Effect of Induced High Linoleic Acid and Tocopherol Content on the Oxidative Stability of Rendered Veal Fat

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ABSTRACT

Veal calves reared by the Animal Physiology and Genetics Institute, USDA, on milk supplemented with tocopherol then on a diet containing encapsulated safflower oil demonstrated increases in the concentrations of linoleic acid (18:2) and tocopherols in their depot fats. The expected decrease in the induction period of oxidation for these fats with 18:2 levels of 7-12.9% was not observed in the fats with increased tocopherol content. The fat of the treated calves was more stable to oxidation than the fat of commercial veal or pork.

INTRODUCTION

Increased evidence that linoleic acid (18:2) is an important factor in human nutrition and tends to favor low concentration of serum cholesterol has given substantial impetus to efforts to increase the level of polyunsaturated acids in the fat of ruminants (1-6). Ca. 14% fat consumed in the average American diet is derived from the meats of these animals in which the polyunsaturated fatty acids (PUFA) seldom exceed 4% of the fat fraction. Ruminant animals, such as sheep, cattle, etc., consume PUFA in grass, forage crops, and grains; but microorganisms in their rumen hydrogenate these fatty acids before they enter the body tissue.

Cook, et al., (2) Scott, et al., (3) and Plowman, et al., (5) reported a new process whereby polyunsaturated oil droplets are protected from ruminal hydrogenation by encapsulation with formaldehyde-treated protein. When these protected oils are fed to ruminants, the formaldehyde complex is hydrolyzed in the abomasum; and the fatty acids are absorbed from the small intestine. This results in a substantial increase in the proportion of PUFA in the triglycerides of milk fat and tissue fat.

Work was undertaken, in cooperation with the Animal Physiology and Genetics Institute, U.S. Department of Agriculture, Beltsville, Md., to determine the effect of feeding calves milk and encapsulated safflower oil with high concentrations of 18:2 upon: (A) the proportion of 18:2 in the triglycerides of veal fat, (B) the palatability of the fresh and frozen veal cuts, and (C) the stability of rendered veal fat.

This report primarily concerns the oxidative stability of the rendered veal fat in relation to changes in the proportions of 18:2 and the tocopherols. Such a study should be of considerable interest to the meat industry and consumer since the induction period of oxidation in rendered fats may be an indicator of the freezer storage life of veal.

EXPERIMENTAL PROCEDURES

Experimental Animals

The experimental animals were fed and reared by the Animal Physiology and Genetics Institute, USDA, Beltsville, Md. (4). A more complete history of these animals will be reported by the authors cited in reference (4) in the near future. Eight 4-day-old bull calves were selected. Four calves (G-1, G-5, G-3, and 5750) were given milk obtained from cows fed safflower oil coated with formaldehyde-treated casein (5) which contained 14% 18:2 in the milk fat for 10 weeks; the other four (G-4, G-8, G-6, and 5751) were fed normal milk, which contained 3.0% 18:2 in the fat. Both milks were supplemented with vitamin E. At the end of 10 weeks, two calves of each group were transferred to an 8 week feeding regimen of formaldehyde-treated casein-coated safflower oil mixed with calf starter, and two from each group were fed casein-coated, but not formaldehyde-treated, safflower oil mixed with calf starter. All eight calves were slaughtered after 8 weeks (18 weeks of age). The carcasses were allowed to hang for 24 hr at 3 C. Samples of round, kidney, and caul fatty tissue then were removed and stored at -18 C for 3 days. Ground fatty tissues, in a beaker, were placed in a 90-95 C water bath and stirred occasionally. The rendered fat, at a temperature

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TABLE I

Fatty Acid Composition of Veal Round Fat[a]

Fatty acid[b]	Experimental animals								Commercial animals	
	Fed milk high in 18:2				Fed normal milk					
	Protected safflower oil[c]		Unprotected safflower oil[c]		Protected safflower oil[c]		Unprotected safflower oil[c]			
	G-1	G-5	G-3	5750	G-4	G-8	G-6	5751	1	2
14:0	4.9	5.2	4.8	3.7	6.2	5.3	6.2	7.2	2.6	2.5
16:0	24.7	24.4	23.5	21.3	30.2	25.6	30.4	32.5	23.5	23.2
16:1	2.6	2.8	2.9	2.7	4.1	3.2	3.2	4.4	4.3	4.3
18:0	18.1	16.0	16.0	18.7	16.4	21.0	18.6	14.2	14.3	14.9
18:1	35.7	36.9	39.9	42.1	34.9	34.1	36.9	37.4	48.0	48.1
18:2	12.2	12.9	10.8	9.6	7.0	9.4	3.2	2.9	5.6	5.4
18:3	1.8	1.8	2.1	1.9	1.2	1.4	1.5	1.4	1.7	1.6

[a]Wt%

[b]Carbon chain length: number of double bonds.

[c]Supplement to milk.

TABLE II

Fatty Acid Composition of Veal Kidney Fat[a]

Fatty acid[b]	Experimental animals								Commercial animals	
	Fed milk high in 18:2				Fed normal milk					
	Protected safflower oil		Unprotected safflower oil		Protected safflower oil		Unprotected safflower oil			
	G-1	G-5	G-3	5750	G-4	G-8	G-6	5751	1	2
14:0	4.0	4.5	3.5	1.7	3.4	3.2	4.8	4.4	2.3	2.1
16:0	24.0	21.7	21.6	15.3	20.6	19.8	24.1	25.0	22.0	21.1
16:1	1.6	1.3	1.9	1.2	1.4	1.4	1.8	2.2	3.5	3.3
18:0	26.7	27.2	24.6	35.5	32.1	30.6	28.5	27.5	18.5	18.9
18:1	30.5	31.5	37.6	37.2	27.6	29.2	35.4	36.1	46.1	47.3
18:2	14.8	12.4	9.2	7.6	13.8	14.7	3.6	3.5	5.9	5.7
18:3	1.4	1.4	1.6	1.5	1.1	1.1	1.8	1.3	1.7	1.6

[a]Wt%.
[b]Carbon chain length: number of double bonds.

of less than 75 C, was filtered through cheesecloth.

Commercial veal samples were obtained fresh from the killing floor of a local slaughter plant. The samples then were treated in the same manner as the ones from the experimental animals.

Beef and pork tissue fats analyzed for comparison also were from Animal Physiology and Genetics Institute, USDA, Beltsville, Md. The samples also were treated in the same manner as the ones from the experimental animals.

Stability Test

The stability of the rendered fat was determined by an oven method at 60 C using 15 mm layers of rendered fat (7).

Peroxides Determination

Peroxides were determined by the iodometric method of Kenaston, et al., (8) and the values expressed as milliequivalents/1000 g fat.

Tocopherols Determination

Total tocopherols in the rendered fat were determined by the column chromatographic method of Erickson and Dunkley (9).

Fatty Acids

Procedure for fatty acids was essentially that of Schmit and Wynne (10). All solvents were redistilled. A mixture of 1 drop of rendered fat (ca. 20 mg), 9 ml ethanol, and 1 ml 33% aqueous KOH (prepared daily) was placed in a 50 ml test tube, flushed with N_2, tightly stoppered, and heated in a water bath at 55 C for 20 min. After cooling in an ice bath, the mixture was acidified with concentrated HCl and extracted with three 10 ml portions of hexane. The combined hexane extracts were washed with two 10 ml portions of H_2O, dried over Na_2SO_4, and filtered into a 50 ml round-bottom flask and the solvent removed with a stream of N_2. To the flask was added 10 ml 5% $HClO_4$ in MeOH. The flask was flushed with N_2, loosely stoppered, and heated in a water bath at 55 C for 20 min. The mixture was cooled in an ice bath and extracted with three 10 ml portions of 1:1 hexane-ether. The combined extracts were washed with three 10 ml portions of H_2O, dried over Na_2SO_4, and filtered. Solvent was removed with a stream of N_2 and 0.2 ml of 500 mg % methyl heptadecanoate in $CHCl_3$ was added. The methyl esters were analyzed using an F&M Model 810 gas chromatograph equipped with a dual hydrogen flame detector. The column was 1/4 in x 6 ft stainless steel tubing packed with 10% DEGS on 60-80 mesh acid-washed, Chromosorb W, treated with dimethyldichlorosilane (DMCS), programed from 100-210 C at 4°/min. Temperature of the injection port was 235 C, of the detector 240 C; and the flow rates were: He, 35 ml/min; H_2, 32 ml/min; and air, 300 ml/min. A standard methyl ester mixture on an equal wt basis was prepared. The peak-height response ratios of the standard C_{17} were determined, and these ratios were used for normalizing the peak height for the sample runs containing the C_{17} internal standard. Numerous minor peaks which represented ca. 5% or less of the total peak heights were not considered in the analysis.

RESULTS AND DISCUSSION

Fatty Acid Composition

The fatty acid compositions of round, kidney, and caul

TABLE III

Fatty Acid Composition of Veal Caul Fat[a]

Fatty acid[b]	Experimental animals							
	Fed milk high in 18:2				Fed normal milk			
	Protected safflower oil		Unprotected safflower oil		Protected safflower oil		Unprotected safflower oil	
	G-1	G-5	G-3	5750	G-4	G-8	G-6	5751
14:0	4.5	4.8	4.4	6.1	3.3	4.9	5.9	7.2
16:0	22.9	22.2	23.0	27.4	19.7	25.5	28.2	29.9
16:1	1.6	1.5	1.9	1.9	1.6	1.9	2.2	2.1
18:0	24.9	25.9	23.6	26.8	27.8	28.0	25.9	23.4
18:1	30.1	32.2	35.4	28.4	36.4	28.8	32.8	32.9
18:2	14.6	12.0	10.1	8.4	9.6	9.8	3.6	3.3
18:3	1.4	1.4	1.6	1.0	1.6	1.1	1.4	1.2

[a]Wt%.
[b]Carbon chain length: number of double bonds.

TABLE IV

Effect of 18:2 and Tocopherols upon Stability of Rendered Fat

	Kidney fat	Round fat		
Animal no.	μg Tocopherols/g fat	μg Tocopherols/g fat	Percent 18:2	Induction[a] period (days)
G-5	32.0	53.0	12.9	19
G-1	30.0	60.0	12.2	27
G-3	18.7	25.4	10.8	17
Pork back fat	---	7.5	9.9	8
5750	20.3	25.7	9.6	21
G-8	28.9	36.3	9.4	25
G-4	21.9	30.5	7.0	28
1	9.4	8.0	5.6	14
2	11.7	9.0	5.4	15
G-6	15.4	25.8	3.2	42
5751	25.0	34.4	2.9	47
Beef fat	10.1	8.6	---	---

[a]Time in days to reach a peroxide value of 15 meq/1000 g at 60 C.

fat from calves fed various diets are shown in Tables I, II, and III. Wrenn, et al., (4) had reported tailhead fat biopsies of these animals at 10 weeks. His data revealed that the 18:2 content of the depot fat of the calves fed high 18:2 milk was 11.9% of the fat fraction compared to 3.0% in the fat from calves fed normal milk. The results in Tables I, II, and III indicate that the proportion of 18:2 in the fat of animals 5751 and G-6 remained at the normal level for ruminants after they were fed normal milk and unprotected safflower oil for 8 weeks. On the other hand, the animals fed normal milk and protected safflower oil (G-4 and G-8) showed two- to threefold increases in the proportion of 18:2 in the triglycerides of the round and caul fats and a fourfold increase in the kidney fat. Furthermore the proportions of 14:0, 16:0, and 16:1 in these animals' kidney and caul fats decreased. Cook, et al., (2) observed similar results in the adipose tissue of lambs fed protected safflower oil for 6 weeks. Results in Tables I, II, and III indicate that variations were relatively small in the proportions of 18:2 among the round, caul, and kidney fats of the animals that were fed both high 18:2 milk and protected safflower oil (G-1 and G-5).

Fat from the commercial veal animals (1 and 2) had higher proportions of 18:1 and 18:2 than fat from the experimental control animals and lower proportions of 14:0 and 16:0 (Tables I and II). The history of these animals prior to slaughter is not known, and literature does not report the normal level of 18:2 in the fat of young veal animals. However, a review of the literature (11) on the composition of natural fat indicates that the proportion of 18:2 in the fat of older ruminants seldom exceeds 3.5%.

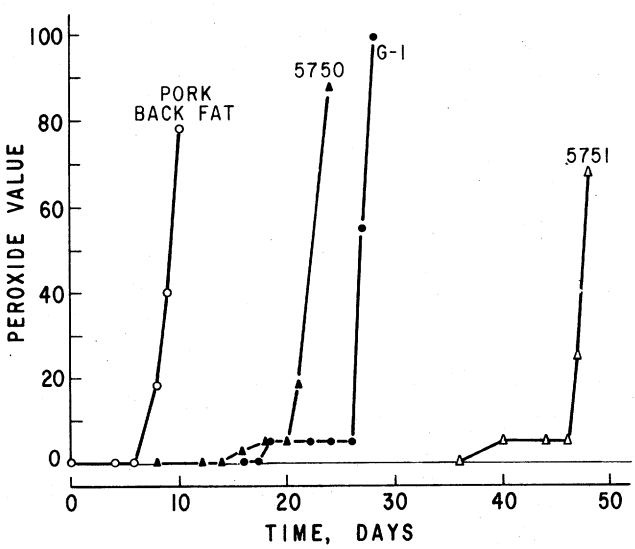

FIG. 1. Autoxidation of rendered pork and veal fats at 60 C. Induction period: time in days to reach a peroxide value of 15 meq/1000 g.

Tocopherols

Tocopherol levels in the depot fat from tne round of the experimental animals were three-seven times the levels of the commercial veal, which were similar to those of pork and beef fats (Table IV). These differences apparently were due to the diet, since milk diets of all experimental animals were supplemented with vitamin E. Safflower oil also contains a relatively high concentration of tocopherols. Herting and Drury (12) reported an average tocopherol content of 340 μg/g for refined safflower oil. The data in Table IV show that the tocopherol levels of the round fat from animals G-3, G-4, 5750, G-8, 5751, and G-6 were quite similar and were three-four times the concentrations found in the commercial animals (1 and 2). The tocopherol levels of the round fat of animals that were fed both high 18:2 milk and protected safflower oil (G-1 and G-5) were ca. double those of the other experimental animals. Scott, et al., (3) suggested that encapsulation may protect soluble lipid substances, e.g. steroids, antioxidants, drugs, etc., which normally would be metabolized by rumen microorganisms. Therefore, the high tocopherol levels for G-1 and G-5 may be explained partly by encapsulation; this explanation, however, is not applicable to G-4 and G-8 since they also were fed encapsulated safflower oil. In all experimental animals, tocopherol levels of the kidney fat were less than those of the round depot fat (Table IV). The commercial veal and beef animals showed a slightly higher proportion of tocopherols in the kidney fat than in the round fat.

Deposition of dietary tocopherol in animal tissue is inefficient, particularly when given to the animal by mouth. Caravaggi, et al., (13) reported that sheep fed α-tocopheryl acetate excreted virtually all of it in the feces in 4 days. However, tissue levels can be increased by increasing tocopherol levels in the diet over a period of time. Buchanan-Smith, et al., (14) reported that an increase in the vitamin E level of the diet resulted in significant increases in the tocopherol content of the skeletal muscle of ewes. Among species, however, differences have been shown in the ability to deposit dietary tocopherol in tissues. In a classical example, Mecchi, et al., (15) reported that the tocopherol of chicken was higher than that of turkey fed the same diet.

The Effect of 18:2 and Tocopherols upon the Stability of Rendered Round Veal Fat

Stability toward oxidation is an important quality factor

in meat. Fats, such as pork, with high levels of PUFA are susceptible to oxidation on frozen storage, with an accompanying decline in palatability. Such fats, when rendered, exhibit a short induction period before rapid oxidation occurs. The induction period for the rendered round veal fats is shown in column 5, Table IV, and in Figure 1. For comparison a stability test with rendered pork back fat was carried out simultaneously with the veal samples.

Oxidative stability of the fat was related inversely to the 18:2 content when the tocopherol levels were similar (Table IV). Commercial veal fat (2), which contains ca. one-half the 18:2 level of, and one-fifth greater, tocopherol than the pork fat, had an induction period twice as long as that observed for pork. Increasing concentration of 18:2 in the fat present in animals G-6, 5750, and G-3 was accompanied by corresponding decrease in the induction period.

The tocopherols appeared to increase substantially the induction period. The fat from animals G-8 and 5750 contained ca. the same level of 18:2 as the pork back fat, but the length of induction period for these veals was ca. three times that for pork. Tocopherol levels, however, were 3.5-5 times the levels in pork fat. The fat of veal animals G-1 and G-5 contained 12.2 and 12.9% 18:2, respectively, and would be expected to have induction periods considerably shorter than those for either the commercial veal or pork back fat, which contained lower concentrations of 18:2. However, the induction periods for G-1 and G-5 were 1.5-3.0 times as long as those for the commercial veal and pork, respectively, apparently because of the high levels of tocopherol.

The activity exhibited by dietary tocopherol on the stability of veal fat appears to be contrary to that reported for hogs. Watts, et al., (16) found that the amount of dietary tocopherol deposited in hog fat was too small to be of practical value. Species difference in ability to deposit dietary tocopherol in tissue already has been cited.

Our results indicate that with an increase in the 18:2, a corresponding increase in the deposition of tocopherols will moderate the expected decrease in stability of the depot fat of veal animals to oxidation. It is suggested that the freeze storage life of meat samples from these animals will be as great as, or greater, than commercial pork meat samples.

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