higher DMI can be attributed to higher moisture and palatability of diet containing AH. Dietary lipid (>8%) has been reported to affect DMI detrimentally due to negative effects of lipids especially PUFA on rumen microbial activity (Rule et al. 1997; Loor et al. 2005) emphasizing altered ruminal infusion of FO caused more reduction in DMI, Ruminal infusion of FO in cows (Doreau and Chilliard 1997; Loor et al. 2005) as an explanation of reduced DMI. Ruminal infusion of FO caused more reduction in DMI comparing to abomasal administration of FO in cows (Doreau and Chilliard 1997; Loor et al. 2005) emphasizing altered rumen metabolism to be involved. In the present study, increasing portion of AH in diet reduced DMI (p < .01) which is in agreement with Shingfield et al. (2006) and Wistuba et al. (2006); however, there was no deleterious effect on DMI when steers fed diet (concentrate to forage ratio of 40:60) containing 3% of FO (Scollan et al. 2001). Dietary lipid (>8%) has been reported to affect DMI detrimentally due to negative effects of lipids especially PUFA on rumen microbial activity (Rule et al. 1989), whereas Nicholson and Omer (1983) suggested post-ruminal effects of PUFA on reticulorumen motility as an explanation of reduced DMI. Ruminal infusion of FO caused more reduction in DMI comparing to abomasal administration of FO in cows (Doreau and Chilliard 1997; Loor et al. 2005) emphasizing altered rumen metabolism to be involved. In the present study, increasing portion of AH in diet reduced DMI (p < .01) which is in agreement with Kowsar et al. (2008) who reported increased DMI when AH was partially replaced with corn silage. The higher DMI can be attributed to higher moisture and palatability of diet containing corn silage. In current study, although water was added during TMR preparation exactly before offering to the bulls in order to minimise the difference in dietary moisture, it was absorbed to the surface of particle and was not effective at maintaining the uniformity of diet. On the other hand, Onetti et al. (2004) eliminated the decline in DMI of dairy cow-fed corn silage-based diet supplemented by tallow through replacing corn silage by alfalfa hay. By increasing the alfalfa silage: corn silage ratio, Onetti et al. (2002) and Ruppert et al. (2003) were successful to alleviate the detrimental effect of tallow supplementation on DMI. These differences in response seems to originate from the shape and quality of the alfalfa they used in their study. The predominant shape of alfalfa usage in Iran is in hay form which caused considerable lose of leaf during dehydration and baling. The alfalfa hay with low leaf proportion wouldn’t be palatable for bulls to stimulate DMI. Average daily gain (ADG) and feed conversion ratio (F:G) were not significantly affected by interaction of AH and FO supplementation (Table 3). Increasing the proportion of AH to replace the corn silage in finishing diet decreased DMI without adversely affecting final BW and ADG, consequently numerically improved (p < .11) F:G. The result suggests that increasing ratio of AH to corn silage may have improved rumen function and feed digestibility. The decreased DMI without affecting the growth performance and F:G is in agreement with Nicholson et al. (1992) and Wistuba et al. (2006). Nicholson et al. (1992) reported that supplementation of fish meal decreased DMI, but did not affect F:G. The present result indicates that increasing FO supplementation in a high-concentrate diet has limited effect on improving growth rate and feed efficiency.

Table 3. Performance of Holstein bulls fed different dietary fish oil level and alfalfa hay ratio.

| % FO | 10% AH | 20% AH | p* < .05 |
|------|--------|--------|----------|
| Initial BW | 340 | 338 | 384 | 342 | 341 | 338 | 17.3 | 0.35 | 0.41 | 0.30 | 0.93 | 0.44 |
| Final BW | 448 | 447 | 452 | 447 | 447 | 436 | 5.2 | 0.19 | 0.70 | 0.26 | 0.89 | 0.74 |
| ADG, kg/day | 1.14 | 1.15 | 1.17 | 1.15 | 1.14 | 1.09 | 0.073 | 0.83 | 0.97 | 0.73 | 0.97 | 0.87 |
| DMI, kg/day | 9.15 | 8.70 | 8.29 | 8.16 | 8.34 | 7.51 | 0.256 | 0.01 | 0.01 | 0.45 | 0.61 | 0.26 |
| F/G, kg/kg | 8.71 | 8.34 | 7.34 | 7.63 | 7.60 | 7.07 | 0.480 | 0.11 | 0.18 | 0.76 | 0.69 | 0.53 |

*AH: portion of alfalfa hay; FO: effect of dietary fish oil; AH × FO: alfalfa hay and fish oil interaction.

Results and discussion

Performance

Dry matter intake was not affected by interaction of AH and FO (Table 3). Inclusion of FO at 2.1% reduced DMI (p < .01) regardless of AH level, which is in agreement with Shingfield et al. (2006) and Wistuba et al. (2006); however, there was no deleterious effect on DMI when steers fed diet (concentrate to forage ratio of 40:60) containing 3% of FO (Scollan et al. 2001). Dietary lipid (>8%) has been reported to affect DMI detrimentally due to negative effects of lipids especially PUFA on rumen microbial activity (Rule et al. 1989), whereas Nicholson and Omer (1983) suggested post-ruminal effects of PUFA on reticulorumen motility as an explanation of reduced DMI. Ruminal infusion of FO caused more reduction in DMI comparing to abomasal administration of FO in cows (Doreau and Chilliard 1997; Loor et al. 2005) emphasizing altered rumen metabolism to be involved. In the present study, increasing portion of AH in diet reduced DMI (p < .01) which is in agreement with Kowsar et al. (2008) who reported increased DMI when AH was partially replaced with corn silage. The higher DMI can be attributed to higher moisture and palatability of diet containing corn silage. In current study, although water was added during TMR preparation exactly before offering to the bulls in order to minimise the difference in dietary moisture, it was absorbed to the surface of particle and was not effective at maintaining the uniformity of diet. On the other hand, Onetti et al. (2004) eliminated the decline in DMI of dairy cow-fed corn silage-based diet supplemented by tallow through replacing corn silage by alfalfa hay. By increasing the alfalfa silage: corn silage ratio, Onetti et al. (2002) and Ruppert et al. (2003) were successful to alleviate the detrimental effect of tallow supplementation on DMI. These differences in response seems to originate from the shape and quality of the alfalfa they used in their study. The predominant shape of alfalfa usage in Iran is in hay form which caused considerable lose of leaf during dehydration and baling. The alfalfa hay with low leaf proportion wouldn’t be palatable for bulls to stimulate DMI. Average daily gain (ADG) and feed conversion ratio (F:G) were not significantly affected by interaction of AH and FO supplementation (Table 3). Increasing the proportion of AH to replace the corn silage in finishing diet decreased DMI without adversely affecting final BW and ADG, consequently numerically improved (p < .11) F:G. The result suggests that increasing ratio of AH to corn silage may have improved rumen function and feed digestibility. The decreased DMI without affecting the growth performance and F:G is in agreement with Nicholson et al. (1992) and Wistuba et al. (2006). Nicholson et al. (1992) reported that supplementation of fish meal decreased DMI, but did not affect F:G. The present result indicates that increasing FO supplementation in a high-concentrate diet has limited effect on improving growth rate and feed efficiency.

Carcass characteristics

There were no interaction between AH and FO on carcase weight and carcase characteristics except CCW and prirenal fat; however, higher CCW in highest FO level and AH proportion was no statistically significant. Prirenal fat increased linearly (p < .05) with increasing FO at low AH, but quadratically decreased (p < .05) with increasing FO at high AH (Table 4). Increasing substitution of AH for corn silage overall did not affect the carcase traits except for LM area which was greater (p < .05) in high AH than with low AH diets. However, increasing FO supplementation quadratically increased HCW (p < .06), CCW (p < .03), carcase percentage (p < .06) and linearly increased carcase length (p < .09). Moreover, FO addition reduced subcutaneous fat thickness in a linear manner (p < .02). Supplementation of FO has been reported to increase insulin sensitivity (Cartiff 2010) and the affinity to insulin is more in visceral adipose tissue depots comparing to subcutaneous adipose tissue and among visceral...
adipose tissue depots, affinity to insulin is not equal (McGrattan et al. 2000). Higher pirenal fat content as a consequence of FO supplementation in low AH level can be related to higher extent of BH of EPA and DHA in high AH level and subsequently lower passage of EPA and DHA from the rumen as AH is not a fermented product and can stimulate rumen fermentation and BH; however, concentration of EPA and DHA in meat was not affected by interaction of AH and FO in current study. Formation of some BH intermediates as a consequence of FO supplementation and low or high AH level can be other explanation leading to accretion of fat in perirenal adipose tissue. The effects of FO supplementation on carcase traits in literature are not consistent. Wistuba et al. (2006) reported that inclusion of FO (3% of dietary DM) in crossbred steers diet reduced HCW, whereas it did not affect carcase percentage, longissimus muscle area and subcutaneous fat thickness. In the study of Scollan et al. (2001), there were no significant changes in CCW as a consequence of FO and Linseed supplementation separately and together in steers diet. The numerical reduction in visceral fat as a consequence of FO supplementation together with lowered subcutaneous fat thickness in treatment with highest level of FO in current study can lead to higher carcase weight and percentage in treatment with higher level of FO.

**Meat quality**

There was no interaction of dietary inclusion of AH and FO on chemical composition of meat and hepatic components including DM, protein, fat and ash (Table 5). The chemical composition of LM and hepatic were not affected by AH level and FO supplementation. These results are in agreement with those of Wistuba et al. (2006) and Najafi et al. (2012) who did not report any significant changes in chemical composition of muscle as a consequence of FO supplementation in steers and goat kids diet, respectively.

Shear force of longissimus dorsi muscle was not affected by AH level, FO supplementation and their interaction. Interaction effect of AH level and FO supplementation on TBARs value was significant ($p < .04$); the linear increase of TBARs value was greater at low AH than at high AH with increasing FO level ($p < .04$). Increasing ratio of AH to CS in diets overall did not affect meat quality of longissimus dorsi muscle except a trend ($p < .10$) of reduction in pH and TBARs value. Increasing FO supplementation increased meat

### Table 4. Carcass characteristics of Holstein bulls fed different dietary fish oil level and alfalfa hay ratio.

| % FO | 10% AH | 20% AH | p$^2$ < .05 | SEM |
|------|--------|--------|-------------|-----|
|      | 0 | 1 | 2.1 | 0 | 1 | 2.1 |      |
| HCW ($\text{kg}$) | 234.1 | 227.5 | 239.2 | 222.0 | 228.3 | 252.8 | 5.63 | 0.87 | 0.01 | 0.18 | 0.98 | 0.06 |
| CCW ($\text{kg}$) | 220.3b | 217.9p | 225.7b | 213.7b | 215.8b | 243.1a | 4.540 | 0.45 | 0.004 | 0.07 | 0.97 | 0.03 |
| C per (%) | 50 | 48 | 51 | 47 | 48 | 54 | 1 | 0.82 | 0.01 | 0.21 | 0.84 | 0.06 |
| Carcass length (cm) | 145.20 | 149.12 | 148.42 | 137.28 | 145.28 | 146.20 | 3.210 | 0.11 | 0.18 | 0.73 | 0.09 | 0.27 |
| LM area ($\text{cm}^2$) | 88.76 | 87.38 | 80.60 | 106.82 | 93.09 | 96.65 | 7.347 | 0.05 | 0.50 | 0.63 | 0.32 | 0.61 |
| Pirenal fat (kg) | 5.70b | 7.94b | 9.34b | 6.86b | 7.29b | 4.68b | 1.011 | 0.13 | 0.43 | 0.06 | 0.21 | 0.25 |
| Visceral fat (kg) | 11.72 | 9.09 | 7.19 | 8.85 | 7.85 | 9.56 | 1.433 | 0.67 | 0.51 | 0.37 | 0.29 | 0.52 |
| Fat thickness (mm) | 4.50 | 3.32 | 3.00 | 3.90 | 3.58 | 3.28 | 0.272 | 0.92 | 0.01 | 0.29 | 0.02 | 0.32 |

| AH: portion of alfalfa hay; FO: effect of dietary fish oil; AH × FO: alfalfa hay and fish oil interaction. |
| AH: portion of alfalfa hay; FO: effect of dietary fish oil; AH × FO: alfalfa hay and fish oil interaction. |

### Table 5. Chemical composition of longissimus dorsi muscle and hepatic of Holstein bulls fed different dietary fish oil level and alfalfa hay ratio.

| % FO | 10% AH | 20% AH | p$^2$ < .05 | SEM |
|------|--------|--------|-------------|-----|
|      | 0 | 1 | 2.1 | 0 | 1 | 2.1 |      |
| LM | Dry matter | 26.16 | 25.66 | 26.00 | 25.66 | 27.33 | 27.66 | 1.231 | 0.55 | 0.64 | 0.39 | 0.56 |
| | Protein | 21.81 | 21.35 | 21.66 | 21.23 | 21.96 | 22.18 | 0.544 | 0.68 | 0.76 | 0.49 | 0.80 | 0.89 |
| | Fat | 3.00 | 3.33 | 3.03 | 2.60 | 2.93 | 2.93 | 0.310 | 0.31 | 0.26 | 0.86 | 0.31 | 0.39 |
| | Ash | 0.99 | 1.03 | 1.24 | 1.08 | 1.24 | 1.03 | 0.133 | 0.77 | 0.72 | 0.32 | 0.48 | 0.67 |
| Hepatic | Dry matter | 27.63 | 27.86 | 27.90 | 27.86 | 27.66 | 27.86 | 0.648 | 0.53 | 0.61 | 0.80 | 0.46 | 0.34 |
| | Fat | 3.80 | 3.26 | 3.00 | 3.63 | 3.76 | 3.50 | 0.678 | 0.62 | 0.79 | 0.85 | 0.77 | 0.95 |
|  | Ash | 1.19 | 1.12 | 1.31 | 1.35 | 1.08 | 1.08 | 0.173 | 0.77 | 0.61 | 0.53 | 0.33 | 0.38 |

| AH: portion of alfalfa hay; FO: effect of dietary fish oil; AH × FO: alfalfa hay and fish oil interaction. |
lightness ($p < .05$), TBARS value ($p < .01$) and cholestrol ($p < .01$), but did not affect other variables of meat quality. These results are in agreement with Wistuba et al. (2006) who reported no difference in these traits when the diet fed to steers was supplemented by FO or the diet fed to goat kids was supplemented with FO and soybean oil (Najafi et al. 2012). It has been mentioned by Priolo et al. (2001) that factors such as carcase fatness, pH and fat content are the main factors influencing meat colour. Anti-oxidant status of meat at slaughter time also has been shown to play a crucial role in meat colour (Ponnampalam et al. 2012). These factors were not affected by treatments in this study, hence, no differences in meat colour can be explained.

The increased MDA value of meat (as a secondary product of oxidation) after 2 months of storage (µg of MDA per g of meat) with highest level of FO in diet is in agreement with Saleh et al. (2010) who reported higher lipid oxidation (MDA concentration) in breast and thigh meat of broilers chicken-fed FO supplement compared to control diet. Haak et al. (2008) reported no difference in pork TBARS value when FO was supplemented to the diet of pigs. This difference can be attributed to length of storage as it was assayed at Day 8 of the storage. Omega-3 sources such as FO are highly unsaturated and elongated which make the meat susceptible to peroxidation in the cases of excessive supplementation as well as insufficient anti-oxidant (Ryu et al. 2005).

The substantially increased concentration of meat cholesterol with 2.1% FO regardless of AH proportion is noticeable. This enhancement in meat cholesterol may have resulted from higher plasma cholesterol content with the high FO supplementation (24.98, 34.80 and 53.04 mg/dl of plasma cholesterol concentration, respectively for 0, 1 and 2.1% FO, $p < .01$).

### Fatty acids profile

Fatty acid composition of longissimus dorsi muscle generally was not interacted by AH and FO levels (Table 7). Increasing AH substitution for CS did not alter the profiles of individual fatty acids, whereas it tended ($p < .06$) to increase the proportion of PUFA and P/S, and increased n-6 fatty acids level ($p < .05$) which can be related to increased concentration of linoleic acid. In the study of Duckett et al. (2013), steers finishing on alfalfa pasture showed higher concentration of linoleic and linolenic acids compared to those finishing on pearl millet and mixed pasture; however, the concentration of PUFA and n-6 to n-3 ratio were not different among forage species. Higher linoleic acid in muscle of bulls consuming diet with higher AH proportion can be related to higher wheat grain and wheat bran proportion in treatments with high AH level and also low grain content of CS used in current study. Addition of FO increased the concentration of C16:0 and C24:0 and decreased the concentration of C18:0 regardless of AH level. Reduction of C18:0 content as a consequence of FO supplementation is in agreement with (Ferreira et al. 2014) who reported reduced C18:0 concentration in response to FO inclusion in lambs diet. There was no difference for oleic acid among dietary treatments. Scollan et al. (2001) and Wistuba et al. (2006) reported an increase in the concentration of C14:0, C16:0, C16:1 and oleic acid and a decrease in stearic acid content by addition of FO to steers diet. It has been reported that supplementation of FO in dairy cows diet inhibit complete rumen BH of unsaturated 18-carbon fatty acids to stearic acid which leads to accumulation of trans fatty acids in rumen (Shingfield et al. 2012). Lough et al. (1992) reported that palmitic acid is not desired due to its hyperlipidemic effect and its contribution to

| Table 6. Meat quality parameters of longissimus dorsi muscle of Holstein bulls fed different dietary fish oil level and alfalfa hay ratio. |
|-----------|-----------|-----------|-----------|-----------|
| % FO      | 10% AH    | 20% AH    | $p^2 < .05$ |
| pH        | 0   | 1   | 2.1  | 0   | 1   | 2.1  | SEM  |
| Shear force| 5.89 | 5.49 | 5.55 | 5.37 | 5.48 | 5.56 | 0.110 |
| Colour$^b$| 32.77 | 39.78 | 44.50 | 30.06 | 35.79 | 41.00 | 3.576 |
| L$^a$     | 12.51 | 12.54 | 13.16 | 12.97 | 13.15 | 11.73 | 0.842 |
| a$^a$     | 3.16  | 5.13  | 3.69  | 4.83  | 4.06  | 4.99  | 1.071 |
| b$^a$     | 14.16 | 22.30 | 15.60 | 20.44 | 17.20 | 22.19 | 4.299 |
| Hue angle | 12.90 | 13.56 | 13.68 | 13.64 | 13.76 | 12.94 | 0.960 |
| Chroma value | 0.14$^a$ | 0.35$^a$ | 1.36$^a$ | 0.13$^b$ | 0.40$^b$ | 0.68$^b$ | 0.141 |
| TBARS value$^c$ | 25.26 | 31.86 | 58.30 | 24.71 | 37.74 | 47.77 | 6.275 |
| Cholesterol (mg/dl) | 0.74 | 0.01 | 0.44 | 0.14 | 0.45 |

$^a$AH: portion of alfalfa hay; FO: effect of dietary fish oil; AH × FO: alfalfa hay and fish oil interaction.

$^b$L: lightness; a: redness; b: yellowness.

$^c$Thio-barbituric acid reactive substances (µg of MDA/g of meat).
enhancement in cholesterol content of meat, whereas, C18:0 and C18:1 benefit meat industry due to their hypolipidemic effects on human health (Rule et al. 1994). Concentration of linoleic acid was not different among dietary treatments, whereas linolenic acids content was increased by FO supplementation which is in agreement with those of Ferreira et al. (2014) and Wistuba et al. (2006). The amount of C20:1, C20:2 and C20:4 increased as dietary FO supplementation increased. The response of intermediate fatty acids with the chain length of 20:0 and 22:0 to FO supplementation is far variable (Ashes et al. 1992; Wonsil et al. 1994; Wistuba et al. 2006). Ruminal BH of EPA and DHA has been reported to be associated with appearance of many 20- and 22-carbon intermediates in the rumen (Shingfield et al. 2012) which can be incorporated in muscle. Higher meat concentration of EPA and DHA in bulls consuming FO-supplemented diet which is in agreement with Scollan et al. (2001), Wistuba et al. (2006) and Ferreira et al. (2014) can be related to lower rate of BH of EPA and DHA comparing to other unsaturated fatty acids (Dohme et al. 2003). The enhancement in linoleic acid, EPA and DHA resulted an increased n-3 fatty acid proportion and consequently a decreased n-6: n-3 in beef which have shown to have suppressive effects on cardiovascular diseases, cancer, and inflammatory and autoimmune diseases (Simopoulos 2002).

Table 7. Fatty acids profile (g/100 g fatty acid) of longissimus dorsi muscle of Holstein bulls fed different dietary fish oil level and alfalfa hay ratio.

| % FO | 10% AH | 20% AH | p<.05 |
|------|--------|--------|-------|
|      | 0      | 1      | 2.1   | 0     | 1      | 2.1   |
| C10:0| 0.96   | 0.80   | 0.65  | 1.41  | 1.06   | 0.56  |
| C12:0| 0.50   | 0.28   | 0.37  | 0.18  | 0.35   | 0.33  |
| C14:0| 3.49   | 4.06   | 3.44  | 3.83  | 3.21   | 3.48  |
| C14:1| 0.73   | 0.59   | 0.54  | 0.81  | 0.71   | 0.57  |
| C16:0| 24.67  | 27.46  | 27.59 | 25.11 | 26.16  | 27.55 |
| C16:1| 3.67   | 3.31   | 3.20  | 3.74  | 3.97   | 3.37  |
| C17:0| 1.33   | 1.16   | 1.16  | 1.58  | 1.29   | 1.08  |
| C18:0| 17.46  | 16.84  | 16.28 | 17.93 | 15.98  | 16.50 |
| C18:1cis-9| 39.42  | 37.12  | 37.20 | 36.83 | 37.95  | 37.12 |
| C18:1trans-11| 1.32  | 1.63   | 2.14  | 1.25  | 1.62   | 2.00  |
| C18:2| 0.15   | 0.23   | 0.28  | 0.17  | 0.22   | 0.27  |
| C20:0| 0.23   | 0.25   | 0.40  | 0.22  | 0.23   | 0.35  |
| C20:1| 0.23   | 0.20   | 0.21  | 0.20  | 0.17   | 0.16  |
| C20:2| 0.23   | 0.31   | 0.37  | 0.22  | 0.29   | 0.38  |
| C20:3| 0.05   | 0.11   | 0.26  | 0.06  | 0.12   | 0.20  |
| C20:5| 0.01   | 0.12   | 0.27  | 0.02  | 0.13   | 0.21  |
| C22:0| 0.13   | 0.11   | 0.09  | 0.18  | 0.14   | 0.09  |
| C22:1| 0.52   | 0.62   | 0.42  | 0.71  | 0.37   | 0.41  |
| C22:6| 0.11b  | 1.26a  | 1.18ab | 1.19ab | 1.09b  | 1.18ab |
| C24:0| 1.75ab | 1.86a  | 1.74b  | 1.84a  | 1.67b  | 1.75ab |

*AH: portion of alfalfa hay; FO: effect of dietary fish oil; AH Æ FO: alfalfa hay and fish oil interaction.  
bSaturated fatty acids.  
cMonounsaturated fatty acids.  
dPolyunsaturated fatty acids.  
eUnsaturated fatty acids.  
fPolyunsaturated to saturated fatty acids ratio.  
gn-6 fatty acids.  
hn-3 fatty acids.  
i-6 to n-3 fatty acids ratio.  
jIndex of atherogenicity: \(\frac{4\ C14:0 + 16\ C16:0 + 18\ C18:0}{MUFA + n-6\ PUFA + n-3\ PUFA}\).  
kIndex of thrombogenicity: \(\frac{C14:0 + C16:0 + C18:0}{0.5\ MUFA + 0.5\ n-6\ PUFA + 3\ n-3\ PUFA + (n-3\ PUFA/n-6\ PUFA)}\).
the rumen as an intermediate in the BH of linoleic acid or in tissues by Δ9-desaturase from VA (trans-11 C18:1), another intermediate in the ruminal BH of oleic, linoleic, and linolenic acids (Grinarii and Bauman 1999; AbuGhazaleh et al. 2005). Offer et al. (1999) and Whitlock et al. (2002) reported that at similar levels, FO was more effective than other polyunsaturated plant oils to increase the accumulation of VA in milk fat, even though FO contains lower proportion of the primary precursors of VA (linoleic and linolenic acid). Fatty acids present in FO specially DHA promote incomplete BH and subsequently VA accumulation. It was hypothesised that DHA may inhibit the bacteria and/or enzymes responsible for the last step of BH which converts VA to C18:0 (AbuGhazaleh and Jenkins 2004). In the present study, the meat concentration of CLA and VA were not affected by the source and ratio of forage comparing to the study of Onetti et al. (2002) which can be due to lower portion of dietary forage as well as lower DMI in Holstein bulls compared to dairy cows. Indexes of atherogenicity and thrombogenicity were affected by interaction of AH proportion and FO levels ($p<.05$). In treatments consuming low proportion of AH, second dietary level of FO increased both atherogenicity and thrombogenicity indexes, whereas in high AH proportion, second dietary level of FO reduced atherogenicity and thrombogenicity indexes. Hence, products with lower value of these index would be of interest. Higher atherogenicity and thrombogenicity indexes in meat of bulls consuming low AH diet containing second level of FO can be the consequence of higher C14:0 and lower MUFA concentration in these treatments, whereas lowered atherogenicity and thrombogenicity indexes in treatments receiving diets with higher proportion of AH supplemented with 1% of FO can be related to lower C14:0 and C16:0 and higher MUFA and PUFA content. Atherogenicity index shows the relationship between the main saturated fatty acids and the main unsaturated fatty acids present in a product, the former being considered pro-atherogenic and the latter anti-atherogenic. Thrombogenicity index shows the tendency to form clots in the blood vessels which is defined by the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFAs, n-6 PUFAs and n-3 PUFAs) (Ulbricht and Southgate 1991). However, these indexes do not properly reflect the health beneficial effect of the meat as dietary inclusion of FO significantly increased vaccenic acid and cis-9, trans-11 CLA concentration in current study which their health beneficial effects are far more comparing to other MUFA and PUFA, respectively but it doesn’t reflected in these indexes.

## Conclusions

Increasing substitution of AH for CS from 10 to 20% and increasing FO supplementation up to 2.1% in finishing diets decreased DMI but did not affect growth performance and feed efficiency. However, carcase traits, meat quality and fatty acid composition were affected with FO with limited effects of AH. The results of this study demonstrate that FO can be used in feedlot diets as a modifier of fat deposition to restrict body fat accumulation. Moreover, FO can be used as a valuable fat supplement to enrich ruminant products from healthy fatty acids as a result of its incomplete rumen BH and its stimulatory effect on CLA and VA accumulation. Furthermore, AH can be replaced by CS to prevent the deleterious effect of fat supplement on feed intake.

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## References

AbuGhazaleh A, Jenkins T. 2004. Short communication: docosahexaenoic acid promotes vaccenic acid accumulation in mixed ruminal cultures when incubated with linoleic acid. J Dairy Sci. 87:1047–1050.

AbuGhazaleh A, Riley M, Thies E, Jenkins T. 2005. Dilution rate and pH effects on the conversion of oleic acid to trans C18:1 positional isomers in continuous culture. J Dairy Sci. 88:4334–4341.

Association of Official Analytical Chemists. 1990. Official methods of analysis. Arlington, VA: W.H. Freeman and Company.

Ashes JR, Siebert BD, Gulati SK, Cuthbertson AZ, Scott TW. 1992. Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. Lipids. 27:629–631.

Cartiff S. 2010. Eicosapentanoic and docosahexanoic acids (EPA; DHA), increase insulin sensitivity in growing steers [master’s thesis]. Raleigh, North Carolina: North Carolina State Univeristy.

CIE. 1986. CIE Publication No. 15.2. Technical report: colorimetry. Commission Internationale de l’éclairage, Vienna, 19–20.

National Research Council. 1996. Nutrient requirements of beef cattle. Washington, DC: National Academy Press.
Dohme F, Fievez V, Raes K, Demeyer DI. 2003. Increasing levels of two different fish oils lower ruminal biohydrogenation of eicosapentaenoic and docosahexaenoic acid in vitro. Animal Res. 52:309–320.

Doreau M, Chilliard Y. 1997. Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows. Reprod Nutr Dev. 37:113–124.

Duckett S, Neel J, Lewis RM, Fontenot J, Clapham W. 2013. Effects of forage species or concentrate finishing on animal performance, carcass and meat quality. J Anim Sci. 91:1454–1467.

Esterbauer H, Cheeseman KH. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 186:407–421.

Ferreira E, Pires A, Susin I, Gentil R, Parente M, Nolli C, Meneghini R, Mendes C, Ribeiro C. 2014. Growth, feed intake, carcass characteristics, and meat fatty acid profile of lambs fed soybean oil partially replaced by fish oil blend. Anim Feed Sci Technol. 187:9–18.

Folch J, Lees M, Sloane SG. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 262:497–509.

Grinarii J, Bauman DE. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Advances in conjugated linoleic acid research. Vol. 1. In: Yurawecz MP, Mossoba MM, Kramer JK, Pariza MW, Nelson GJ, editors. Champaign, IL: AOCS Press; p. 180–200.

Haak L, De Smet S, Fremaut D, Van Wellegehm K, Raes K. 2008. Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. J Animal Sci. 86:1418–1425.

Hoffman L, Muller M, Cloete S, Schmidt D. 2003. Comparison of six crossbred lamb types: sensory, physical and nutritional meat quality characteristics. Meat Science. 65:1265–1274.

Hunter R, Harold R. 1987. Uniform color scales. The measurement of appearance, 2nd ed. VA: Hunter Association Laboratory; p. 135–148.

Ichihara K, Fukubayashi Y. 2010. Preparation of fatty acid methyl esters for gas-liquid chromatography. J Lipid Res. 51:635–640.

Janssen GB, Meijer GW. 1995. Enzymatic determination of lipids in liver extracts. Clin Biochem. 28:312.

Kowser R, Ghorbani G, Alikhani M, Khvorvash M, Nikkhah A. 2008. Corn silage partially replacing short alfalfa hay to optimize forage use in total mixed rations for lactating cows. J Dairy Sci. 91:4755–4764.

Kris-Etherton PM, Harris WS, Appel LJ. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 106:2747–2757.

Loor J, Doreau M, Chardigny J, Ollier A, Sebedio J, Chilliard Y. 2005. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of trans-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. Anim Feed Sci Technol. 119:227–246.

Lough D, Solomon M, Rumsby T, Elsasser T, Slyter L, Kahl S, Lynch G. 1992. Effects of dietary canola seed and soy lecithin in high-forage diets on cholesterol content and fatty acid composition of carcass tissues of growing ram lambs. J Animal Sci. 70:1153–1158.

McGrattan P, Wylie A, Nelson J. 2000. Tissue-specific differences in insulin binding affinity and insulin receptor concentrations in skeletal muscles, adipose tissue depots and liver of cattle and sheep. Animal Sci. 71:501–508.

Najafi M, Zeinolaeldini S, Ganjkhaniou M, Mohammadi H, Hopkins D, Ponnampalam E. 2012. Performance, carcass traits, muscle fatty acid composition and meat sensory properties of male Mahabadi goat kids fed palm oil, soybean oil or fish oil. Meat Sci. 92:848–854.

Nicholson J, Charmley E, Bush R. 1992. The effect of supplemental protein source on ammonia levels in rumen fluid and blood and intake of alfalfa silage by beef cattle. Canadian J Animal Sci. 72:853–862.

Nicholson T, Omer SA. 1983. The inhibitory effect of intestinal infusions of unsaturated long-chain fatty acids on for- estomach motility of sheep. Br J Nutr. 50:141–149.

Offer N, Marsden M, Dixon J, Speake B, Thacker F. 1999. Effect of dietary fat supplements on levels of n-3 polyunsaturated fatty acids, trans acids and conjugated linoleic acid in bovine milk. Animal Sci. 69:613–625.

Onetti S, Reynal S, Grummer R. 2004. Effect of alfalfa forage preservation method and particle length on performance of dairy cows fed corn silage-based diets and tallow. J Dairy Sci. 87:652–664.

Onetti S, Shaver R, McGuire M, Palmquist D, Grummer R. 2002. Effect of supplemental tallow on performance of dairy cows fed diets with different corn silage-alfalfa silage ratios. J Dairy Sci. 85:632–641.

Parodi PW. 2003. Conjugated linoleic acid in food. Advances in conjugated linoleic acid Research. Vol. 2. Sebedio J-L, Christie WW, Adlof RO, editors. Champaign, IL: AOCS Press; p. 101–122.

Peyron-Caso E, Fluteau-Nadler S, Kabir M, Guerre-Millo M, Quignard-Boulange A, Slama G, Rizkalla S. 2002. Regulation of glucose transport and transporter 4 (GLUT-4) in muscle and adipocytes of sucrose-fed rats: effects of N-3 poly- and monounsaturated fatty acids. Horm Metab Res. 34:360–366.

Ponnampalam EN, Butler KL, McDonagh MB, Jacobs JL, Hopkins DL. 2012. Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and functionality (retail colour) of meat in lambs. Meat Sci. 90:297–303.

Priolo A, Micol D, Agabriel J. 2001. Effects of grass feeding systems on ruminant meat colour and flavour. A review. Animal Res. 50:185–200.

Rule D, Busboom J, Kercher C. 1994. Effect of dietary canola on fatty acid composition of bovine adipose tissue, muscle, kidney, and liver. J Animal Sci. 72:2735–2744.

Rule D, Wen-Hsin W, Busboom J, Hinds F, Kercher C. 1989. Dietary canola seeds alter the fatty acid composition of bovine subcutaneous adipose tissue. Nutr Rep Int. 39:781–786.

Ruppert L, Drackley J, Bremmer D, Clark J. 2003. Effects of tallow in diets based on corn silage or alfalfa silage on digestion and nutrient use by lactating dairy cows. J Dairy Sci. 86:593–609.

Ryu Y, Rhee M, Lee K, Kim B. 2005. Effects of different levels of dietary supplemental selenium on performance, lipid oxidation, and color stability of broiler chicks. Poultry Sci. 84:809–815.
SAS. 2002. PROC user’s manual, version 9.1. NC: SAS Institute Cary.
Saleh H, Rahimi S, Torshizi MK, Golian A. 2010. Effect of dietary fish oil on oxidative stability and lipid composition of broiler chickens breast and thigh meat. J Animal Vet Adv. 9:2877–2882.
Scollan ND, Choi NJ, Kurt E, Fisher AV, Enser M, Wood JD. 2001. Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. Br J Nutr. 85:115–124.
Shingfield KJ, Kairenius P, Årolä A, Paillard D, Muetzel S, Ahvenjärvi S, Vanhatalo A, Huhtanen P, Toivonen V, Griinari JM, et al. 2012. Dietary fish oil supplements modify ruminal biohydrogenation, alter the flow of fatty acids at the omasum, and induce changes in the ruminal Butyrivibrio population in lactating cows. J Nutr. 142:1437–1448.
Shingfield KJ, Reynolds CK, Hervás G, Griinari JM, Grandison AS, Beever DE. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J Dairy Sci. 89:714–732.
Simopoulos AP. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 56:365–379.
Ulbricht T, Southgate D. 1991. Coronary heart disease: seven dietary factors. Lancet. 338:985–992.
Whitlock L, Schingoethe D, Hippen A, Kalscheur K, Baer R, Ramaswamy N, Kasperon K. 2002. Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. J Dairy Sci. 85:234–243.
Wistuba T, Kegley E, Apple J. 2006. Influence of fish oil in finishing diets on growth performance, carcass characteristics, and sensory evaluation of cattle. J Animal Sci. 84:902–909.
Wonsil BJ, Herbein JH, Watkins BA. 1994. Dietary and ruminally derived trans-18:1 fatty acids alter bovine milk lipids. J Nutr. 124:556.