International Journal of Animal and Veterinary Advances 3(5): 379-385, 2011
ISSN: 2041-2908

Submitted: August 12, 2011 Accepted: September 17, 2011 Published: October 15, 2011

Effect of Different Oil Sources on Muscle Fatty Acid Composition and Serum Lipoproteins Levels in Sarabi Beef Steer

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Abstract: This study examined the effects of different vegetable oil sources on the Fatty Acid (FA) composition of muscle and performance of beef steer (Sarabi strain). Twenty one steers (384±17 kg BW) were assigned in seven treatment that fed diets containing 0% oil (control), 2 and 4% of Canola Oil (CO), Sunflower Oil (SO) and Restaurant Waste Oils (RWO). Ribeye steaks from steers fed CO, SO and RWO for 90 days of experiment were used after slaughtering to evaluate the effects of oil source on fatty acid composition. Amounts of muscle saturated FAs decreased and polyunsaturated FAs increased in both 2% CO and 2% SO groups. The highest contents of Total n-3, n-6 and n-7 FAs were significantly ($p<0.05$) obtained with 2% CO, 2% SO and control groups, respectively. Animals fed 2% CO had the lowest content of total n-9 FAs in compared to other groups. Control and 2% SO dietary groups presented lowest total cholesterol and 4% CO group resulted in a lowest triglycerides ($p<0.01$). The high and low-density lipoprotein (HDL and LDL) was highest in 2 and 4% RWO group, respectively and animals fed 4% SO and 4% CO had the lowest LDL and very low-DL (VLDL), respectively. Control animals and those fed 2% oils tended to have higher dry matter intake (DMI, <0.05). The best Daily Weight Gain (DWG) was related to 2% RWO dietary group and followed by 2% SO dietary groups; however, differences were not significant.

Key words: Beef steer and performance, fatty acid, vegetable oil

INTRODUCTION

Enhancing the n-3 Polyunsaturated Fatty Acid (PUFA) level of beef is important in view of the generally saturated nature of FA in ruminant muscles and the negative effect this can have on human health (Rule *et al*., 1994; Mandell *et al*., 1997). In ruminants, altering tissue fatty acid composition is difficult because unsaturated FAs are extensively biohydrogenated by rumen microorganisms (Demeyer and Doreau, 1999). It was demonstrated that incorporation of dietary PUFA into edible tissues indicate that at least a portion of fatty acids can escape biohydrogenation by ruminal microorganisms (Rule *et al*., 1994; Lough *et al*., 1992). Casutt *et al.* (2000) observed that concentrations of n-3 series such as α-linolenic acid in adipose tissue of Brown Swiss bulls fed flaxseed were increased.

Inclusion of Polyunsaturated Fatty Acid (PUFA)-rich plant oils or whole seeds in ruminant rations was shown in several studies (Scollan *et al*., 2001a; Mir *et al*., 2002; Mir *et al*., 2003) to increase the concentration of CLA and PUFA in meat, despite the extensive bio-hydrogenation of dietary lipids within the rumen. Notable effects of PUFAs in human diets include improved immunity (Harbige, 1998), reduced risk of cardiovascular disease (Alexander, 1998), and anti-inflammatory relief for rheumatoid arthritis (Belch and Muir, 1998; Pariza *et al*., 2001). Focant *et al.* (1998) demonstrated that feeding Linseed Oil (LO) to lactating dairy cows increased omega-3 (n-3) fatty acids deposition in soft tissues. N-3 fatty acids have been reported to exert beneficial effects on cardiovascular health and subsequently on performance (Simopoulos, 1999; Lee and Lip, 2003).

There is much interest in adaptation of the FA profile of meat, eggs and milk fats, due to a greater consumer demand for dietary mono or poly-unsaturated FA, which are perceived to be healthier than saturated FA (SFA) (Elgersma *et al*., 2003). SFA - mainly lauric, myristic and palmitic FAs - are reported to be responsible for increasing total plasma and low density lipoprotein-cholesterol (LDL-Chol) concentrations, while the other major SFA, stearic acid, has been shown not to increase total cholesterol or LDL-cholesterol concentrations (Bonanome and Grundy, 1988). MUFA and certain PUFAs play an important role in the prevention and treatment of Cardiovascular Diseases (CVD),

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Fig. 1: Sarabi beef steer

hypertension, diabetes, arthritis and other inflammatory or autoimmune disorders, and cancer (Simopoulos, 1999). The most important and necessary factors that can affecting FA composition are nutrition; season and breed; appropriate supplements and correct feed rations can increase the proportion of USFA and decrease the SFA level at the same time (Delbecchi *et al*., 2001; Ward *et al*., 2002; Stockdale *et al*., 2003). Dietary cholesterol is one of the factors reported to elevate serum cholesterol. Each 100mg increase in dietary cholesterol elevates the serum cholesterol level by 4-5 mg/dL (Randall *et al*., 1993). Although the role of dietary cholesterol on human health has been discussed, it is important to determine cholesterol level and composition of FA in food of animal origin including meat, egg, milk and their products.

Many studies are necessary for better understanding the efficacy of oil supplementation in beef steer. This study examined the effects of different oil sources on to the muscle n-3 PUFA concentrations, serum lipoproteins levels muscle and performance of beef steer (Sarabi strain).

MATERIALS AND METHODS

Animal, housing and feeding: The present study was carried out in summer of 2009 to 2010 in East Azarbayjan Province, Tabriz, Iran. A total of 21 Sarabi steers (384 kg BW±17 kg, Fig. 1) were adapted to a common diet based on dry-rolled corn for 17 d prior to beginning trial. Hence, differences in gastrointestinal fill were minimized. Animals were divided by initial BW and allotted, within strata, to 7 experimental treatments, with a total of 3 steers per treatment. The experiment was conducted as a randomized complete-block design with each animal as the experimental unit. Dietary treatments were: 0% oil (control), 2% canola oil (CO), 2% sunflower oil (SO), 2% restaurant waste oils (RWO), 4% CO, 4% SO and 4% RWO. The experimental oils added to concentrates of the same diet containing 5.2 kg hay, 1.2 kg corn silage and 2 kg concentrate (171 g CP/Kg DM) that were fed to each of steer in each day. Corn silage and hey was fed at 14:00 h daily, and the concentrates in two equal portions at 09:00 h and 16:30 h.

Steers were implanted with Component-TES (Vetlife, Inc., Norcross, GA) and placed into individual pens and fed their respective diets once daily to allow *ad libitum* consumption for 90 d. Bulls were weighed every week and data on weekly food intake, Food Conversion Ratio (FCR) and Daily Weight Gain (DWG) were recorded in each replicate group and the Body Weight (BW) presented as growth performance at the end of trial. On day 90, steers were individually weighed and transported to a commercial slaughter facility and after which carcass data were obtained. Following a 24 h chill, wholesale ribs were removed from the left side of each carcass. Ribs were sealed in vacuum packages and aged for 2 d at 4ºC. Steaks (1 steak per side; 2.54 cm thick) were cut from the posterior end of the rib, and were used for the following analyses: fatty acid composition or other meat characteristics. Similar locations within the steaks were utilized for common assay.

Chemical analysis: A ribeye steak from each steer was coarsely ground through a plate grinder and sub-sampled. FA composition were extracted in ribeye samples (1 g) with a 1 chloroform: 2 methanol (v:v) according to the method of Folch *et al.* (1957) Separation of fatty acid methyl esters in meat samples was performed by a Gas Chromatography (Italy), equipped with a flame ionization detector, data processor (GC-1000, Dany), hydrogen generator (Glaind-2200, Italy) and a split-splitless injector on an Altech Econo-Cap, EC-1000 capillary column (30 m × 0.25 mm i.d., film thickness of 0.25 μm). The methyl esters were extracted in 0.5 mL of 3× *n*-heptane, and 1 μL was injected into the gas chromatograph using helium as the carrier gas at a flow rate of 1.2 mL/min. Initial temperature was 75°C for 1 min, and this was followed by an increase of 30°C/min to a final temperature of 182ºC that held for 8 min at this temperature and then increased further to 200ºC and held for 1 min. Serial To measurement of high-density lipoprotein–cholesterol (HDL-C), low and very low density lipoprotein cholesterol (LDL-C and VLDL-C) levels, days 42 and 49 blood samples were collected from steers via indwelling jugular catheter and serums was obtained by centrifugation at 5000 rpm for a period of ten minutes. An ALCYON-300, automated analyzer (American) was used according to fully enzymatic methods using commercial kits (Kone kit, Japan).

Table 1: Detected fatty acid methyl esters of experimental oils[1]

FA Methyl Esters (% wt/wt of total lipids)	CO	SO	RWO
C14:0	0.126667	0.103704	0.763232
C16:0	6.333333	6.5925934	0.7046
C18:0	1.666667	3.925926	5.358419
C20:0	0.193333	0.481481	0.476513
C22:0	ND	0.733333	ND
∑ Saturated FAs	8.319999	11.837037	47.302764
C14:1 (-5)	ND	ND	0.038042
C16:1 (-7)	0.25	0.22963	0.138356
C18:1 (-9)	51.06667	37.77778	40.04317
C18:1 (-7)	8.666667	3.703704	0.768938
∑ Monounsaturated FAs	59.98333	741.711114	40.950464
C18:2 (n-6) cis	22.535	41.629631	0.03548
C18:3 (n-3) (ALA)	8.115	2.740741	0.200794
C18:3 (-6)	0.425	0.622222	0.180284
C18:4 (-3)	ND	0.422222	ND
C20:2 (-6)	ND	ND	ND
C20:3 (-6)	ND	ND	0.111644
C20:3 (-3)	ND	0.618519	ND
C20:4 (-3)	ND	ND	0.385792
C20:4 (n-6) (AA)	ND	ND	ND
C20:5 (n-3) (EPA)	ND	ND	ND
C22:5 –6	ND	ND	ND
C22:5 (n-3) (DPA)	ND	0.257037	0.090969
C22:6 (n-3) (DHA)	ND	ND	ND
∑ Polyunsaturated Fas	31.075	46.290371	11.004963
Total FA	99.37833	99.83852	99.29623

[1]: Values represent the means of two determinations; [2]: CO: canola oil; SO: sunflower oil, [3]: RWO: restaurant waste oils; [4]: Other abbreviations; FA: fatty acid; ALA: α-linolenic acid; LA: linoleic acid; AA: arachidonic acid; EPA: eicosapentanoic acid; DPA: dicosapentanoic acid; DHA: docosahexanoic acid; ND: not detected

Table 2: The fatty acid composition of beef steer's muscle

Treatments	∑ n-3 FA	∑ n-6- FA	∑ n-7 FA	∑ n-9 FA
Control	0.43^{a}	3.68^{c}	3.39^{b}	30.80^{b}
2 %	2.77^{a}	11.93^{ab}	0.73^{b}	22.77^{b}
4 %	1.36^{b}	4.43^{b}	1.55^{b}	32.74^{ab}
SO				
2 %	1.82^{ab}	16.84^{a}	$0.90^{b}3$	2.82^{ab}
4 %	0.83^{b}	$9.96a^{b}$	1.55^{b}	30.82^{ab}
RWO				
2 %	0.54^{b}	6.42^{b}	1.43^{b}	34.58^{a}
4 %	0.34^{b}	6.47^{b}	1.49^{b}	32.57^{a}
SEM	0.23	1.20	0.42	1.11
p-value	**	**	**	**

Values are expressed as the mean and their SEM for three animals in each group; TSFA: total saturated fatty acid; TPUFA: total polyunsaturated fatty acid ratio; CO: canola oil; SO: sunflower oil; RWO: restaurant waste oil; *: $p<0.05$; **: $p<0.01$

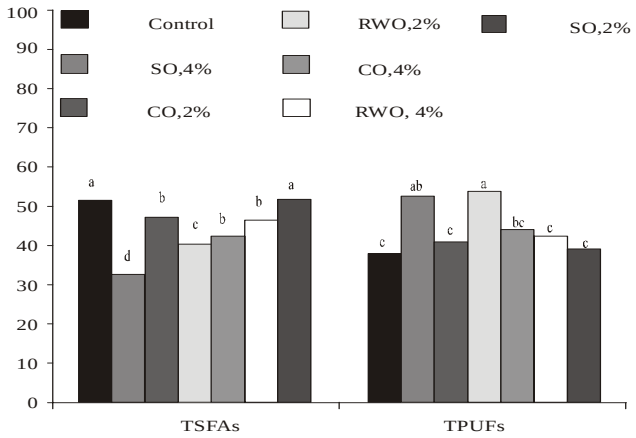

Fig. 2: Values of figures are correspondent to those shown in Table 2. TSFA: total saturated fatty acid, TPUFA: total polyunsaturated fatty acid; CO = canola oil; SO = sunflower oil; RWO = restaurant waste oil

Table 3: Blood lipoproteins levels of beef steers

Treatments	T-CHOL	T-TG	HDL	LDL	VLDL
Control	103.1[c]	38.47[ab]	69.80[a]	24.48[a]	8.18[ab]
CO2 %	143.9[ac]	37.27[ab]	82.53[a]	51.87[bc]	7.32[abc]
4 %	149.8[b]	23.32[b]	82.20[a]	71.97[ab]	4.71[c]
SO					
2 %	115.9[bc]	28.50[b]	83.90[a]	26.80[cd]	5.20[bc]
4 %	128.6[bc]	48.80[a]	80.36[b]	20.050[d]	9.40[a]
RWO2 %	140.8[ab]	32.60[b]	101.50[a]	30.80[cd]	6.53[bc]
4 %	175.1[a]	35.73[ab]	88.36[a]8	5.99[a]	6.76[abc]
SEM	11.55	7.251	0.18	5.87	2.11
p-value	**	**	**	**	**

Values are expressed as the mean and their SEM for three animals in each group; TSFA: total saturated fatty acid; TPUFA: total polyunsaturated

Statistical analysis: Performance and fatty acid data were analyzed by analysis of variance as a 2´2+1 randomized complete-block design using the MIXED procedure (SAS Institute, 2000. The GLM procedure of SAS was used for performance and FA data. Means and contrasts were considered significantly different when the F-test<0.05.

RESULTS

Fatty acid composition: The FA profiles of the experimental oils, Canola Oil (CO), Sunflower Oil (SO) and Restaurant Waste Oil (RWO), are shown in Table 1. CO, SO and RWO contained 8.319, 11.837037 and 47.302764% ∑ Saturated FAs, respectively. ∑ Monounsaturated FAs contents of CO, SO and RWO were 59.983337, 41.711114 and 40.950464%, respectively. While amounts of ∑ Polyunsaturated FAs of these supplemental oils were 31.075, 46.290371 and 11.004963, respectively. Predominant FA methyl esters in CO were palmitic acid (C16:0 = 6.333333%), oleic acid (C18:1n9 = 51.06667%), linoleic acid (C18:2 n-6, LA = 22.535%) and linolenic acid (C18:3 n-3, ALA = 8.115%) without a detected LC n-3 PUFA series. SO presented a same predominant C16:0, lower C18:1n9 (37.77778%) and C18:3 n-3, ALA (2.740741), higher C18:2 n-6, LA (41.62963) with detected LC n-3 PUFAs (C20:3, n-3 = 0.618519% and C20:5, n-3, DPA = 0.257037%). Predominant palmitic acid of RWO was much more than CO and SO (C16:0 = 40.7046%) with a 40.04317% C18:1n9 content while amounts of C18:3 (n-3, ALA) and C18:2 (n-6, LA) were lower than other experimental oils (10.03548 and 0.200794%, respectively).

The fatty acid composition is shown in Table and Fig. 2. Intramuscular saturated and unsaturated FA contents were significantly (p<0.05) different among treatments 2 and 4 % of oil origins (CO, SO and RWO). The TSFA and TPUFAs ratios were therefore, affected in groups adjusted to oils sources (Fig. 2). The SFAs contents of muscles in steers fed dietary 2% CO did showed the lowest value and followed by 2% SO dietary group. Similar to total SFA content of steaks of groups 2% CO and 2% SO, their total PUFA content did showed the significant different than other groups, but unlike SFA, the highest values of PUFA were observed in these groups (p<0.01). The lower SFA and higher PUFA level in tissue caused a reduced ratio of TSFA: TPUFA of muscle of beef steer fed dietary 2% canola or sunflower oils. 2% CO dietary groups presented the highest content of ∑ n-3 FAs, followed by 2% SO, while it was observed for ∑ n-6 FAs contents in reverse (p<0.05). Control group animals were significantly presented the highest level of ∑ n-7 FAs and animals fed 2% CO had the lowest content of ∑ n-9 FAs in compared to other groups. Therefore, muscle from beef steers fed 2% CO or 2% SO could decrease saturated/polyunsaturated FAs ratio.

Serum lipoproteins levels: Determined serum lipoproteins levels are shown in Table 3. Serum of animals fed on 2 and 4% dietary RWO did showed highest levels of high and low-density lipoproteins (HDL and LDL), respectively. Steers fed 4% SO and 4% CO had the lowest LDL and very low-DL (VLDL), respectively. Control animals and those fed 2% dietary SO presented lowest total cholesterol and 4% CO group resulted in a lowest triglycerides (p<0.01).

Performance: According results presented in Table 4, dry matter intake (IDM) significantly (p<0.05) decreased in groups supplemented with 4% oil sources in compared to control. DMI of animals fed from 2% canola or sunflower oils were not significantly decreased than the control group (p<0.01). Lipid source did not significantly influence growth rate but, DWG tended to be improved for steers fed diets containing 2% RWO, followed by 2% so compared with performance of steers fed other experimental diets.

DISCUSSION

Fatty acid composition: According to results from the presented study, groups 2% CO and SO have lowest SFAs, highest PUFA levels and also the highest content of ∑ n-3 FAs. Despite the high degree of bio-hydrogenation of dietary PUFA reported by Scollan *et al.* (2001b) and by Doreau and Ferlay (1994), supplementation with PUFA-rich rations (2% oil level) in the present experiment resulted in a decrease in the SFA and an increase in the PUFA proportion in the muscle and

n-3 enriched by oil rich in n-3 series. This decrease in SFA suggests an increase in the incorporation of PUFA in muscle at the expense of SFA, due to the different proportions of fatty acids and oil levels in the non-supplemented and supplemented diets.

Control group animals had the highest levels of ∑ n-7 FAs and those fed 2% CO had the lowest content of ∑ n-9 FAs in compared to other groups. Oil supplementation decreased further the proportion of palmitic acid as a reflection of the greater level of PUFA in the diet. This represents a nutritional improvement compared with the tissues from animals fed exclusively on pasture. Mir *et al.* (2002) reported an increase in PUFA and a decrease in SFA such that the SFA: PUFA ratio in muscle decreased when steers were supplemented with sunflower oil on a DM basis. Similarly, Madron *et al.* (2002) reported that the SFA: PUFA ratio in muscle decreased by feeding extruded soybeans rich in C18:2n-6. The SFA: PUFA ratio in muscle achieved in this experiment with the SO treatment, although lower than current recommendations (Department of Health, 1994) is among the greatest reported for cattle fed unprotected fat sources. Realini *et al.* (2004) and Duckett *et al.* (1993) recognized that the fat concentration of muscle has a major influence on the SFA: PUFA ratio because PUFA are mainly found in the polar lipid fraction, which is diluted by the growth in the neutral lipid fraction as animals accrete lipid. Mir *et al.* (2003) also found a significant increase in the total PUFA in muscle due to the increased incorporation of C18:2n-6 in the muscle for cattle supplemented with sunflower oil. Feeding LO similar to CO from the point of view PUFA and n-3 series levels, however, increased the n-3 PUFA proportion in muscle. Comparisons of the PUFA: SFA ratio across studies should therefore be made with caution because lean animals will have a lesser SFA: PUFA ratio irrespective of ration composition (Raes *et al*., 2003). In the presented study, the lower SFA and higher PUFA levels in tissue cause a reduced ratio of TSFA: TPUFAs ratio of bull steaks with diet supplementation by 2% of canola or sunflower oils (Fig. 2). It is recognized that an increase in the concentration of PUFA was achieved in the current study by CO and SO in 2% oil levels that could ensure the recommended consumption of PUFA from animal tissues, daily institute of medicine of the national academies (Institute of Medicine of the National Academies, 2002). Thus, 200 g of fresh muscle produced from bulls supplemented with canola oil or sunflower oil in the current study would provide with consider to recommendations of International Life Sciences Institute (International Life Sciences Institute, 1995) in the human daily requirements to PUFAs intake.

Blood lipoprotein level: Citing the results, it was evident that 2 and 4% RWO dietary groups did showed highest levels of high and low-density lipoproteins (HDL and LDL), respectively; while 2 and 4% SO dietary groups resulted in a lowest LDL and total cholesterol and a high HDL levels. Likewise, animals fed on 2% SO and 4% CO, presented lowest TG compared to other groups. Results of this study are agreement with findings of Randall *et al*. (1993), Elgersma *et al*. (2003) and Ward *et al*. (2002).

Table 4: Performance of steers fed different vegetable oils

Treatments	IDM (g)	DWG (g)
Control	6067[a]	1040
CO		
2 %	5903[a]	1021
4 %	5433[b]	1002
SO		
2 %	5800[a]	1080
4 %	5344[b]	954
RWO		
2 %	6070[a]	1121
4 %	5400[b]	950
SEM	152	84
p-value	*	ns

Values are expressed as the mean and their SEM for three animals in each group; DMI: dry matter intake; DWG: daily weight gain; CO: canola oil; SO: sunflower oil; RWO: restaurant waste oil; *: p<0.05fatty acid ratio; CO: canola oil; SO: sunflower oil; RWO: restaurant waste oil; *: p<0.05, **: p<0.01

Based on results of Table 1, it was revealed that RWO contained higher total SFAs (nearly 4 fold, 47.302764%) compared to CO and SO (8.319% and 11.837037%, respectively). Predominant SFAs such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) FAs, have been identified as a factor increasing Low Density Lipoprotein-cholesterol (LDL-Chol) concentrations (Bonanome and grundy, 1988). Hence, diet supplemented with RWO caused a high LDL in animals. RWO contained nearly same total MUFAs with SO and lower vs. CO (40.950464% compared to 41.711114% and 59.983337%). This result could explain the high HDL of steers serum caused by RWO dietary group (Table 3). Amounts of total PUFAs of CO, SO and RWO were 31.075, 46.290371 and 11.004963, respectively. Therefore, a lowest LDL-Chol and TG and also, a highest HDL or good cholesterol achieved by CO or SO dietary groups. MUFA and certain PUFAs play an important role in the prevention and treatment of Cardiovascular Diseases (CVD), hypertension, diabetes, arthritis and other inflammatory or autoimmune disorders, and cancer (Simopoulos, 1999). Dietary cholesterol (contained high SFAs) is one of the factors reported to elevate serum cholesterol (Stockdale *et al*., 2003). Although the role of dietary cholesterol on human health has been discussed, it is important to determine cholesterol level and composition of FA in food of animal origin including meat, egg, milk and their products.

Performance: Incorporation of lipid into beef steer diet did not influence DM intake, although there was a tendency for intake of the 4% oils diet to be lower.

Chilliard and Doreau (1997) reported a reduction of 1.6 kg DM per d when dairy cows fed on maize and concentrates were supplemented with 300 ml of fish oil (menhaden type) and similar effects have been observed elsewhere (Mandell *et al*., 1997; Wonsil *et al*., 1994). It is thought that these effects are mediated by specific fatty acids produced as a result of rumen bio-hydrogenation rather than a negative effect of the fatty acids of fish oil on rumen function, since the fish oil does not disturb ruminal fibre digestion (Chilliard and Doreau, 1997). Since intakes were similar across treatments of similar nutrient balance, no significant differences in daily weight gain were expected (Table 4). From the results in presented study it is concluded that supplementation of steers with vegetable oils rich in PUFAs in 2% levels led to a substantial increase in PUFA in compared with control or other groups by different oil or in 4% levels.

ACKNOWLEDGMENT

This study was funded by Department of Animal Science, Islamic Azad University, Heris Branch, Heris, Iran. The workers are thankful to Dr. Amir A. Matin for his attempts in conduction of laboratory assays.

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