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Registry No. Ca, 7440-70-2; lipase, 9001-62-1; sodium taurocholate, 145-42-6.

Effect of Dietary Iron Level on in Situ Turkey Muscle Lipid Peroxidation†

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The main object of this study was to evaluate the effect of removing the iron (Fe) supplement from the diet fed prior to slaughtering on the stability of turkey muscle tissue stored at 5 °C. The results demonstrate that a decrease in nutrititional Fe 3–7 weeks prior to slaughtering could result in a reduction of more than 50% in lipid peroxidation in in situ turkey dark muscle. Turkey light muscle, which is by its nature more stable to lipid peroxidation, was affected during the first experiment but not during the second. Removal of the Fe supplement from the diet fed during 3–7 weeks prior to slaughtering did not affect body weight or blood hematocrit, although the latter was slightly decreased.

It is becoming increasingly apparent that in vivo lipid peroxidation and several other tissue injuries derived from oxidative processes are accompanied by iron (Fe) toxicity (Halliwell and Gutteridge, 1984; Aust et al., 1985; Jacobs, 1977; Beanish et al., 1974).

Iron overload is a medical problem. The potential toxicity associated with excessive iron intake is exemplified by the tissue damage observed in human patients suffering from disorders commonly called hemochromatoses and those receiving blood transfusions for treatment of β-thalassemia (Jacobs, 1977; Beanish et al., 1974).

Goldberg's group (1962) performed some of the first studies linking iron-loading to lipid peroxidation. These authors demonstrated that iron–dextran injections caused accumulation of Fe in certain organelles and the changes noted closely resembled those observed in animals deficient in vitamin E. It has been shown similarly that a vitamin E deficiency increases lipid peroxidation induced by Fe–dextran (Dillard et al., 1984).

Lipid peroxidation is one of the primary reactions leading to quality deterioration in stored foods and especially in muscle tissues (Wilson et al., 1976). One of the main problems with regard to lipid peroxidation in muscle tissue was identification of the nature of the predominant form of Fe catalysts, e.g., myoglobin, non-heme iron or "free" iron ions involved in the initiation of this process. Most recently, it was found by us that free iron ions are the most important catalysts of in situ muscle lipid peroxidation (Kanner et al., 1988a–c). These results led us to develop the recent study.

The Fe requirement of turkeys at age 16–24 weeks is a 50 mg/kg diet (National Research Council, 1984). According to Scott et al. (1976) the Fe content of normal feedstuffs should be sufficient to meet the nutritional requirements of chickens without the use of iron supplements. Nevertheless, commercial diets are usu-

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Table I. Composition of the Basal Diets (g/kg)

ingredient[a,b]	age period, weeks 16–20	18–25
sorghum grain	408.5	490.8
yellow corn	250	250
soybean oil meal (44.5% protein)	244	166
DL-methionine	1	0.7
soybean oil soapstock	59	55
constant ingredients[c]	37.5	37.5
total	1000	1000
calcd compn		
metab energy, kcal/kg	3220	3282
crude protein, %	16.7	14

[a] Vitamin mix (mg/kg of diet): choline chloride, 400; riboflavin, 5.0; Ca-pantothenate, 12; pyridoxine, 1.5; folic acid, 0.5; menadione, 2; niacin, 60; vitamin B_{12}, 0.01; zinc bacitracin, 25; ethoxyquin, 125. Additional components (per kg): vitamin A, 10 000 IU; α-tocopherol, 10 IU; vitamin D_3 2000. [b] Mineral mix (g/kg): sodium chloride, 2.5; sodium sulfate, 1. Additional components (mg/kg): Mn, 80; Zn, 80; Fe, 25; Cu, 2; I, 1.2; Co, 0.2; Se, 0.2. [c] Dicalcium phosphate, 20; ground limestone, 10; vitamin mix, 2.5; mineral mix, 4 g/kg.

ally supplemented with Fe at a level of ca. 25 mg/kg to ensure adequate Fe content.

This study was conducted to evaluate whether the stability of turkey muscle to the process of lipid peroxidation could be improved when the Fe supplement is omitted from the diet fed during the last growth period.

MATERIALS AND METHODS

Sixteen-week-old male, British United turkeys, kept in individual cages in a temperature-controlled room (24 °C) under continuous artificial illumination, were used. Up to that age, the birds were fed commercial diets formulated according to the recommendations of the National Research Council (1984) for the 0–4-, 4–8-, 8–12-, and 12–16-week age groups. At the beginning of the experiment, the birds were individually weighed, and approximately one-third of them, the lightest and the heaviest, were discarded. The remainder were assigned to two and three groups of six birds each (experiments 1 and 2, respectively), with similar average weight and weight distribution. One group (treatment 1) was fed ab libitum the diets detailed in Table I (in mash form) and served as the control. The other groups were fed the same diets, but their mineral mixture did not contain the Fe supplementation. The turkeys were weighed biweekly. At the termination of the experiment, at 25 (experiment 1) or 23 (experiment 2) weeks of age, blood was taken with a heparinized syringe from the brachial vein and the birds were then slaughtered. Breasts and thighs were removed and immediately cooled on ice.

The hematocrit was determined with a Hettich centrifuge and a Clay Adams micro-hematocrit reader.

Fresh, dark (thighs) and white (breasts) muscles of six turkeys from each treatment were stored at 4 °C in polyethylene bags and tested periodically for lipid peroxidation.

Thiobarbituric acid reactive compounds (TBA-RC) were determined by a method developed by Witte et al. (1970) and calculated as malonodialdehyde (MDA) with an extinction coefficient of $\epsilon_{532} = 1.55 \times 10^5\ M^{-1}\ cm^{-1}$ (Buege and Aust, 1978). Iron was determined by wet digestion with nitric and perchloric acids, as recommended by Schricker et al. (1982), and measured on a Perkin-Elmer atomic absorption spectrophotometer and an air acetylene flame at 248.3 nm.

The Hornsey (1956) method was used to determine heme iron. Nitric oxide heme compounds were extracted by acidified acetone, dehydrated, and then wet digested and determined as iron with use of an atomic absorption spectrophotometer.

Results were subjected to analysis of variance (Snedecor and Cochran, 1967) and to a multiple-range test (Duncan, 1955). In the figures, each bar denotes the standard deviation.

Table II. Effect of Diet without Iron Supplementation on Body Weight and Blood Hematocrit of Turkey (Experiments 1 and 2)[a]

	treatment			
		without Fe		
	control	20–25 weeks	16–23[c] weeks	SE[b]
Experiment 1				
body wt, 16 weeks, g	10156	10083		201
body wt, 20 weeks, g	13239	13713		307
body wt, 25 weeks, g	15739	15707		511
hematocrit, 25 weeks, %	41.7	38.4		1.3
Experiment 2				
body wt, 16 weeks, g	9788	9647	9777	143
body wt, 20 weeks, g	13488[a]	12822[b]	13548	201
body wt, 23 weeks, g	14645	13958	14857	320
hematocrit, 23 weeks, %	41.1[ab]	42.4[a]	36.6[b]	1.8

[a] Average of six birds per treatment. [b] SE = standard error. Calculated from the error term of the analysis of variance. [c] 23 weeks in experiment 2. (a, b) Within each age group, values followed by different superscripts differ significantly ($P < 0.05$).

RESULTS AND DISCUSSION

The main object of this study was to evaluate the effect of removing the Fe supplement from the diet fed during the last stage of growth on the stability of turkey muscle tissue. Therefore, the effect of such removal on growth performance was not investigated adequately (a small number of birds per treatment and without replicates). Nevertheless, the data obtained can be used as evidence that removal of the Fe supplement from the diet fed during 20–25 weeks of age (first experiment) did not affect body weight or blood hematocrit significantly, although the latter was slightly decreased (Table II).

During the second experiment, removal of the Fe supplement from the diet fed either during 20–23 or 16–23 weeks of age did not affect body weight significantly (Table II). However, feeding the Fe-unsupplemented diets from 16 to 23 weeks of age resulted in a marked decrease in the hematocrit. These data agree with those of Morck and Austic (1981) who reported that the decrease in hematocrit in white Leghorn laying hens, due to Fe deficiency was not followed by a decrease in body weight and egg production during the early stage of the experiment. Calculations carried out according to the data of Scott et al. (1976) show that the grains and the soybean oil meal contributed 66.9 and 59.6 mg of Fe/kg of the diets fed during 16–20 and 20–25 weeks of age, respectively. The decrease in the hematocrit values of birds fed these diets suggests that the Fe from these sources was not completely available, probably due to the presence of phytic acid.

The results demonstrate (Figures 1 and 2) that a decrease in nutritional iron 3–7 weeks prior to slaughtering could result in a reduction of more than 50% in lipid peroxidation in in situ turkey dark muscle. Turkey light muscle, which is by its nature more stable to lipid peroxidation, was affected during the first experiment (Figure 1) but not during the second.

In muscle tissues, myoglobin is the most abundant iron compound. In turkey dark and white muscles, the total iron ions are at an average of 12–13 and 4–5 μg/g wet weight, respectively. Iron connected with myoglobin in dark and white muscles contains about 8–9 and 2–3 μg/g wet weight, respectively (Table III). The remainder of the Fe ions are connected with non-heme proteins and nonprotein-bound iron. A small pool of nonprotein-bound iron at a level of 0.5–2 μg/g wet weight was found previously by us (Kanner et al., 1988b).

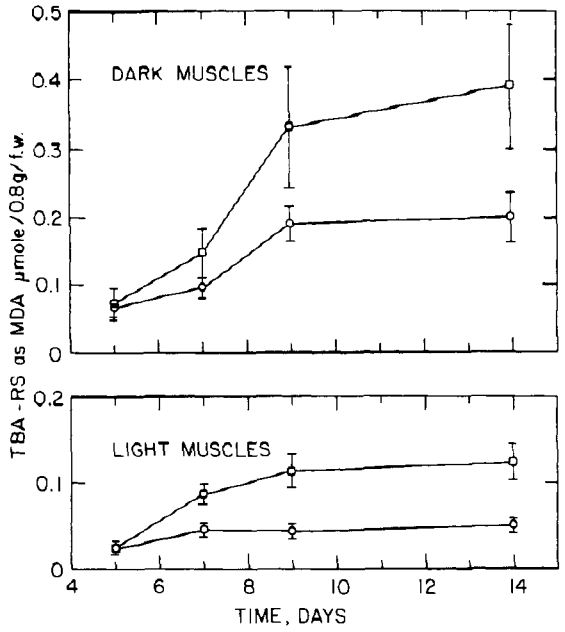

Figure 1. Effect of removal of iron supplementation, 5 weeks prior to slaughtering, on turkey muscle lipid peroxidation: □, control; ○, without Fe (5 weeks) supplementation.

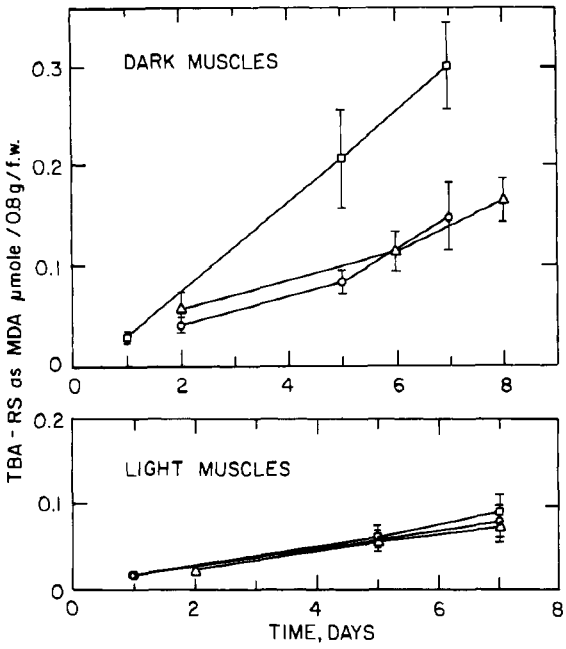

Figure 2. Effect of removal of iron supplementation, 3 and 7 weeks prior to slaughtering, on turkey muscle lipid peroxidation: □, control; △, 3 weeks prior to slaughtering; ○, 7 weeks prior to slaughtering.

Table III. Effect of Removal of Iron Supplementation on Total Iron, Heme Proteins, and Non-Heme Iron in Turkey Muscle Tissues

treatment	heme Fe, μg/g wet wt	total Fe,[a] μg/g wet wt
	Dark Muscle	
control	8.5 ± 0.6	11.8 ± 0.8
without Fe (5 weeks)	8.8 ± 0.4	12.7 ± 0.7
	Light Muscle	
control	2.8 ± 0.3	4.6 ± 0.6
without Fe (5 weeks)	2.7 ± 0.5	4.7 ± 0.5

[a] The results are means from three different birds.

The results found by us show that removal of iron supplementation 5 weeks prior to slaughtering did not change significantly the amount of total iron and iron heme proteins (Table III). More work needs to be carried out in order to identify the changes in muscle cell molecular iron distribution, and especially of the nonprotein-bound iron (chelatable iron), which we suggest affects mainly the initiation of muscle lipid peroxidation (Kanner et al., 1988a–c). It will also be necessary in the future for the identification of changes in the small pool of nonprotein-bound iron to adopt or develop more sensitive methods for determining free iron ions.

CONCLUSIONS

Our results demonstrated that the normal turkey feedstuff contains Fe at a high concentration and this seems to affect meat quality and its stability during storage. The removal of Fe supplementation did not have a significant effect on the body weight or total iron of the birds we tested, but it did significantly decrease muscle lipid peroxidation during storage at 4 °C. In order to improve meat quality and storage capacity, a reduction in iron supplementation of the turkey diet is suggested.

The effect of Fe supplementation to other animal feedstuffs should be determined carefully in order to evaluate its impact on muscle quality and stability during storage.

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Characterization of Hake (*Merluccius merluccius* L.) and Trout (*Salmo irideus* Gibb) Collagen

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An analysis of the different types of collagen present in various body structures, the amino acid composition of the collagen, and the degree of collagen aggregation for hake and trout is presented. Type I collagen was found at all body sites, and its amino acid profile and degree of hydroxylation are considered. Hydroxyproline values, the ratio of hydroxyproline to the other amino acids, and the proportions of alanine, tyrosine, and methionine are also discussed. Values for collagen solubility in salt and acid indicated a lower insolubility rate for skin collagen than for muscle collagen. The proportions of the α, β, and γ components in the acid-soluble fractions of skin and muscle collagen are also examined; the proportion of γ components is lower in hake muscle collagen than in hake skin collagen, whereas the converse holds true in trout.

The study of such biochemical characteristics of fish collagen as amino acid composition, collagen types, cross-linking, and the like is an essential basis for further investigation into technical aspects of more widespread industrial applications for collagen.

The characteristics of collagen at different body sites in food animals and other higher vertebrates have been studied in some detail (Bailey and Sims, 1977; Sims and Bailey, 1981; Bailey et al., 1984). However, except for gaping, which has received considerable attention (Love, 1970; Love and Haq, 1970; Love and Lavety, 1972), only a few studies have dealt with collagen in fish and other marine organisms (Yamaguchi et al., 1976; Sikorski et al., 1984). Published results concerning collagen types in fish are limited (Bogason, 1984; Almas, 1986), and we were unable to discover in the literature any references to collagen types according to the anatomical location of the connective tissue.

The object of the present study was to ascertain the biochemical characteristics of fish collagen in order to increase our understanding of this protein and thus lay the groundwork for future research into industrial technology and uses for the functional properties of fish collagen. To this end, collagen types, amino acid composition, and degree of aggregation in the skin and in the connective tissue in various muscle structures were determined for two different fish species.

MATERIALS AND METHODS

Hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb) were the species used. The hake were caught by long-lining on the continental shelf off Galicia, Spain, in March; total sample weight was 20.1 kg. The trout were reared on a fish farm and hence were all similar in size, and total sample weight was 17.7 kg. Mean individual weight was 2.1 kg for the hake and 1.7 kg for the trout; mean length was 65 cm for the hake and 45 cm for the trout. Specimens were kept refrigerated from the time of capture until use at the laboratory some 24 h later.

Extraction of Connective Tissue. Specimens were headed, gutted, and filleted, and the fillets were skinned.

Before the collagen or connective tissue was separated from the dermis, any remnants of muscle tissue or fat adhering to the dermis or epidermis were eliminated by hand.

Connective tissue was removed from the following sites: the fasciae surrounding the fillet; the myocommata separating the myotomes; the interior of the myotomes.

The connective tissue was separated out by dissection with a surgical knife. The types of collagen present in each body structure were then determined.

Separation and Purification of the Connective Tissue. The connective tissue was separated and purified by the method described by Borderías and Montero (1985).

Purification and Fractionation of the Different Collagen Types. A modified version of the fractionation method of Timpl et al. (1975) was used, as illustrated in Figure 1.

Electrophoresis on Polyacrylamide Gel Containing Sodium Dodecyl Sulfate. Electrophoresis on polyacryla-

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