EFFECTS OF TIME-ON-FEED AND COOKING ON THE NUTRIENT COMPOSITION OF BEEF LONGISSIMUS MUSCLES

By

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NOMENCLATURE

AOAC	Association of Official Analytical Chemists
^{o}C	degrees Celcius
cm	centimeters
d	days
FAME	fatty acid methyl esters
g	grams
hr	hours
m	meters
mm	millimeters
MUFA	monounsaturated fatty acids
NL	neutral lipid
oz	ounces
PhL	phospholipid
PL	polar lipid
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
TL	total lipid
TOF	time-on-feed
TRT	treatment
ul	microliters
um	micrometers

CHAPTER I

INTRODUCTION

Consumption of beef continues to decline as Americans change their lifestyles and diets. Recommendations from health officials to lower saturated fat content in the diet persist as cardiovascular disease remains the number one leading cause of death in the United States (CDC, 1985). The American Heart Association (1985) even advocates using poultry and fish more often in meals than red meat.

As the concerns over dietary fat mount, consumers are demanding leaner beef products with no waste fat. Retailers have responded to the consumers' demands by completely trimming nearly 40% of all beef cuts. This practice of physically trimming the external fat has done little, however, to reduce the nearly two billion pounds of excess fat produced each year. Current marketing systems for high dressing, extensively marbled carcasses actually encourage the overfattening of cattle. In order to meet the demands of the health-conscious consumers, the cattle industry must begin to market leaner cattle immediately. One means by which to avoid overfattening of cattle is to identify the optimum time cattle need to receive a high concentrate diet.

As an optimum time-on-feed is identified, changes that may occur in the nutrient composition of beef during those feedlot months need to be assessed.

Consumers are also demanding prepackaged, kitchen-ready beef products. Relatively little research has been conducted on cooking trimmed, three ounce beef cuts. As the industry starts to develop and market these products, there is a great need to map the effect of cooking on subsequent composition.

The objectives of this research were: 1) to assess the effect time-on-feed has on the nutrient composition of beef longissimus muscles and 2) to evaluate the effect of cooking on the fatty acid composition of beef longissimus muscle lipids.

CHAPTER II

LITERATURE REVIEW

Beef in the American Diet

Consumption of Beef

Consumption of beef reached its peak in 1976 but has since declined 24% in the last 14 years. In 1976, per capita disappearance of beef (Figure 1), edible weight basis, was 89.0 pounds compared to 67.8 pounds in 1990 (USDA, 1990). Americans are changing their diets as consumption of red meat declines and poultry consumption continue to rise (NRC, 1989). The perception of red meat as high in cholesterol and saturated fat has had a negative effect on the purchase of red meat (Savell et al., 1987).

In a 1983 survey of supermarket shoppers, Farr (1987) reported that 14% of consumers polled said they were concerned about cholesterol and fat, compared to 23% in 1985 and 30% in 1986. The Economics and Statistics Service of the United States Department of Agriculture conducted a nationwide survey to obtain data linking consumers health and nutrition concerns (Jones and Weimer, 1981). They found that about 28% of households making a change in food use for

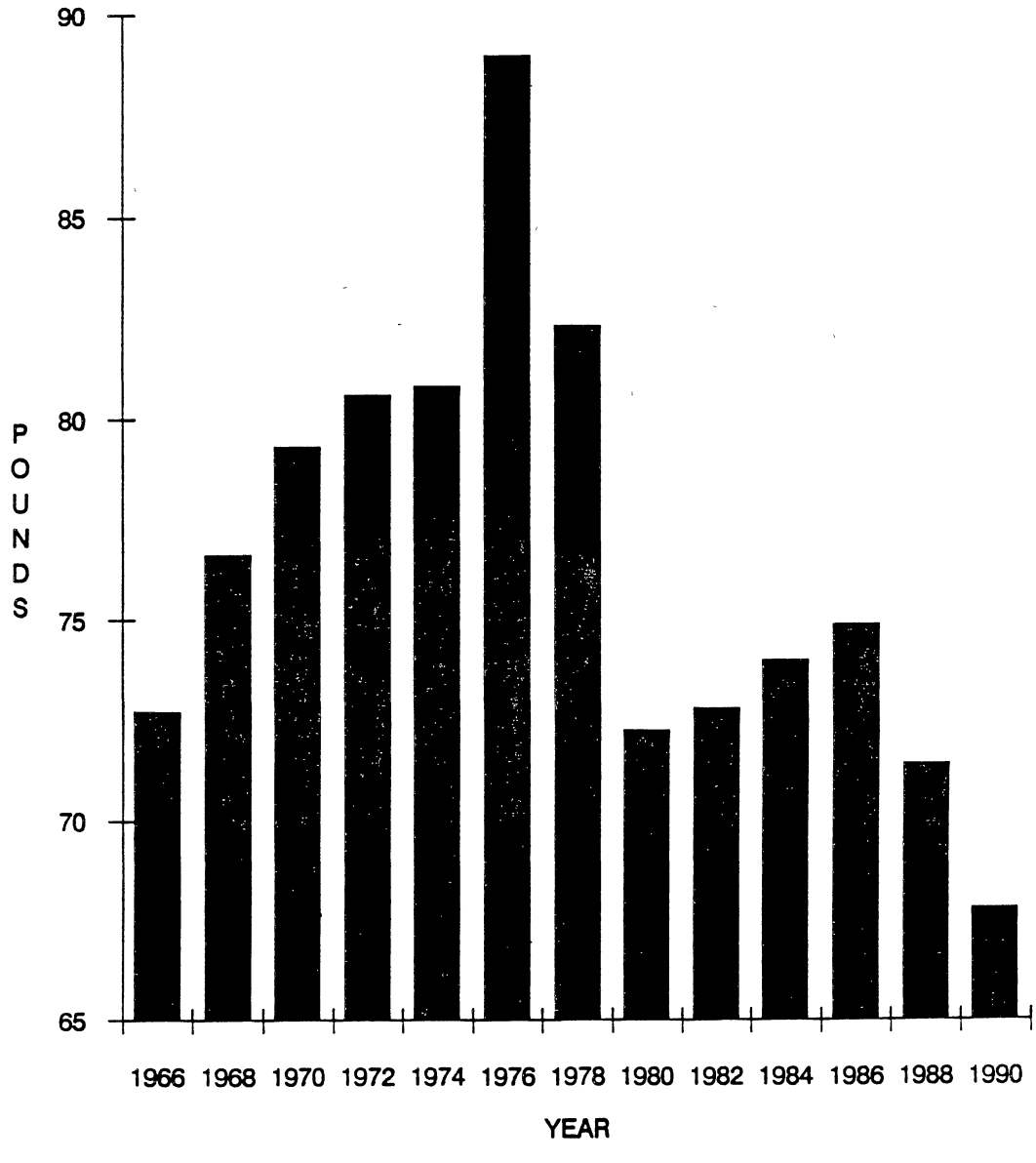

FIGURE 1. Per capita disappearance of beef, edible weight basis.

health or nutrition reasons cited a concern about fat intake; 23% were concerned about cholesterol; and 43% wanted to lose weight. Americans are becoming increasingly health conscious, as nearly one million Americans lose their lives each year due to cardiovascular disease (CDC, 1986). Despite a two percent annual decline in its prevalence since 1968, cardiovascular disease remains the leading cause of death in the United States (CDC, 1986). The American Heart Association (1986) and National Institutes on Health (1984) have chosen the following target levels for adults to have a healthy diet: (1) caloric intake matched to individual needs and appropriate to achieve and maintain desirable body weight, (2) 30 percent or less of calories from fat, (3) ten percent or less of calories from saturated fat, (4) ten percent or less of calories from polyunsaturated fat, (5) 15 percent or less of calories from monounsaturated fat, (6) 300 mg or less of cholesterol per day. To follow these guidelines, the American Heart Association in their eating plan for healthy Americans (1985) suggests that adults need no more than six ounces (about two small, 3 ounce servings) of meat, poultry or seafood per day, and that poultry or fish should be used more often than red meat. Thus Americans are expressing debate about the inclusion of beef into their diets, mainly due to its saturated fat content.

Fat in the diet

The concern about overconsumption of dietary fats may be linked to their concentrated energy content (9 kcal/g) and implications to coronary heart disease. More than one-third of the calories consumed by most people in the United States are provided by fat (RDA, 1989). Half of the total fat, three-fourths of saturated fat and all the cholesterol in the U.S. diet is contributed by animal products (NRC, 1988). In a Nationwide Food Consumption Survey, the USDA (1984) found red meat to provide the major source of fat to Americans of all age groups.

Populations consuming high amounts of saturated fatty acids have been found to have higher cholesterol levels and higher prevalence of coronary heart disease (Keys, 1970). The cholesterol raising effect of dietary saturates has been quantified by Keys et al. (1965) who determined an increase in plasma cholesterol equal to 2.7 mg/dl for each one percent of the total calories supplied by saturated fat. The suggested mechanism by which saturated fatty acids increase plasma cholesterol is that these fats cause a reduction in the clearance of low-density lipoprotein receptor system (Spady et al., 1985). According to these authors, this effect would be related to the decreased esterification rate of intracellular cholesterol, which would increase the content of free cholesterol. The increased amount of free cholesterol would be responsible

for the lower synthesis of low density lipoprotein receptors. The predominant saturated fatty acids are lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids. These saturated fatty acids are responsible for the hypercholesterolemic effect, however, the efficacy of this effect is not identical for all of them. Stearic (C18:0) acid, one of the saturated fatty acids, has been shown to have little if any effect on plasma cholesterol (Hegsted et al., 1965; Keys, 1965). Keys and Hegsted (1965) recommended that for the most accurate prediction of the increase in plasma cholesterol, only lauric (C12:0), myrisitic (C14:0) and palmitic (C16:0) acid concentrations should be considered. Bonanome and Grundy (1988) reported that a diet high in stearic (C18:0) acid lowered plasma cholesterol 14% when compared to a diet high in palmitic acid. These authors believe that a possible mechanism for the cholesterol lowering effect of stearic acid, as compared to palmitic acid, may be that stearic acid is rapidly converted to oleic acid upon ingestion.

Dietary recommendations suggest lowering consumption of saturated fatty acids and increasing the ingestion of polyunsaturated fatty acids and monounsaturated fatty acids through proper food selection (LRCP, 1984). The monounsaturated fatty acids and polyunsaturated fatty acids have been found to be equally effective in lowering plasma total cholesterol and low density lipoprotein cholesterol (Mattson et al., 1985; Grundy, 1986). The polyunsaturated

fatty acids, however, also tend to lower high density lipoprotein cholesterol, whereas the monounsaturated fatty acids do not (Shepherd et al., 1980; Mattson et al., 1985). The predominant polyunsaturated fatty acid is linoleic (C18:2) acid, an essential fatty acid that is a precursor of arachidonic (C20:4) acid. The mechanism underlying the effect of linoleic acid seems to consist of an increased clearance of the low density lipoprotein particles (Shepherd et al., 1980). Oleic (C18:1) acid is the principle monounsaturated fatty acid. Diets high in oleic acid have been compared to low fat diets, both diets lowered cholesterol; however, the low fat diet increased plasma triglycerides and decreased high density lipoprotein cholesterol (Grundy, 1986; Mensink et al., 1987).

Although both saturated and unsaturated fatty acids are present in beef, the common tendency is to categorize all animal fat as "saturated fat". Beef contains approximately 48%, 48% and 4%, respectively of its fatty acids as saturated, monounsaturated and polyunsaturated (USDA, 1990). The most abundant fatty acid in beef is oleic (C18:1) acid, a monounsaturated fatty acid. Beef contains approximately 13 percent of its fatty acids as stearic (C18:0) acid and thus the cholesterol-elevating saturated fatty acid content in beef is approximately 35 percent.

Impeding Changes for Beef

Research findings (Savell et al., 1987) led to the conclusion that consumers still want enough fat for taste and palatability but do not want waste fat. When external fat was trimmed, demand for beef increased and consumer's perceptions of diet/health traits improved. Unfortunately, this trimming of external fat has done little to reduce the two billion pounds of excess fat produced each year. The inefficiencies of the cattle industry are estimated to be $12 billion per year, of which $ 4.4 billion is due to excess fat production (BEEF, 1991). This excess fat production constitutes 20 percent of the inefficiency of the cattle industry and if cattle producers would correct this, choice beef could sell cheaper and still return the same dollars to the industry. In order to meet demands for nutrition and palatability of the health-conscious consumers, the beef industry must produce leaner animals immediately, with intramuscular adipose tissue that has less cholesterol-elevating saturated fatty acids (Dikeman, 1982).

Production Factors Affecting the Nutrient Composition of Beef

Time-on-Feed

Increasing time-on-feed on a high concentrate diet increases fat deposition in cattle which results in

carcasses with higher quality and yield grades (Wheeler et al., 1989; Williams et al., 1989; Greene et al., 1989; May, 1987; Zinn et al., 1970). In these trials, yield grades increase linearly, however, quality grades and percent total lipid in the longissimus muscle increases quadratically with increased time-on-feed. This quadratic increase in total lipid resulted in marbling scores that increased up to 112 days on feed when the choice quality grade was reached and then remained relatively constant with increased time-on-feed (Williams et al., 1989; Greene et al., 1989; May, 1987). Wheeler and co-workers (1989) in a comparison of late and early maturing cattle found that marbling scores in late maturing cattle increased up to 77 days on feed and then remained the same, in contrast to early maturing cattle that had increased marbling scores up to 180 days on feed. In a comparison of pre-finishing diets, Miller and co-workers (1987), reported that the cattle on a high energy pre-finishing diet at 0 or 56 days on feed had higher quality grades, however by day 112, quality grades for both groups consuming the high energy and low energy pre-finishing diets were similar. Williams et al. (1989) looked at hot fat trimming of carcasses and found that total fat trim (hot fat trim plus fat removed during fabrication) increased as time-on-feed increased. Thus approximately 112 days on a high concentrate diet is needed to achieve the choice quality grade and increasing time-on-feed past 112 days results in carcasses with relatively little improvement

in quality grade but increased yield grades (Williams et al., 1989; Greene et al., 1989; May, 1987).

Age and Growth

Several researchers have investigated compositional changes that occur during growth and with advancing age. Hecker et al. (1975) and Link et al. (1970a) observed that as the intramuscular lipid content of the muscle increases with advancing age and growth, this increase is largely due to a proportional increase in triglycerides and decrease in phospholipid concentration. Hecker et al. (1975) suggests that the increase in triglyceride concentration is probably due to adipocyte infiltration into the muscle. The phospholipids are essential cell constituents that are associated with muscle leanness and membrance structure, and whose concentration is related to the physiological activity of that muscle's fibers (Bloor et al., 1934; Terrell et al., 1969; Turkki et al., 1969). The phospholipid fraction remains constant during growth and its contribution to total lipid decreases as the amount of triglycerides increase (Link et al., 1970a; O'Keefe et al., 1968). Hecker et al. (1975) and Turkki et al. (1967) also suggest that fiber type (red versus white) plays an important role in determining the amount of phospholipid in the muscle. These authors have observed that red fibers in comparison to white fibers contain 50% more phospholipids.

Age has been suggested to be the primary contributor toward changes in the fatty acid composition of adipose tissue (Clemens et al., 1973). Clemens et al. (1973) found myristic (C14:0), palmitoleic (C16:1), stearic (C18:0) and linoleic (C18:2) acids to be associated with animal age, however, no apparent trends could be drawn. Oleic (C18:1) acid and age have a highly significant positive association (Waldman et al., 1965; Clemens et al., 1973). With respect to the polyunsaturated fatty acids, Link et al. (1970b) reported that since the polyunsaturated fatty acids are located predominantly in the phospholipid fraction, they are diluted, especially arachidonic acid (C20:4), with the fatty acids from the neutral lipid with advancing age. Concentrations of linoleic, linolenic (C18:2) and arachidonic acids decreased from the first biopsy period to the second, a difference of 60 days. Link et al. (1970b) found that polyunsatrated fatty acids per unit weight of muscle remain about the same during growth. Terrell et al. (1968) found palmitic and stearic to be negatively associated with days of age in the polar lipid fraction, which lead to decreased saturated fatty acid concentrations in the polar lipid with advancing age.

Diet

In a comparison of forage versus grain finishing diets, Williams et al. (1983) reported that consumption of the

grain diet resulted in increased marbling scores, quality grades and percent fat in the soft tissue. This increase in fat content in the soft tissue resulted in decreased crude protein, moisture and ash concentrations. In comparison of mineral concentrations, cattle on the forage diets had increased concentrations of zinc, phosphorus, magnesium and potassium. Concentrations of zinc, iron, phosphorus, sodium and potassium were negatively correlated with fat content. As the total lipid content in the muscle increased, a proportional increase in triglyceride content was noted (Williams et al., 1983; Miller et al., 1981). The phospholipid content decreased and was inversely related with fat content (Williams et al., 1983).

The difference between the forage and grain finishing diets resulted in differences in the fatty acid composition of the lipid. Grain feeding leads to increases in oleic (C18:1) acid and decreases in stearic (C18:0), linoleic (C18:2) and linolenic (C18:3) acids (Williams et al., 1983). Sumida et al. (1972) found that cattle fed high concentrate feedlot diets had increased amounts of myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1) and oleic (C18:1) acids when compared to cattle on pasture diets. Cattle on forage diets have increased concentrations of saturated and polyunsaturated fatty acids (Williams et al., 1983; Westerling et al., 1979; Marmer et al., 1984). Marmer et al. (1984) found increased amounts of the branched chain fatty acids in cattle fed forage diets. This increase

in branched fatty acids is believed to be due to the digestion of microorganisms from the rumen. These fatty acids are prevalent in forage fed diets because the contribution from dietary lipids is minimal. The increase in polyunsaturated fatty acids of forage fed cattle is primarily due to an increase in linoleic (C18:2) acid (Larick et al. 1989; Williams et al. 1982; Marmer et al., 1984; Miller et al.,1981).

Cooking Effects on the Composition of Beef

Cooking increases the total lipid content in beef (Smith et al. 1989; Gilpin et al. 1965). Gilpin et al. (1965) attributed this increase in lipid content to moisture loss incurred during cooking. If the muscle is broiled, the loss in moisture is believed to be due to evaporation (Gilpin et al. 1965). The weight loss during cooking usually averages about 30% (Ono et al., 1985; Janicki et al., 1974; Smith et al., 1989). In comparisons of cooking with or without subcutaneous fat trim, Berg et al. (1985) found that cooking with fat resulted in lower moisture losses and beef less calorically dense than beef cooked with the fat off. However, Smith et al. (1989) found no significant difference in moisture content between cuts cooked with external fat trim and cuts without external fat trim.

Limited research is available as to the effects of cooking on the composition of the lipid. Janicki et al. (1974) found increase in oleic (C18:1) acid, linolenic (C18:3) acid and the unsaturated to saturated ratio when ground beef was broiled. Ono et al. (1985) also found an increase in the polyunsaturated to saturated ratio when cooking ground beef. Terrell et al. (1968) looked at the effect of broiling on the neutral and polar lipid in longissimus muscle. They found that in the neutral lipid, linolenic (C18:3) acid decreased with cooking, whereas in polar lipid, myristic (C14:0) and pentadeconic (C15:0) acids decreased with broiling. These authors believe that since the phospholipids serve as an inherent function in cellular membranes, they are tightly bound and are not easily released during broiling. Conversely, Larick and Turner (1989 & 1990) found the polyunsaturated fatty acids to be extremely reactive and through thermal and autoxidative degradation, they give rise to a number of carbonyl compounds that greatly influence flavor. To date, relatively little or no differences in fatty acid concentrations between raw and cooked cuts of meat have been reported (Terrell et al., 1968; Anderson et al., 1971, 1975; Janicki and Appledorf, 1974; Smith et al., 1989).

CHAPTER III

EFFECT OF TIME-ON-FEED ON THE NUTRIENT COMPOSITION OF BEEF LONGISSIMUS MUSCLES.[1]

S.K. Duckett, D.G. Wagner, H.G. Dolezal, L.D. Yates, A.C. Clutter and S.G. May

ABSTRACT

Forty-eight Angus x Hereford steers were used to assess the effect of time-on-feed (TOF) on the nutrient composition of beef longissimus muscle (LM). The steers were fed a high concentrate diet and serially slaughtered at 28 d intervals over a 196 d feeding period. Day 0 served as a grass-fed control. At 72 hr postmortem, steaks were removed from the tenth rib and trimmed of exterior fat and epimysial connective tissue for chemical analysis. Intramuscular fat content increased cubically ($P<.05$) with TOF. The most substantial increase came between 84 and 112 d. As fat content increased, moisture and protein concentration in the LM decreased linearly ($P<.05$) and ash content decreased cubically ($P<.05$). Mg and K did show a tendency ($P<.10$) for a cubic effect over TOF. The increase in the total lipid (TL) content of the LM stemmed from a proportional increase

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(P<.05) of the neutral lipid (NL) as the polar lipid (PL) remained constant throughout TOF. The NL and TL became more unsaturated as TOF increased, primarily due to a linear (P<.01) increase in oleic (C18:1) acid. The PL became more saturated with increased TOF due to linear (P<.01) decreases in polyunsaturated fatty acid concentration.

(Key Words: Beef, Time-on-Feed, Fatty Acids, Proximate Composition)

INTRODUCTION

Consumer's want enough fat for taste and palatability but do not want waste fat (Savell et al., 1987). When external fat thickness was reduced, the demand for beef increased, and consumer's perceptions of fatty acid/cholesterol levels and diet/health traits improved (Savell et al., 1987). However, this practice of trimming external fat has done little to reduce the two billion pounds of excess fat produced each year. In order to meet demands of the health-conscious consumer, the beef industry must produce leaner animals, either through genetic selection and/or altering current management practices, with residual adipose tissue containing increased amounts of monounsaturated and polyunsaturated fatty acids at the expense of the hypercholesterolemic saturated fatty acids

Increasing time-on-feed (TOF) on a high concentrate

diet results in cattle with carcasses with higher quality and less desirable yield grades (Wheeler et al., 1989; Greene et al., 1989; Zinn et al., 1970). Yield grade numbers and fat thickness increase linearly, while, quality grade and marbling scores increase quadratically with increased finish (Williams et al., 1989; Greene et al., 1989). Williams et al. (1989) found total fat trim (hot fat trimming plus fat removed during fabrication) increased as TOF increased. In order to market leaner cattle with less excessive fat trim, cattle producers need to identify the optimum TOF on a high concentrate diet to obtain adequate intramuscular fat deposition for palatability while not overfinishing and adding unnecessary deposition of external fat. As this optimum TOF is identified, there is a great need to understand how protein, mineral and fatty acid composition will be affected. The objective of this research was to assess the effect of TOF on the nutrient composition of beef longissimus muscles.

MATERIALS AND METHODS

Forty-eight Angus x Hereford steers, approximately 16 months of age, were obtained from a native range stocker operation in northwestern Oklahoma. Steers were blocked by weight into eight equally sized groups to be serially slaughtered at 28 d intervals (0 - 196 d). Steers were fed a high concentrate diet (87.5% DM, 1.84 Mcal/kg NEm, 1.19

Mcal/kg NEg) except for the day 0 slaughter group which served as a grass-fed control. All steers were slaughtered at the Oklahoma State University Meat Laboratory. A 2.5 cm steak corresponding to the 10th rib was removed, vacuum packaged and stored at -20°C. After removal of all exterior fat and epimysial connective tissue, the lateral half of each longissimus muscle (LM) was pulverized in liquid nitrogen and stored at -20°C until chemical analyses.

Duplicate 2 g samples were analyzed for nitrogen by the Kjeldahl procedure, moisture by weight loss after drying at 100°C for 24 hr and mineral content by ashing at 600°C for 8 hr (AOAC, 1984). Mineral analyses were conducted by acidifying ash (AOAC, 1984) and then analyzing for Mg, Ca, Fe, K, Zn and Na a Perkin-Elmer Model 403 Atomic Absorption Spectrometer. Samples were extracted using the dry column method (Marmer and Maxwell, 1981) which allows for the sequential elution for neutral (NL) and polar (PL) lipids. Aliquots of NL and PL were freed of solvent and dried at 95°C for 24 hr to determine dry lipid weight (AOAC, 1984). The lipid weight of the NL and PL were added for each sample to obtain a total lipid (TL) weight. Phospholipid (PhL) content was calculated by determining P content of PL by the modified Fiske-Subbarow procedure (Bartlett, 1959) and multiplying by 25. Both the NL (Slover and Lanza, 1979) and PL (Maxwell and Marmer, 1983) were esterified to yield fatty acid methyl esters (FAME). The FAME were analyzed using a HP5890A gas chromatograph equipped with a flame-ionization

detector and HP7673A automatic sampler. Separations were accomplished on a 60 m SP2340 capillary column with a 0.25 mm internal diameter and 0.2 um film thickness. The injector and detector were maintained at 280°C. Column oven temperature was programmed at 155-165°C at 0.5°C/minute, 165-167°C at 0.2°C/minute, 167-200°C at 1.5°C/minute and held at 200°C for 18 minutes. Sample injection volume was 2 ul. Data were collected and integrated by HP3365 ChemStation software. Identification of peaks was based on retention times of reference compounds from Alltech. Total lipid fatty acid profiles were calculated by multiplying the percent of NL and PL in TL by each fatty acid.

The General Linear Model procedure of SAS was used to test the effect of time-on-feed (TOF). Differences between means were compared using Tukey's t-tests. Orthogonal polynomials were computed to determine linear, quadratic and cubic effects.

RESULTS and DISCUSSION

Carcass weight, fat thickness, longissimus muscle area and yield grade (Table 1) increased linearly ($P<.01$) over the 196 d feeding period. Marbling score showed quadratic trends ($P<.05$) across days-fed. The carcasses from steers fed 112 d were the first to attain the mean marbling score (small) required for the U.S. Choice quality grade.

Marbling scores did not ($P<.05$) increase after 112 d on a high concentrate ration.

Increased time-on-feed resulted in cubic increase in crude fat content (Table 2) with a concomitant linear decreases in moisture and protein content ($P<.01$) in the LM. Total lipid values were 0.6% higher than crude fat values on the average. This difference is due to the fact that organic solvents are more efficient at extracting phospholipids compared to petroleum ether (Lewis et al., 1990). The most substantial increase in lipid content was noted between 84 and 112 d. Lipid content did not differ ($P>.05$) from 0 to 84 d or from 112 to 196 d. Several researchers (Williams et al., 1989; Greene et al., 1989; Miller et al., 1981) also noted that intramuscular fat deposition does not proceed in a linear manner but occurs in a stepwise manner such that approximately 112 d on a high concentrate diet is needed before sufficient intramuscular lipid is deposited to obtain the choice quality grade. The NL increased proportionally with the increase in TL ($P<.05$). Concentrations of PL and PhL in the LM did not differ ($P>.05$) across TOF. Link et al. (1970), Hecker et al. (1975) and Miller et al. (1981) have also shown that the PhL fraction remains constant throughout growth. The increase in TL with TOF is largely due to the increase in triglycerides. As TOF increased, the percent PhL on a TL basis decreased from 25% at 0 d to 4% at 196 d. The

increase in TL as TOF increased resulted from proportional increases in NL and a diluting of the PhL.

Ash content in the LM decreased cubically ($P<.05$) as TOF increased; however, individual mineral concentrations did not differ ($P>.05$). Mg and K (Table 3) did show a tendency ($P<.10$) for a cubic effect. Williams et al. (1983) reported that consumption of a grain diet resulted in increased fat content and decreased ash content in the LM.

The most prevalent fatty acids in the NL (Table 4) were oleic (C18:1), stearic (C18:0) and palmitic (C16:0) acids, the sum of which accounted for over 80% of the total fatty acids in the NL. The NL was composed of approximately 51% SFA, 47% MUFA and 2% PUFA. The SFA content in the NL decreased ($P<.01$) 5.2% as TOF increased from 0 to 196 d. The decrease in SFA concentration was mainly due to a 5.7% decrease ($P<.01$) in stearic (C18:0) acid. The odd chain fatty acids (C15:0, C17:0, C19:0) showed a quadratic ($P<.05$) response to increased TOF. Myristic (C14:0) acid showed a linear ($P<.05$) increase and arachidic (C20:0) acid showed a linear decrease ($P<.01$). Palmitic (C16:0) acid did not differ ($P>.05$) across TOF. The MUFA showed a linear increase ($P<.05$) of 5.3% with the increase in TOF. The increase was due in part to a linear increase ($P<.01$) of 3.8% in oleic (C18:1) acid. Palmitoleic (C16:1) acid and C17:1 also increased linearly ($P<.01$) with TOF. Myristoleic (C14:1) acid showed a cubic decrease ($P<.05$). The percent PUFA in the NL showed a cubic effect ($P<.05$) over the 196 d

feeding period. Linoleic (C18:2) acid differed cubically ($P<.01$) as TOF increased. Linolenic (C18:3) acid and C20:3 did show small increases ($P<.05$) in concentration over TOF.

In the PL (Table 5), the most predominant fatty acids were oleic (C18:1), palmitic (C16:0) and linoleic (C18:2), and accounted for approximately 60% of the total fatty acids. The PL was comprised of approximately 38% SFA, 30% MUFA and 32% PUFA. Over two-thirds of the PUFA in the TL were from the PL. Link et al. (1970b) also found that the PUFA are predominantly located in the PL. Increased TOF from 0 to 196 d resulted in a linear increase ($P<.05$) of 6.4% in the SFA concentration of the LM. The largest increase in the SFA concentration came between 0 and 28 d. Myristic (C14:0), pentadecyclic (C15:0), palmitic (C16:0) and margaric (C17:0) acids all showed linear increases ($P<.05$) across TOF. The MUFA concentration in the LM showed a quadratic effect ($P<.01$). The increase in TOF resulted in cubic increases ($P<.01$) in myristioleic (C14:1) acid, and quadratic ($P<.05$) increases in oleic (C18:1) acid and C17:1. Palmitoleic (C16:1) acid showed a quadratic decrease ($P<.01$) over TOF. The PUFA in the PL decreased linearly ($P<.01$) resulting in a 11% decrease in concentration from 0 to 196 d. Arachidonic (C20:4) acid showed the largest linear decrease ($P<.01$) of 3%. C22:4 and C22:6 decreased linearly ($P<.01$) while linolenic (C18:3) acid, linoleic (C18:2) acid and C20:5 showed quadratic ($P<.01$) effects over TOF. C20:2 and C22:5 did not differ ($P>.05$) as TOF increased.

The SFA content in the TL (Table 6) changed cubically ($P<.05$) as TOF increased. Myrisitic (C14:0) acid increased linearly ($P<.01$) and pentadecyclic (C15:0), palmitic (C16:0), C19:0 and arachidic (C20:0) acids showed cubic ($P<.05$) decreases. Stearic (C18:0) acid had a 3.46% linear ($P<.01$) decrease. The MUFA demonstrated a 8.8% linear increase ($P<.01$) increase from 0 to 196 d. This increase was largely due to a 7% linear increase ($P<.05$) in oleic (C18:1) acid. Other researchers (Waldman et al., 1965; Clemens et al., 1973) have found a significant positive association with age and oleic (C18:1) acid concentration. Myristoleic (C14:1) and gadoleic (C20:1) also showed linear ($P<.05$) increases. Palmitoleic (C16:1) changed quadratically ($P<.05$) over TOF. C17:1 demonstrated a cubic ($P<.05$) response with increasing TOF. The PUFA content changed quadratically ($P<.05$) over TOF resulting in a 8.9% decrease from 0 to 196 d. Linoleic (C18:2), C20:3 and clupadonic (C22:5) acids showed linear ($P<.05$) responses over TOF. C20:2, arachidonic (C20:4) acid, C22:4 and C22:6 decreased quadratically ($P<.05$). Linolenic (C18:3) and timnodonic (C20:5) acids showed a cubic ($P<.05$) response to increasing TOF.

IMPLICATIONS

Increased TOF on a high concentrate diet results in cattle with increased quality and yield grades. However,

the greatest increase in intramuscular fat deposition occurred between 84 and 112 d. Thus, it appears that cattle need to be fed a high concentrate diet approximately 112 d to reach the choice grade and by extending the feeding period past 112 d does relatively little to increase intramuscular fat deposition but does increase excess fat trim and yield grades. The increase in intramuscular fat is largely a reflection of increases in the NL, predominantly triglycerides. The increase in lipid also results in a product with decreased moisture, protein and mineral composition. Increasing TOF results in increases in the MUFA concentration and decreases in the PUFA concentration. These changes result in a SFA:UNSFA ratio that changes cubically over TOF.

TABLE 1. MEAN VALUES FOR CARCASS CHARACTERISTICS ACROSS TIME-ON-FEED.

	Time-on-feed, days								
	0	28	56	84	112	140	168	196	SEM
Marbling score[a]**	252.2^{f}	299.0^{ef}	336.0^{def}	372.8^{cde}	472.2^{b}	428.3^{bcd}	471.7^{b}	464.2^{bc}	21.4
Fat thickness, mm*	3.05^{e}	4.11^{e}	6.82^{de}	9.78^{d}	14.60^{c}	15.03^{c}	18.20^{bc}	21.08^{b}	2.0
Longissimus muscle area, cm^2*	63.3^{e}	69.8^{e}	78.6^{cd}	76.3^{cd}	82.8^{bc}	85.7^{bc}	84.5^{bc}	93.2^{b}	2.8
Carcass weight,kg*	196.6^{g}	236.7^{fg}	263.7^{ef}	295.8^{de}	327.2^{cd}	353.0^{c}	364.7^{c}	417.4^{b}	9.1
Yield grade*	1.4^{f}	1.7^{ef}	1.7^{ef}	2.4^{de}	2.9^{d}	3.2^{cd}	3.7^{bc}	4.0^{b}	0.2

[a] Marbling score: small=400-499; slight=300-399; traces=200-299

^{bcdefg} Means with different superscripts in the same row differ (P<.05).

* Linear effect (P<.01)

** Quadratic effect (P<.05)

TABLE 2. MEAN VALUES FOR PROXIMATE COMPOSITION OF THE LONGISSIMUS MUSCLE ACROSS TIME-ON-FEED.

	Time-on-Feed, days								
%	0	28	56	84	112	140	168	196	SEM
Moisture*	74.58^{c}	74.08^{c}	73.32^{bc}	73.64^{c}	70.45^{ab}	68.93^{a}	68.59^{a}	67.47^{a}	0.70
Crude Protein*	21.71^{a}	21.83^{a}	20.51^{ab}	21.41^{ab}	19.50^{b}	20.33^{ab}	20.58^{ab}	20.07^{ab}	0.41
Ash***	1.09^{a}	1.08^{a}	1.07^{ab}	1.08^{a}	0.99^{bc}	0.99^{bc}	0.99^{bc}	0.98^{c}	0.02
Crude Fat***	2.09^{b}	2.62^{b}	4.10^{b}	4.02^{b}	8.67^{a}	9.35^{a}	9.64^{a}	10.14^{a}	0.80
Total Lipid*	2.52^{b}	3.06^{b}	4.96^{b}	4.09^{b}	9.48^{a}	9.73^{a}	9.83^{a}	11.65^{a}	0.90
Neutral Lipid*	1.84^{b}	2.45^{b}	4.34^{b}	3.44^{b}	8.88^{a}	9.03^{a}	9.14^{a}	10.95^{a}	0.89
Polar Lipid	0.68	0.61	0.61	0.65	0.62	0.70	0.69	0.70	0.04
Phospholipid	0.56	0.47	0.50	0.46	0.47	0.54	0.51	0.51	0.05

abc Means with different superscripts in the same row differ (P<.05).

* Linear effect (P<.05).

*** Cubic effect (P<.05).

TABLE 3. MEAN VALUES FOR MINERAL CONTENT IN THE LONGISSIMUS MUSCLE ACROSS TIME-ON-FEED.

Mineral[a]	Time-on-Feed, days								
	0	28	56	84	112	140	168	196	SEM
Mg*	17.78	20.96	19.31	20.75	21.97	15.44	17.71	19.66	1.95
Na	85.35	86.33	92.76	100.65	91.91	92.40	107.85	89.00	14.01
K*	329.83	315.23	321.79	336.31	324.85	295.27	283.72	332.97	13.49
Ca	19.55	24.98	23.45	23.17	30.50	15.05	21.02	24.33	6.38
Fe	5.36	5.08	5.12	5.30	5.37	4.97	5.76	5.68	0.23
P	226.00	188.73	198.93	185.43	189.07	216.47	204.47	202.00	21.61
Zn	5.66	6.70	8.11	12.17	8.43	7.48	8.94	9.41	1.86

[a] mg/100g of tissue.

* Cubic effect (P<.10).

TABLE 4. MEAN VALUES FOR FATTY ACID COMPOSITION OF THE NEUTRAL LIPID ACROSS TIME-ON-FEED.

	Time-on-Feed, days								
%	0	28	56	84	112	140	168	196	SEM
14:0*	3.41	2.34	3.28	3.59	3.64	3.79	3.85	3.70	0.07
14:1***	1.10^{ab}	1.01^{ab}	0.80^{b}	0.88^{b}	0.86^{b}	0.97^{ab}	1.31^{a}	1.02^{ab}	0.04
15:0**	0.76	0.96	0.88	0.92	1.08	0.99	0.93	0.85	0.03
16:0	27.57	28.22	27.77	29.63	27.51	27.52	27.22	27.40	0.20
16:1*	3.55^{ab}	3.55^{ab}	3.27^{b}	3.48^{ab}	3.67^{ab}	3.90^{ab}	4.28^{a}	4.21^{a}	0.08
17:0**	1.47^{c}	1.88^{bc}	2.22^{ab}	2.30^{ab}	2.63^{a}	2.33^{ab}	2.12^{ab}	2.02^{bc}	0.06
17:1**	0.59^{c}	0.97^{bc}	1.17^{ab}	1.31^{ab}	1.52^{a}	1.44^{a}	1.46^{a}	1.40^{a}	0.05
18:0*	19.63^{a}	18.42^{ab}	18.68^{ab}	16.10^{bc}	15.62^{c}	14.26^{c}	13.41^{c}	13.92^{c}	0.38
18:1*	38.66	38.97	39.22	40.29	40.23	41.48	42.53	42.44	0.35
18:2***	2.08	1.71	1.81	2.05	2.28	2.26	2.15	1.96	0.06
18:3*	0.00	0.00	0.07	0.00	0.07	0.05	0.03	0.06	0.01
19:0**	0.13^{ab}	0.26^{a}	0.14^{ab}	0.02^{b}	0.11^{ab}	0.19^{ab}	0.18^{ab}	0.24^{ab}	0.02
20:0*	0.51^{a}	0.48^{ab}	0.29^{bc}	0.25^{c}	0.30^{bc}	0.22^{c}	0.20^{c}	0.16^{c}	0.02
20:3*	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.02^{b}	0.02^{b}	0.03^{ab}	0.06^{a}	0.00
SFA*	53.49^{a}	53.45^{a}	53.26^{a}	51.80^{ab}	50.89^{ab}	47.32^{ab}	47.91^{b}	48.29^{b}	0.45
MUFA*	44.22^{b}	44.80^{b}	44.74^{b}	46.10^{a}	46.66^{a}	48.23^{a}	50.00^{a}	49.56^{a}	0.44
PUFA***	2.08	1.71	1.87	2.05	2.37	2.34	2.21	2.08	0.06

abc Means with different superscripts in the same row differ (P<.05).

* Linear effect (P<.05).

** Quadratic effect (P<.05).

*** Cubic effect (P<.05).

TABLE 5 MEAN VALUES FOR THE FATTY ACID COMPOSITION OF THE POLAR LIPID ACROSS TIME-ON-FEED

	Time-on-Feed, days								
%	0	28	56	84	112	140	168	196	SEM
14:0*	0.54^{c}	0 67^{bc}	0.71^{bc}	0 99^{abc}	1 02^{abc}	1.11^{ab}	1 34^{a}	1.20^{ab}	0.06
14:1***	0 03^{cd}	0 00^{d}	0 01^{cd}	0 04^{cd}	0 07^{bcd}	0 13^{bc}	0.25^{a}	0 17^{ab}	0.01
15:0*	0.34^{b}	0 47^{ab}	0 46^{ab}	0 48^{ab}	0 57^{a}	0.51^{ab}	0.48^{ab}	0.53^{ab}	0 02
16:0***	19 14	22 50	23 03	22 63	23 47	21 53	21 77	23.68	0 39
16:1**	2.59^{a}	2 23^{ab}	1 52^{bcd}	1 45^{cd}	1 29^{d}	1 60^{bcd}	2 04^{ab}	2 12^{ab}	0.08
17.0*	0.84	0 99	0.99	1 08	1 20	1.07	1 07	1 07	0 03
17:1***	0.63	0 63	0 58	0 60	0 56	0.62	0 78	0.79	0 02
18:0	12.52	13 29	13 05	13 82	14 00	13.60	13.19	13.78	0.20
18:1**	26.77^{b}	27.42^{ab}	23 96^{b}	26 33^{b}	25 77^{b}	26 50^{b}	29.47^{ab}	33.16^{a}	0.58
18:2**	16.24	16.19	20 31	20 35	20.66	22 14	19.22	15.32	0 61
18:3**	2 97^{a}	2 05^{b}	1 45^{c}	0 92^{cd}	0.52^{de}	0.32^{e}	0.26^{e}	0.04^{e}	0.14
19.0***	0.12^{a}	0.00^{b}	0 00^{b}	0 00^{b}	0 00^{b}	0 00^{b}	0 00^{b}	0 00^{b}	0.01
20·0**	0 38^{a}	0 29^{a}	0 08^{b}	0 06^{b}	0 00^{b}	0.04^{b}	0.06^{b}	0 00^{b}	0 02
20:2	2.12	1.74	1 92	1 71	1 90	1.94	1.77	1.58	0 06
20:4*	7.39^{a}	5 36^{ab}	5 98^{ab}	5 68^{ab}	5 63^{ab}	5.52^{ab}	5.17^{ab}	4.39^{b}	0 21
20.5**	0 54^{a}	0 34^{b}	0 15^{bc}	0 05^{c}	0 00^{c}	0.00^{c}	0.00^{c}	0 00^{c}	0 03
22:4*	1 77^{a}	1 36^{ab}	1 40^{ab}	1 07^{bc}	0 99^{bc}	0 68^{cd}	0 56^{cd}	0.25^{d}	0 08
22:5	0.74	1.13	0.57	0 54	0.46	0 65	0 94	0 59	0.09
22:6*	3 46^{a}	3·01^{ab}	2 99^{ab}	2 44^{abc}	2 06^{bcd}	1.72^{bcd}	1.48^{cd}	1.07^{d}	0 15
U*	0.12	0.18	0.16	0 04	0.00	0 03	0.00	0.00	0 02
SFA*	33 87	38 22	38 32	39 05	40.26	37 86	37.91	40.26	0 55
MUFA**	30 03^{ab}	30 28^{ab}	26 08^{a}	28 42^{a}	27.69^{a}	28.85^{a}	32.55^{ab}	36.23^{a}	0 65
PUFA*	35.22^{a}	31 18^{ab}	34.78^{a}	32 76^{ab}	32 23^{ab}	32 96^{ab}	29.39^{ab}	23 24^{b}	0 98

^{abcde} Means with different superscripts in the same row differ (P < 05)

* Linear effect (P < 05)

** Quadratic effect (P < 05)

*** Cubic effect (P < 05)

TABLE 6 MEAN VALUES FOR FATTY ACID COMPOSITION OF THE TOTAL LIPID ACROSS TIME-ON-FEED

	Time-on-Feed, days								
%	0	28	56	84	112	140	168	196	SEM
14 0*	2 52^{c}	2 68^{bc}	2 88^{abc}	3 15^{abc}	3 45^{ab}	3 56^{a}	3 67^{a}	3 54^{ab}	0 09
14 1*	0 78^{b}	0 79^{b}	0 69^{b}	0 74^{b}	0 78^{b}	0 91^{ab}	1 23^{a}	0 96^{ab}	0 04
15 0**	0 66^{b}	0 86^{ab}	0 82^{ab}	0 85^{ab}	1 03^{a}	0 98^{a}	0 90^{ab}	0 83^{ab}	0 03
16 0***	24 84^{b}	26 97^{ab}	26 96^{ab}	27 66^{a}	27 59^{a}	26 70^{ab}	26 80^{ab}	27 16^{ab}	0 21
16 1**	3 32^{c}	3 27^{c}	3 01^{c}	3 15^{c}	3.52^{abc}	3 66^{abc}	4 12^{a}	4 08^{bc}	0 08
17 0**	1 28^{c}	1 69^{bc}	2 04^{ab}	2 10^{ab}	2 48^{a}	2 33^{a}	2.04^{ab}	1 97^{ab}	0 06
17 1**	0 63^{c}	0 89^{bc}	1 08^{ab}	1 20^{ab}	1.41^{a}	1 42^{a}	1 41^{a}	1 37^{a}	0 05
18 0***	17 38^{a}	17 38^{a}	17 80^{a}	15 72^{ab}	15 71^{ab}	14 29^{b}	13 38^{b}	13 92^{b}	0 29
18 1*	34 95^{c}	36 40^{bc}	36 85^{bc}	38 04^{abc}	38 92^{abc}	40 31^{ab}	41 58^{a}	41 86^{a}	0 46
18 2*	6 46^{a}	4 94^{ab}	4 61^{ab}	5 13^{ab}	3 56^{b}	3 96^{ab}	3.36^{b}	2 81^{b}	0 24
18 3***	0 93^{a}	0 45^{b}	0.28^{bc}	0 15^{cd}	0 10^{bc}	0 08^{c}	0 05^{c}	0 06^{c}	0 05
19 0**	0 13^{a}	0 21^{a}	0 00^{a}	0 02^{b}	0 08^{a}	0 18^{ab}	0 17^{ab}	0 22^{a}	0 02
20 0**	0 49^{a}	0.43^{a}	0 26^{b}	0 22^{b}	0 27^{b}	0.22^{b}	0 20^{b}	0 15^{b}	0 02
20·1*	0 23^{a}	0 26^{ab}	0.25^{ab}	0 11^{a}	0 36^{ab}	0 39^{a}	0 40^{a}	0 46^{a}	0 02
20 2**	0 66^{a}	0 39^{ab}	0 28^{bc}	0 28^{bc}	0 14^{bc}	0 16^{bc}	0 13^{bc}	0 10^{c}	0 03
20·3*	0 00^{a}	0 00^{a}	0 00^{a}	0 00^{a}	0 02^{ab}	0 02^{ab}	0 03^{ab}	0 05^{b}	0 00
20 4**	2 27^{a}	1 15^{b}	0 91^{ab}	0 94^{bc}	0 40^{bc}	0 45^{bc}	0 38^{bc}	0 28^{c}	0 11
20 5***	0 17^{a}	0 07^{b}	0 02^{bc}	0 01^{c}	0 00^{c}	0 00^{c}	0 00^{c}	0 00^{c}	0 01
22 4**	0 54^{a}	0 30^{b}	0 21^{bc}	0 17^{bc}	0 07^{c}	0 06^{c}	0 04^{c}	0 02^{c}	0 03
22 5*	0 22	0 21	0 09	0 09	0 05	0 06	0 03	0 04	0 02
22 6**	1 06^{a}	0 64^{b}	0 44^{bc}	0 39^{bc}	0 14^{c}	0 14^{c}	0 11^{c}	0 07^{c}	0 06
U*	0 03	0 04	0 02	0 00	0 00	0 00	0 00	0 00	0 00
SFA***	47 30	50 24	50 90	49 70	50 61	48 26	47 14	47 79	0 35
MUFA*	39 91^{d}	41 60^{cd}	41 89^{bcd}	43 24^{bcd}	44 98^{bcd}	46 69^{ab}	48 75^{a}	48 73^{a}	0 58
PUFA**	12 31^{a}	8 15^{ab}	6 58^{bc}	7 16^{bc}	4 48^{bc}	4 93^{bc}	4 18^{bc}	3 42^{c}	0 51

^{abcde} Means with different superscripts in the same row differ (P < 05)

* Linear effect (P < 05)

** Quadratic effect (P < 05)

*** Cubic effect (P < 05)

CHAPTER IV

EFFECT OF COOKING ON THE FATTY ACID COMPOSITION OF BEEF LONGISSIMUS MUSCLE LIPIDS

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ABSTRACT

Forty-eight ribeye steaks (2.5 cm thick) from steers serially slaughtered at 28 d intervals (0-196 d on a high concentrate diet) were used to assess the effect of cooking on the fatty acid composition of beef longissimus muscle (LM) lipids. After removal of all exterior fat and epimysial connective tissue, the LM was sectioned as: lateral half for raw analysis (R) and dorsal half for cooked analysis (C). Dorsal halves, broiled to an internal temperature of 70°C, shrunk 34.5% and approximated a 66 g cooked serving size. As expected, solvent extraction of R and C to obtain neutral (NL) and polar lipid (PL) fractions revealed that cooking increased ($P<.01$) the total lipid (TL), NL and PL concentrations. Cooking increased ($P<.05$) the saturated fatty acid (SFA) and polyunsaturated fatty acids (PUFA) and decreased ($P<.05$) the monounsaturated fatty acid (MUFA) concentrations in the NL. In the PL, cooking

increased (P<.05) the SFA and decreased (P<.05) PUFA concentrations. In the TL, SFA concentrations increased (P<.05) while MUFA and PUFA decreased (P<.05) with cooking. The increases in the SFA content were the result of the increased (P<.05) concentration of stearic (C18:0) acid with cooking. The decreases in the MUFA and PUFA were attributed to losses in oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids during cooking.

(KEY WORDS: Beef, Cooking, Lipids)

INTRODUCTION

Health-conscious consumers are demanding leaner beef products with no waste fat. These responses have resulted from the recommendations by the dietitians and health organizations that intake of total and saturated fat in the diet should be limited to 30% and 10% of total caloric intake, respectively. The American Heart Association (1985) advocates that Americans should consume no more than six ounces (about two small, three ounce servings) of meat, poultry and seafood per day, and that poultry and fish should be used more often than red meat. Retailers have responded to consumers' demands by completely trimming excess fat on nearly 40% of all retail cuts of beef. However, relatively little research has been conducted as to

the effects of cooking smaller portion sizes with no external fat trim.

Berg et al. (1985) found that cooking beef with the fat on resulted in lower moisture losses and beef less calorically dense than beef cooked with fat removed. Conversely, Smith et al. (1989) found no significant differences in moisture content between cuts cooked with external fat trim or without. To date, small, nonsignificant differences have been found in fatty acid distributions between raw and cooked beef samples (Terrell, et al., 1968; Anderson et al., 1971; Janicki and Appledorf, 1974; Smith et al., 1989). However, most of these experiments have been conducted with a limited number of samples by comparing adjacent, eight to ten ounce steaks. The objective of this experiment was to assess the effect of cooking on the fatty acid composition of beef longissimus muscle lipids.

MATERIALS and METHODS

Forty-eight ribeye steaks (2.5 cm thick) were obtained from steers serially slaughtered at 28 d intervals (0-196 d on a high concentrate diet). After removal of all exterior fat and epimysial connective tissue, the longissimus muscle (LM) was sectioned as: lateral half for raw analysis (R) and dorsal half for cooked analysis (C). COOK steaks were thawed for 24 h in a 5^{o}C cooler and broiled on Farberware

Open Hearth Broilers (model 450A) to an internal temperature of 70°C. An Omega (model OM 302-10) temperature logger equipped with copper constantan probes was used on all steaks to ensure the desired internal endpoint temperature. C were weighed before and after cooking to determine the weight loss during cooking. RAW and COOK portions were individually pulverized in liquid nitrogen and stored at -20°C until subsequent analyses.

Duplicate 2 g samples were dried at 100°C for 24 h and then extracted with petroleum ether to determine moisture and crude fat content (AOAC, 1984). Samples were extracted using the dry column method (Maxwell and Marmer, 1984) which allows for the sequential elution for neutral (NL) and polar (PL) lipids. Aliquots of NL and PL were freed of solvent and dried at 95°C for 24 hr to determine dry lipid weight (AOAC, 1984). The lipid weight of the NL and PL were added for each sample to obtain a total lipid (TL) weight. Neutral lipid (Slover and Lanza, 1979) and PL (Maxwell and Marmer, 1984) were esterified to yield fatty acid methly esters (FAME). The FAME were analyzed using a HP5890A gas chromatograph equipped with a flame-ionization detector and HP7673A automatic sampler. Separations were accomplished on a 60 m SP2340 capillary column with a 0.25 mm internal diameter and 0.2 um film thickness. The injector and detector were maintained at 280°C. Column oven temperatures was programmed at 155-165°C at 0.5°C/minute, 165-167°C at 0.2°C/minute, 167-200°C at 1.5°C/minute and held at 200°C

for 18 minutes. Sample injection volume was 2 ul. Data were collected and integrated by HP3365 ChemStation software. Identification of peaks was based on retention times of reference compounds from Alltech . Fatty acids were quantified by using an internal standard, methyl heneicosanoic acid (C21:0). Total lipid fatty acid profiles were calculated by multiplying the percent of NL and PL in TL by each fatty acid.

Data were analyzed using the split-plot analysis of variance with animal considered the whole plot and steak as the sub-plot.

RESULTS

Moisture and lipid contents of the LM before and after cooking are reported in Table 1. Cooking loss averaged 34.5%. COOK portions averaged 100g before cooking and 66g after cooking, approximately 2.3 oz. Moisture content decreased ($P<.05$) 20.4% with cooking which resulted in greater concentrations of lipid in the LM. TL, NL and PL all increased ($P<.05$) in concentration from cooking.

Cooking increased ($P<.05$) the SFA concentration 2.7% in the NL (Table 2). This increase was attributed to the 2.7% increase in the stearic (C18:0) acid concentration after cooking. Myristic (C14:0) acid concentration was not different ($P>.05$) between raw and cooked samples. Palmitic (C16:0) acid decreased ($P<.05$) slightly in concentration

after cooking. A treatment (TRT) x TOF interaction for palmitic acid showed that cooking had a greater effect from 0 to 84 d compared to 112-196 d (Figure 1). The MUFA concentration decreased ($P<.05$) 3.8% in the NL after cooking. The 2% decrease in the concentration of oleic (C18:1) acid with cooking was largely responsible for the decline in the MUFA. Palmitoleic (C16:1) acid also showed a slight reduction after cooking ($P<.05$). The PUFA in the NL increased ($P<.05$) in concentration with cooking. Linoleic (C18:2) acid concentration increased slightly with cooking. A TRT x TOF interaction for linoleic acid revealed that R and C responses differed in direction at 0 d (Figure 2). Linolenic (C18:3) acid was not detected in the cooked samples. Arachidonic (C20:4) acid increased ($P<.05$) in concentration after cooking.

In the PL (Table 3), the SFA concentration increased ($P<.05$) 2.5% after cooking. Stearic (C18:0) acid increased 3.4% while palmitic (C16:0) acid decreased 2.4% in concentration after cooking. Myristic (C14:0) acid did show a slight increase ($P<.05$) in concentration after cooking. MUFA and oleic (C18:1) acid concentrations did not differ ($P>.05$) between raw and cooked samples. Palmitoleic (C16:1) acid showed a slight increase ($P<.05$) with cooking. Cooking reduced ($P<.05$) the PUFA concentration 3.4% in the PL. Linoleic (C18:2) acid concentration did not differ ($P>.05$) between R and C. Linolenic (C18:3) acid was not detected in

cooked samples. Arachidonic (C20:4) acid increased (P<.05) 1% in concentration with cooking.

In the TL (Table 4), cooking increased (P<.05) SFA content 3.9%. Stearic (C18:0) acid increased (P<.05) 3.1% with cooking. A TRT x TOF interaction for stearic acid and SFA revealed that cooking had a greater effect at 0 and 28 d compared to 56-196 d (Figure 3). Palmitic (C16:0) acid did not differ (P>.05) in concentration between raw and cooked samples. Myristic (C14:0) did show a slight increase (P<.05) in concentration with cooking. The TRT x TOF interaction of myristic acid had a similar response to that of palmitic (C16:0) in the NL (Figure 1). The MUFA concentration decreased 2.1% with cooking. Palmitoleic (C16:1) acid did not differ (P>.05) in concentration between raw and cooked samples. A TRT x TOF interaction for palmitoleic acid revealed that R and C responses differed in direction between 84 and 112 d (Figure 4). The PUFA concentration decreased (P<.05) 2% after cooking. Linoleic (C18:2) acid and arachidonic (C20:4) acid decreased (P<.05) 1% and 0.4% in concentration, respectively, after cooking. The TRT x TOF interaction was significant for linoleic acid, arachidonic acid and PUFA in the TL. The response was similar to that of palmitic acid in the NL (Figure 1) in which cooking had a greater effect from 0-84 d compared to 112-196 d. Linolenic (C18:3) acid was not detected in cooked samples.

DISCUSSION

The moisture loss and lipid values reported in this experiment exceed those published by the USDA (1990) and other researchers (Smith et al., 1989; Terrell et al., 1968; Anderson et al., 1971; Janicki and Appledorf, 1974). The 20.4% decrease in moisture content observed in this experiment resulted in a greater concentration of lipid in the LM. This difference is believed to be due to cooking smaller portion sizes with less surface area and/or the removal of the epimysial connective tissue. Another factor important in comparing raw and cooked samples is whether there is more variation in lipid distribution within a steak, i.e. lateral versus dorsal halves, or with adjacent steaks. After quantification of the fatty acids in the cooked samples, only 13.4% of the TL could be accounted for. Adding 10% to this for the weight of glycerol would leave a remaining 6% extracted by both petroleum ether and organic solvents that would be non-fatty acid compounds. A small percentage of this would be cholesterol, however the remaining percentage is probably degradative products that resulted from cooking, such as carbonyl compounds from the autoxidation of lipids and/or thermally-induced heterocyclic amines. Meats are believed to particularly susceptible to fatty acid oxidation due to their relatively high content of unsaturated membrane phospholipid species and of both heme and non-heme iron (Hotchkiss & Parker, 1990). These

compounds are believed to be important in the flavor of meats. Further investigation into cooking smaller portion sizes, different sections of the muscle and denuded muscle are greatly needed.

The cooking of the LM resulted in increased concentrations of SFA at the expense of MUFA and PUFA. The increase in the SFA concentration resulted from the increase in stearic (C18:0) acid with cooking. Stearic acid has been shown to have little or no effect on plasma cholesterol levels (Hegsted et al., 1965; Keys, 1965). Thus cooking resulted in a product with a lower hypercholesterolemic fatty acid concentration. The MUFA decrease was due to losses in oleic (C18:1) acid with cooking. The decrease in linoleic (C18:2) and linolenic (C18:3) acids were responsible for the loss of PUFA during cooking. Fatty acid melting points decrease with increasing degree of unsaturation. The losses of oleic, linoleic and linolenic acids and increase of stearic acid would be due to the lower melting point of the unsaturates, thus making them more susceptible to autoxidation during cooking. The interactions between treatment and time-on-feed demonstrated that the polyunsaturated fatty acids in the NL and TL had a greater susceptibility to autoxidation during cooking at 0 to 84 d due to their greater concentration in grass-fed cattle. This resulted in larger increases in the SFA concentration in the early slaughter periods.

TABLE 1. TREATMENT MEANS FOR MOISTURE AND LIPID CONTENT OF THE LONGISSIMUS MUSCLE.

	Raw %	Cook %	SE
Moisture[ab]	71.38	51.00	1.13
Crude fat[ab]	6.33	21.62	0.99
Total lipid[ab]	6.92	20.83	0.94
Neutral lipid[ab]	6.26	19.63	0.91
Polar lipid[a]	0.66	1.20	0.03
Cooking loss		34.49	1.78

[a] Treatment effect (P<.05).
[b] Time-on-Feed effect (P<.05).
[c] Treatment x Time-on-Feed interaction (P<.05).

TABLE 2. TREATMENT MEANS FOR FATTY ACID COMPOSITION OF THE NEUTRAL LIPID.

Fatty acid	Raw %	Cook %	SE
14:0[b]	3.56	3.49	0.05
16:0[abc]	27.73	27.13	0.14
16:1[b]	3.73	3.62	0.05
18:0[ab]	16.28	18.97	0.31
18:1[ab]	40.45	38.52	0.29
18:2[abc]	2.04	2.39	0.05
Other[d]	6.17	5.81	
SFA[abe]	51.08	53.74	0.33
MUFA[abe]	46.76	43.09	0.36
PUFA[abce]	2.09	2.78	0.05
SFA:UNSFA[e]	1.04	1.17	

[a] Treatment effect (P<.05)

[b] Time-on-Feed effect (P<.05).

[c] Treatment x Time-on-Feed interaction (P<.05).

[d] Pooled sum of 14:1, 15:0, 17:0, 17:1, 19:0, 20:0, 20:1, 20:2, 20:3, 20:5, 22:4, 22:5, 22:6 and Unidentified.

[e] SFA=Saturated fatty acids; MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids and SFA:UNSFA=Ratio of saturated fatty acids to unsaturated fatty acids.

TABLE 3. TREATMENT MEANS FOR FATTY ACID COMPOSITION OF THE POLAR LIPID.

Fatty acid	Raw %	Cook %	SE
14:0[ab]	0.95	1.23	0.04
16:0[a]	22.22	19.78	0.25
16:1[ab]	1.86	2.06	0.05
18:0[a]	13.40	16.85	0.22
18:1[b]	27.42	28.44	0.35
18:2[b]	18.80	17.52	0.40
18:3[abc]	1.07	0.00	0.09
20:4[a]	5.64	6.56	0.15
Other[d]	8.64	7.56	
SFA[ae]	38.22	40.73	0.35
MUFA[be]	30.02	30.73	0.39
PUFA[ae]	31.47	28.11	0.61
SFA:UNSFA[e]	0.62	0.69	

[a] Treatment effect (P<.05)

[b] Time-on-Feed effect (P<.05).

[c] Treatment x Time-on-Feed interaction (P<.05).

[d] Pooled sum of 14:1, 15:0, 17:0, 17:1, 19:0, 20:0, 20:1, 20:2, 20:3, 20:5, 22:4, 22:5, 22:6 and Unidentified.

[e] SFA=Saturated fatty acids; MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids and SFA:UNSFA=Ratio of saturated fatty acids to unsaturated fatty acids.

TABLE 4. TREATMENT MEANS FOR FATTY ACID CONTENT OF THE TOTAL LIPID.

Fatty acid	Raw %	Cook %	SE
14:0[abc]	3.18	3.35	0.06
16:0[b]	26.84	26.66	0.14
16:1[bc]	3.51	3.52	0.05
18:0[abc]	15.70	18.80	0.29
18:1[ab]	38.62	37.90	0.31
18:2[abc]	4.35	3.35	0.14
18:3[abc]	0.26	0.00	0.03
20:4[abc]	0.85	0.50	0.06
Other[d]	6.69	5.92	
SFA[abce]	49.00	52.89	0.32
MUFA[abe]	44.47	42.33	0.38
PUFA[abce]	6.43	4.43	0.26
SFA:UNSFA[e]	0.96	1.13	

[a]Treatment effect (P<.05)

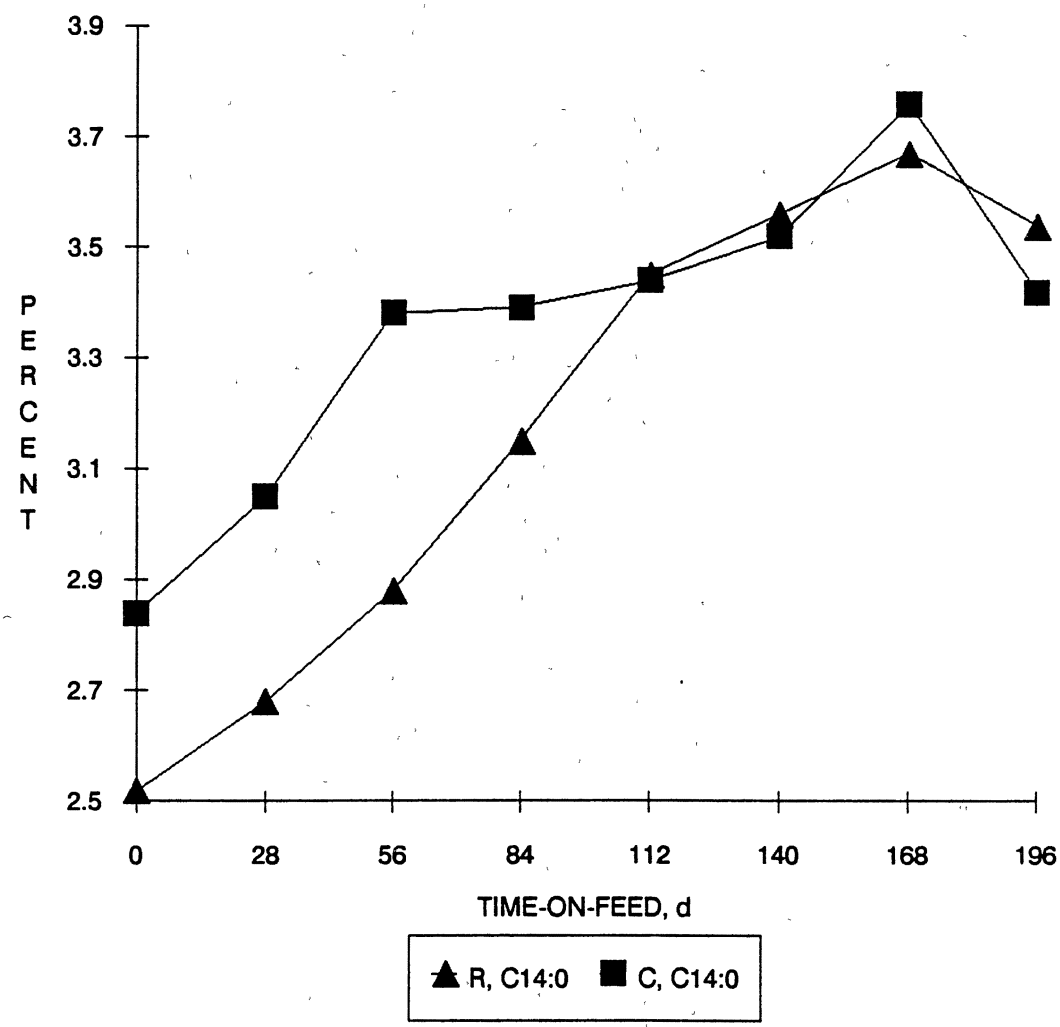
[b]Time-on-Feed effect (P<.05).

[c]Treatment x Time-on-Feed interaction (P<.05).

[d] Pooled sum of 14:1, 15:0, 17:0, 17:1, 19:0, 20:0, 20:1, 20:2, 20:3, 20:5, 22:4, 22:5, 22:6 and Unidentified.

[e] SFA=Saturated fatty acids; MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids and SFA:UNSFA=Ratio of saturated fatty acids to unsaturated fatty acids.

FIGURE 1. TREATMENT X TIME-ON-FEED INTERACTION FOR C14:0 IN THE TOTAL LIPID.[a]



[a]C16:0 and PUFA in the NL, and C18:2, C20:4 and PUFA in the TL all have a similar response as C14:0 in the TL.

FIGURE 2. TREATMENT X TIME-ON-FEED INTERACTION FOR C18:2 IN THE NEUTRAL LIPID.

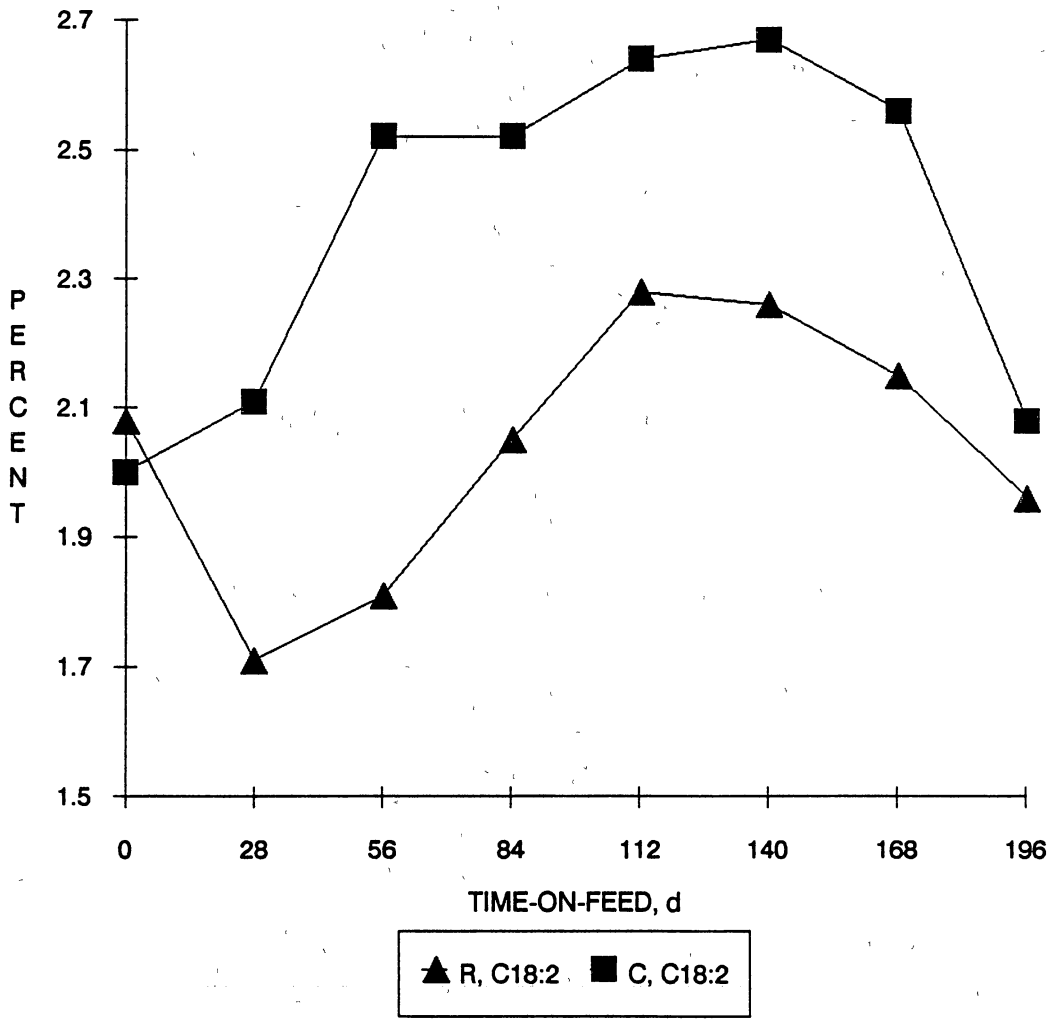
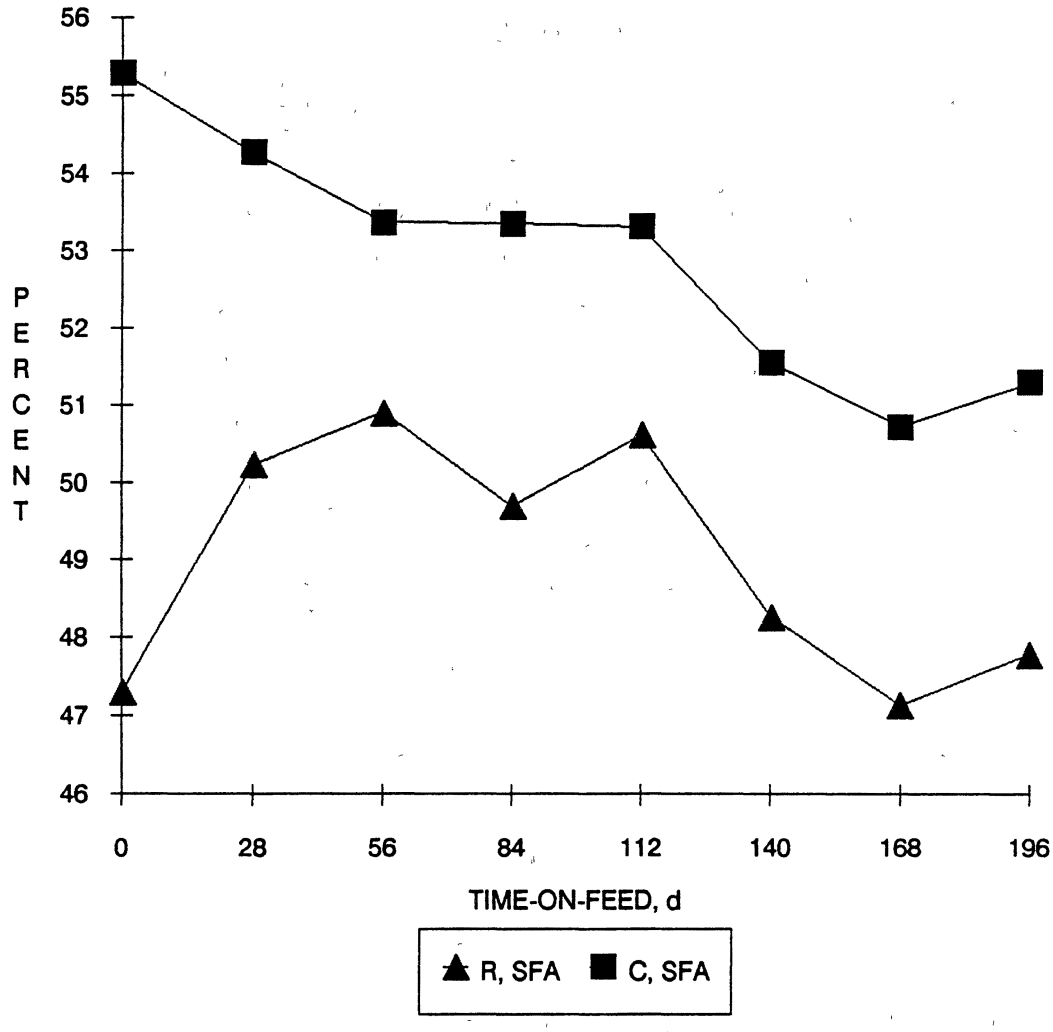
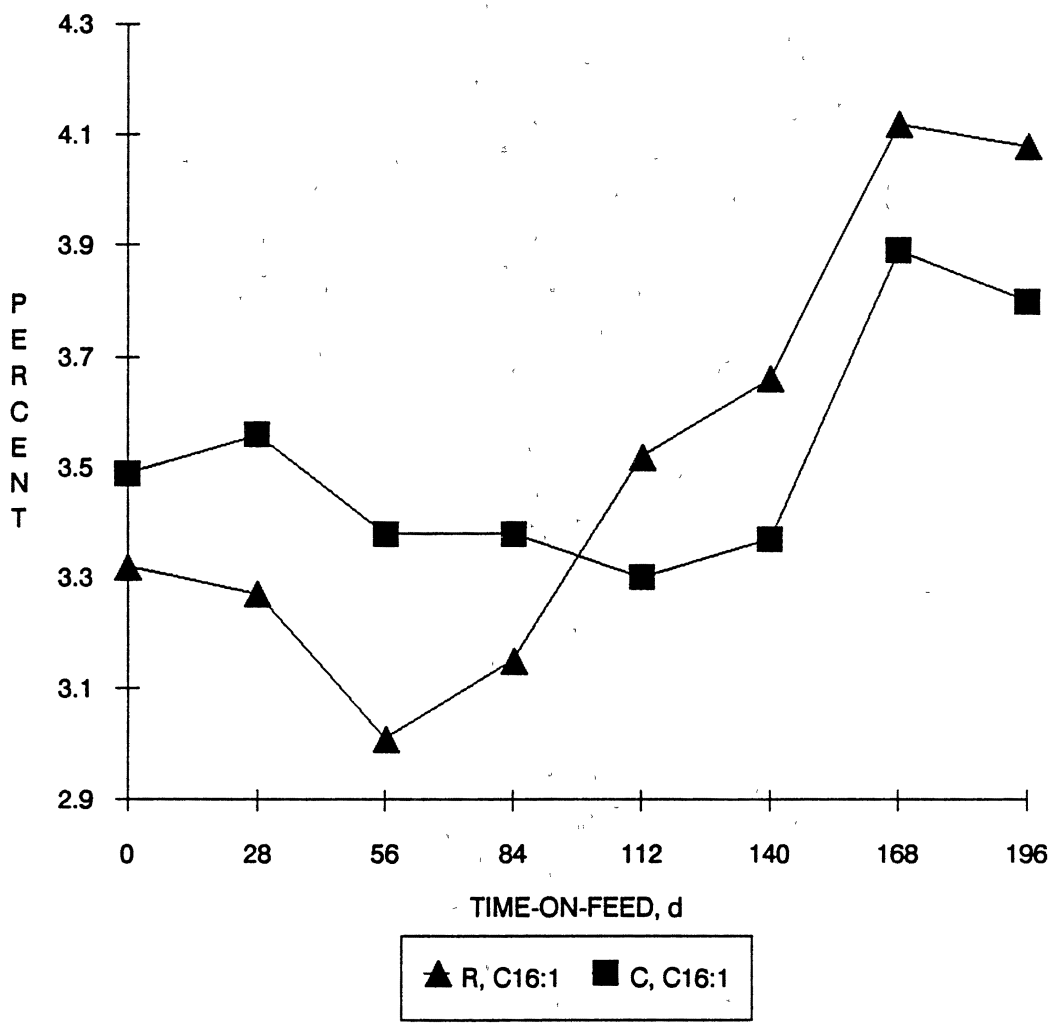


FIGURE 3. TREATMENT X TIME-ON-FEED INTERACTION FOR SATURATED FATTY ACID CONCENTRATION IN THE TOTAL LIPID.[a]



[a]C18:0 in the TL also follows a similar response.

FIGURE 4. TREATMENT X TIME-ON-FEED INTERACTION FOR C16:1 IN THE TOTAL LIPID.



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APPENDIX A

COMMON NAMES AND MELTING POINTS OF FATTY ACIDS

TABLE 1. COMMON NAMES AND MELTING POINTS OF FATTY ACIDS.[a]

Common name	IUPAC[b] name	Carbons (number)	Double bonds (number)	M.P. (°C)
Lauric	Dodecanoic	12	0	44.2
Myristic	Tetradecanoic	14	0	54.4
Myristoleic	Tetradecenoic	14	1	18.5
Pentadecylic	Pentadecanoic	15	0	52.1
Palmitic	Hexadecanoic	16	0	62.9
Palmitoleic	Hexadecenoic	16	1	21.0
Margaric	Heptadecanoic	17	0	61.3
Stearic	Octadecanoic	18	0	69.6
Oleic	Octadecenoic	18	1	16.3
Linoleic	Octadecadienoic	18	2	-5.0
Linolenic	Octadecatrienoic	18	3	-10.0
Nonadecylic	Nonadecanoic	19	0	68.7
Arachidic	Eicosanoic	20	0	75.4
Gadoleic	Eicosenoic	20	1	22.0
Arachidonic	Eicosatetraenoic	20	4	-49.5
Timnodonic	Eicosapentaenoic	20	5	NA
Behenic	Docosanoic	22	0	80.0
Stearidonic	Docosatetraenoic	22	4	NA
Clupandonic	Docosapentaenoic	22	5	-78.0

[a]Adapted from Small, 1986
[b]International Union of Pure and Applied Chemists

APPENDIX B

COOKING EFFECTS ON THE FATTY ACID COMPOSITION OF THE LONGISSIMUS MUSCLE ACROSS TIME-ON-FEED

TABLE 1. MOISTURE AND LIPID CONTENT OF THE LONGISSIMUS MUSCLE ACROSS TIME-ON-FEED.

TOF	TRT	MOISTURE[ac]	TL[ab]	NL[ab]	PL[a]	COOKING LOSS
d		%	%	%	%	%
0	R	74.59	2.52	1.84	0.68	
	C	57.55	11.82	10.57	1.25	34.33
28	R	74.08	3.06	2.45	0.61	
	C	55.11	15.32	14.20	1.17	35.89
56	R	73.32	4.96	4.34	0.61	
	C	53.40	16.60	15.49	1.11	33.38
84	R	73.64	4.09	3.44	0.65	
	C	52.80	18.42	17.23	1.19	35.66
112	R	70.45	9.48	8.88	0.62	
	C	47.96	25.50	24.28	1.23	34.34
140	R	68.93	9.73	9.03	0.71	
	C	45.47	28.42	27.15	1.28	33.57
168	R	68.59	9.83	9.14	0.69	
	C	47.83	24.89	23.68	1.21	34.02
196	R	67.47	11.65	10.95	0.70	
	C	48.4.	25.64	24.42	1.19	34.69
SE		1.13	0.94	0.91	0.03	1.78

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

TABLE 2. SATURATED FATTY ACID CONCENTRATIONS OF THE NEUTRAL LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	14:0 %	15:0[ac] %	16:0[ab] %	17:0[a] %	18:0[a] %	19:0[ac] %	20:0[a] %	SFA[a] %
0	R	3.41	0.76	27.57	1.47	19.63	0.13	0.51	53.49
	C	3.06	1.62	25.98	1.92	23.55	0.00	0.81	56.95
28	R	2.34	0.96	28.22	1.88	18.42	0.26	0.48	53.46
	C	3.19	1.49	27.00	2.07	20.78	0.00	0.56	55.08
56	R	3.28	0.88	27.77	2.22	18.68	0.14	0.29	53.26
	C	3.55	1.30	27.36	2.20	19.32	0.00	0.57	55.08
84	R	3.59	0.92	28.63	2.30	16.10	0.02	0.25	51.80
	C	3.56	1.24	27.47	2.51	19.12	0.00	0.45	54.34
112	R	3.64	1.08	27.51	2.63	15.62	0.11	0.30	50.89
	C	3.55	1.34	27.54	2.74	18.29	0.00	0.44	53.90
140	R	3.79	0.99	27.52	2.33	14.26	0.19	0.22	40.30
	C	3.62	1.18	26.77	2.63	17.43	0.50	0.21	52.13
168	R	3.85	0.93	27.22	2.12	13.41	0.18	0.20	47.91
	C	3.88	1.08	27.46	2.30	16.14	0.00	0.47	51.33
196	R	3.70	0.85	27.40	2.02	13.92	0.24	0.16	48.29
	C	3.54	1.00	27.46	2.24	17.14	0.00	0.50	51.88

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

TABLE 3. SATURATED FATTY ACID CONCENTRATIONS OF THE POLAR LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	14:0[a] %	15:0[b] %	16:0[ad] %	17:0[ab] %	18:0[ab] %	19:0[ac] %	20:0[ac] %	SFA[ab] %
0	R	0.54	0.34	19.14	0.84	12.52	0.12	0.38	33.87
	C	0.99	0.67	18.85	1.33	17.30	0.00	2.75	41.89
28	R	0.67	0.47	22.50	0.99	13.29	0.00	0.29	38.22
	C	1.16	0.60	20.17	1.32	18.00	0.00	1.92	43.17
56	R	0.71	0.46	23.03	0.99	13.05	0.00	0.08	38.32
	C	0.98	0.38	19.30	1.08	17.22	0.00	1.32	40.30
84	R	0.99	0.48	22.63	1.07	13.82	0.00	0.06	39.05
	C	1.20	0.46	19.51	1.29	16.76	0.00	1.02	40.23
112	R	1.02	0.57	23.47	1.20	14.00	0.00	0.00	40.26
	C	1.42	0.51	20.42	1.46	17.20	0.00	0.73	41.79
140	R	1.11	0.51	21.53	1.07	13.60	0.00	0.04	37.86
	C	1.37	0.50	19.48	1.38	16.28	0.00	0.59	39.58
168	R	1.34	0.48	21.77	1.07	13.19	0.00	0.06	37.91
	C	1.53	0.41	20.24	1.07	15.74	0.00	0.37	39.36
196	R	1.20	0.53	23.68	1.07	13.78	0.00	0.00	40.26
	C	1.22	0.37	20.26	0.98	16.28	0.00	0.44	39.55

[a]C differed from R ($P < .05$).

[b](C-R): linear effect over TOF ($P < .05$).

[c](C-R): quadratic effect over TOF ($P < .05$).

[d](C-R): cubic effect over TOF ($P < .05$).

TABLE 4. SATURATED FATTY ACID CONCENTRATION OF THE TOTAL LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	14:0[ab] %	15:0[ac] %	16:0[c] %	17:0[ac] %	18:0[ac] %	19:0[ac] %	20:0[ab] %	SFA[ad] %
0	R	2.52	0.66	24.84	1.28	17.38	0.13	0.49	47.30
	C	2.84	1.51	25.18	1.86	22.83	0.02	1.04	55.28
28	R	2.68	0.86	26.97	1.68	17.38	0.21	0.43	50.24
	C	3.05	1.42	26.52	2.01	20.59	0.00	0.68	54.27
56	R	2.88	0.82	26.96	2.04	17.80	0.13	0.26	50.90
	C	3.38	1.24	26.82	2.12	19.17	0.00	0.63	53.36
84	R	3.15	0.85	27.66	2.10	15.72	0.02	0.22	49.70
	C	3.39	1.19	26.90	2.42	18.96	0.00	0.49	53.34
112	R	3.45	1.03	27.59	2.48	15.71	0.08	0.27	50.61
	C	3.44	1.30	27.19	2.68	18.23	0.00	0.46	53.30
140	R	3.56	0.98	26.70	2.33	14.29	0.18	0.22	48.26
	C	3.52	1.14	26.44	2.57	17.38	0.00	0.50	51.55
168	R	3.67	0.90	26.80	2.04	13.38	0.17	0.20	47.14
	C	3.76	1.05	27.10	2.24	16.12	0.00	0.46	50.72
196	R	3.54	0.83	27.16	1.97	13.92	0.22	0.15	47.79
	C	3.42	0.98	27.12	2.19	17.10	0.00	0.50	51.30

[a]C differed from R ($P < .05$).

[b](C-R): linear effect over TOF ($P < .05$).

[c](C-R): quadratic effect over TOF ($P < .05$).

[d](C-R): cubic effect over TOF ($P < .05$).

TABLE 5. MONOUNSATURATED FATTY ACID CONCENTRATIONS IN THE NEUTRAL LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	14:1[ad] %	16:1[ab] %	17:1[ac] %	18:1[a] %	20:1[ab] %	MUFA[a] %
0	R	1.10	3.55	0.59	38.66	0.30	44.22
	C	0.36	3.60	0.48	34.58	0.45	39.46
28	R	1.01	3.55	0.97	38.97	0.30	44.80
	C	0.48	3.65	0.22	37.11	0.37	41.82
56	R	0.80	3.27	1.17	39.27	0.28	44.74
	C	0.61	3.50	0.01	37.85	0.28	42.25
84	R	0.88	3.48	1.31	40.29	0.13	46.10
	C	0.60	3.49	0.00	38.02	0.24	42.36
112	R	0.89	3.67	1.52	40.23	0.38	46.66
	C	0.53	3.38	0.00	38.60	0.20	42.71
140	R	0.97	3.90	1.44	41.48	0.43	48.23
	C	0.67	3.44	0.00	40.18	0.21	44.50
168	R	1.31	4.28	1.46	42.53	0.43	50.00
	C	0.81	4.00	0.00	40.86	0.07	45.73
196	R	1.02	4.21	1.40	42.44	0.49	49.56
	C	0.95	3.88	0.00	40.97	0.08	45.88

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

TABLE 6. MONOUNSATURATED FATTY ACID CONCENTRATIONS OF THE POLAR LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	14:1[ac] %	16:1[ac] %	17:1[ab] %	18:1 %	20:1[a] %	MUFA[b] %
0	R	0.03	2.59	0.63	26.77	0.00	30.03
	C	0.11	2.59	0.10	28.58	0.14	31.56
28	R	0.00	2.23	0.63	27.42	0.00	30.28
	C	0.15	2.37	0.07	29.02	0.06	31.67
56	R	0.01	1.52	0.58	23.96	0.00	26.08
	C	0.14	1.72	0.00	26.07	0.01	27.94
84	R	0.04	1.45	0.60	26.33	0.00	28.42
	C	0.18	1.91	0.01	26.92	0.00	29.02
112	R	0.07	1.29	0.56	25.77	0.00	27.69
	C	0.19	1.92	0.00	27.88	0.00	29.99
140	R	0.13	1.60	0.62	26.50	0.00	28.85
	C	0.23	1.38	0.00	28.53	0.00	30.66
168	R	0.25	2.04	0.78	29.47	0.00	32.55
	C	0.24	1.89	0.00	29.61	0.00	31.75
196	R	0.17	2.12	0.79	33.16	0.00	36.23
	C	0.20	2.22	0.00	30.87	0.00	33.28

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

TABLE 7. MONOUNSATURATED FATTY ACID CONCENTRATIONS OF THE TOTAL LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	14:1[ac] %	16:1[d] %	17:1[ac] %	18:1[ab] %	20:1[ac] %	MUFA[ab] %
0	R	0.78	3.32	0.63	34.95	0.23	39.94
	C	0.34	3.49	0.44	33.90	0.41	38.57
28	R	0.79	3.27	0.89	36.40	0.26	41.60
	C	0.46	3.56	0.20	36.53	0.34	41.09
56	R	0.69	3.01	1.08	36.85	0.25	41.89
	C	0.58	3.38	0.01	37.05	0.27	41.29
84	R	0.74	3.15	1.20	38.04	0.11	43.24
	C	0.58	3.38	0.00	37.23	0.22	41.41
112	R	0.78	3.52	1.41	38.92	0.36	44.98
	C	0.52	3.30	0.00	38.07	0.19	42.08
140	R	0.91	3.66	1.42	40.31	0.39	46.69
	C	0.65	3.37	0.00	39.64	0.20	43.86
168	R	1.23	4.12	1.41	41.58	0.40	48.75
	C	0.78	3.89	0.00	40.30	0.07	45.04
196	R	0.96	4.08	1.37	41.86	0.10	48.73
	C	0.92	3.80	0.00	40.49	0.02	45.29

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

TABLE 8. POLYUNSATURATED FATTY ACID CONCENTRATIONS OF THE NEUTRAL LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	18:2[ac] %	18:3[ab] %	20:2[ad] %	20:3[ab] %	20:4[ac] %	20:5[a] %	22:4[a] %	PUFA[ac] %
0	R	2.08	0.00	0.00	0.00	0.00	0.00	0.00	2.08
	C	2.00	0.00	0.24	0.24	0.11	0.00	0.00	2.60
28	R	1.71	0.00	0.00	0.00	0.00	0.00	0.00	1.71
	C	2.11	0.00	0.12	0.29	0.08	0.00	0.00	2.60
56	R	1.81	0.07	0.00	0.00	0.00	0.00	0.00	1.88
	C	2.52	0.00	0.12	0.22	0.04	0.01	0.02	2.97
84	R	2.05	0.00	0.00	0.00	0.00	0.00	0.00	2.05
	C	2.52	0.00	0.08	0.19	0.06	0.01	0.01	2.86
112	R	2.28	0.04	0.00	0.02	0.00	0.00	0.00	2.37
	C	2.64	0.00	0.08	0.18	0.04	0.01	0.01	2.95
140	R	2.26	0.05	0.00	0.02	0.00	0.00	0.00	2.34
	C	2.67	0.00	0.05	0.18	0.03	0.00	0.01	2.97
168	R	2.15	0.03	0.00	0.03	0.00	0.00	0.00	2.21
	C	2.56	0.00	0.04	0.18	0.03	0.00	0.00	2.85
196	R	1.96	0.06	0.00	0.06	0.00	0.00	0.00	2.08
	C	2.08	0.00	0.02	0.21	0.10	0.00	0.00	2.41

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

TABLE 9. POLYUNSATURATED FATTY ACID CONCENTRATIONS IN THE POLAR LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	18:2[b] %	18:3[ad] %	20:2[ac] %	20:3[a] %	20:4[ab] %	20:5[ab] %	22:4[a] %	22:5[ab] %	22:6[ab] %	PUFA[ab] %
0	R	16.24	2.97	2.12	0.00	7.39	0.54	1.77	0.74	3.46	35.22
	C	13.49	0.00	0.18	0.28	7.36	0.96	2.01	0.00	1.42	25.71
28	R	16.19	2.05	1.74	0.00	5.36	0.34	1.36	1.13	3.01	31.18
	C	14.01	0.00	0.13	0.21	6.41	0.67	1.75	0.00	1.39	24.60
56	R	20.31	1.45	1.92	0.00	5.98	0.15	1.40	0.57	2.99	34.78
	C	19.39	0.00	0.16	0.12	7.42	0.90	2.04	0.00	1.37	31.40
84	R	20.35	0.92	1.71	0.00	5.68	0.05	1.07	0.54	2.44	32.76
	C	19.35	0.00	0.11	0.10	6.40	0.71	1.82	0.00	1.16	27.90
112	R	20.66	0.52	1.90	0.00	5.63	0.00	0.99	0.46	2.06	32.23
	C	18.40	0.00	0.09	0.14	5.82	0.67	1.90	0.00	0.89	27.90
140	R	22.14	0.32	1.94	0.00	5.52	0.00	0.68	0.65	1.72	32.96
	C	19.23	0.00	0.13	0.16	6.31	0.87	2.00	0.00	0.74	29.45
168	R	19.22	0.26	1.77	0.00	5.17	0.00	0.56	0.94	1.48	29.39
	C	19.08	0.00	0.06	0.18	6.33	0.64	1.90	0.00	0.62	28.82
196	R	15.32	0.04	1.59	0.00	4.39	0.00	0.25	0.59	1.07	23.24
	C	16.71	0.00	0.03	0.28	6.45	0.84	1.83	0.13	0.58	26.84

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

TABLE 10. POLYUNSATURATED FATTY ACID CONCENTRATIONS OF THE TOTAL LIPID ACROSS TIME-ON-FEED.

TOF d	TRT	18:2[ac] %	18:3[ab] %	20:2[ad] %	20:3[ab] %	20:4[ac] %	20:5[a] %	22:4[a] %	22:5[ac] %	22:6[ac] %	PUFA[ac]
0	R	6.46	0.93	0.66	0.00	2.27	0.17	0.54	0.22	1.06	12.31
	C	3.29	0.00	0.23	0.25	0.94	0.12	0.29	0.03	0.07	5.22
28	R	4.94	0.45	0.39	0.00	1.15	0.07	0.30	0.21	0.64	8.15
	C	3.08	0.00	0.12	0.29	0.60	0.06	0.20	0.00	0.04	4.38
56	R	4.61	0.28	0.28	0.00	0.91	0.02	0.21	0.09	0.44	6.85
	C	3.65	0.00	0.12	0.21	0.57	0.08	0.20	0.01	0.02	4.87
84	R	5.13	0.15	0.28	0.00	0.93	0.01	0.17	0.09	0.39	7.16
	C	3.75	0.00	0.08	0.19	0.50	0.06	0.19	0.00	0.02	4.80
112	R	3.56	0.10	0.14	0.02	0.40	0.00	0.07	0.03	0.14	4.48
	C	3.42	0.00	0.08	0.17	0.33	0.04	0.17	0.00	0.02	4.22
140	R	3.96	0.08	0.16	0.02	0.45	0.00	0.06	0.05	0.14	4.93
	C	3.43	0.00	0.08	0.18	0.32	0.04	0.14	0.00	0.01	4.21
168	R	3.36	0.05	0.13	0.03	0.38	0.00	0.04	0.06	0.11	4.18
	C	3.38	0.00	0.01	0.18	0.35	0.04	0.14	0.00	0.00	4.10
196	R	2.81	0.06	0.10	0.05	0.28	0.00	0.02	0.04	0.07	3.42
	C	2.79	0.00	0.02	0.21	0.41	0.04	0.14	0.00	0.00	3.62

[a]C differed from R ($P<.05$).

[b](C-R): linear effect over TOF ($P<.05$).

[c](C-R): quadratic effect over TOF ($P<.05$).

[d](C-R): cubic effect over TOF ($P<.05$).

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