Nutritional Effects on Beef Collagen Characteristics and Palatability

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NUTRITIONAL EFFECTS ON BEEF COLLAGEN CHARACTERISTICS AND PALATABILITY[1]

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Summary

To determine effects of different nutritional regimens on beef palatability and collagen characteristics, we randomly assigned 18 Hereford steers of similar age and nutritional background to three treatment groups: group 1 was slaughtered directly off pasture at about 19 to 20 months of age; group 2, composed of animals the same age as those in group 1, was slaughtered after being fed a high energy diet for 120 days, and group 3, 23 to 24 months of age, was slaughtered after 126 days on a high energy diet. Feeding the high energy diet increased USDA quality and yield grades. *Longissimus* (LD) steaks from the three groups did not differ in total collagen content, sarcomere length, Warner-Bratzler shear force, cooking loss or sensory panel ratings for tenderness, juiciness and flavor intensity. However, LD samples from group 3 animals had a higher ($P<.05$) percentage of salt plus acid soluble collagen and less organoleptically detectable connective tissue than LD samples from group 1. LD samples from group 2 animals had a higher ($P<.05$) percentage of salt soluble collagen than those from group 1. *Biceps femoris* (BF) steaks from group 1 steers were juicier than those from groups 2 and 3. Feeding regimen did not consistently affect BF collagen solubility, taste panel tenderness or shear force. Beef from grass-finished cattle received acceptable taste panel scores. The effects of the high energy diet on plasma nonprotein hydroxyproline content and live animal weight were monitored in group 3. Plasma nonprotein hydroxyproline content (an indicator of collagen degradation) was highest at approximately 42 days on feed and corresponded well with weight gain. These results suggest that collagen turnover is accelerated during the rapid growth phase of cattle fed a high energy diet.
(**Key Words:** Beef, Nutrition, Meat Palatability, Collagen Solubility.)

Introduction

Variation in beef palatability due to nutritional treatment has been reported by Jacobson and Fenton (1956), Garrigus *et al.* (1969), Dube *et al.* (1971), Kropf *et al.* (1975), Bowling *et al.* (1977), Smith *et al.* (1977), Smith *et al.* (1979) and Burson *et al.* (1980). These authors noted a decrease in beef palatability due to a lower plane of nutrition.

Grass-finished cattle have less marbling and subcutaneous fat than do grain-fed cattle (Godbey *et al.*, 1959; Klosterman *et al.*, 1965; Kropf *et al.*, 1975). Smith *et al.* (1974) suggested that increasing quantities of either subcutaneous fat or marbling may insulate muscle fibers and decrease cold shortening during postmortem chilling, and thus improve tenderness.

Meat tenderness is determined primarily by two factors: (1) the nature and state of the contractile protein and (2) the content and properties of connective tissue (Dutson, 1974). McClain (1977) reported that the type and extent of cross-linking in intramuscular collagen are influenced by animal age, and possibly by nutritional status of the animals, as well. Harris (1976) stressed the importance of avoiding myofibrillar contraction when looking for treatment effects other than those directly related to cold shortening. The specific objective of this study was to determine effects of high energy and grass diets on beef collagen characteristics and palatability.

Experimental Procedure

Eighteen Hereford steers about 15 to 16 months of age were grazed on Kansas flint hill pasture for 60 to 80 days before the experi-

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ment. The cattle were then divided into three groups of six each (figure 1). Animals in group 1 were slaughtered in October after grazing bluestem pasture for another 120 days. Animals in group 2 were removed from grass at the beginning of the experiment and fed a high energy diet (table 1) for 120 days before slaughter. Animals in group 3 were pastured until October, then fed a high energy diet (table 1) for 126 days before slaughter. Because of extremely cold weather, animals in group 3 lost some weight during the last 3 weeks of the experiment.

The effects of the high energy diet on animal growth and collagen metabolism were monitored in group 3. At 3-week intervals during the feeding period for this group, body weights were taken and blood samples obtained for analyses of nonprotein hydroxyproline, an indicator of collagen degradation.

Cattle were slaughtered in the Kansas State University Meat Laboratory. Since this study was concerned primarily with the influence of nutritional regimen on connective tissue and tenderness, precautions were taken to avoid cold shortening during postmortem chilling. Carcasses were retained on the slaughter floor (approx. 20 C) until 3 hr postmortem before being chilled at 3 C. At 24 hr postmortem, *longissimus* (LD) and *biceps femoris* (BF) muscles were removed from the right side of each carcass, vacuum-packaged and stored at 3 C for 5 days before being cut into steaks (2.5 cm thick). Steaks were packaged, frozen and stored at −26 C for later evaluation. USDA yield and quality grade factors were determined approximately 48 hr postmortem on the left side of each carcass. Samples for taste panel and shear force determination were thawed at 4 C for 18 hr and then cooked to an internal

TABLE 1. INGREDIENT COMPOSITION OF HIGH ENERGY DIET[a]

Ingredient	%
Cracked corn (IFN 4-02-931)	85.0
Corn silage (IFN 3-08-153)	10.0
Supplement[b]	5.0

[a]Metabolizable energy = 3.14 Mcal/kg.

[b]Soybean oilmeal (IFN 5-04-604) supplement plus rolled milo, limestone, urea, salt, animal fat, dynak, trace mineral and vitmain A.

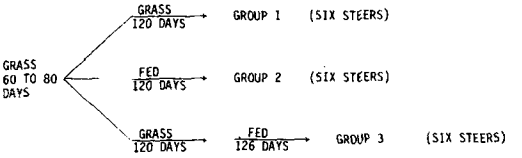

Figure 1. Schematic presentation of the feeding regimens.

temperature of 70 C in a 165 C gas oven. Thermocouples were used to monitor steak and oven temperature. Samples of the cooked muscles were scored by a six-member trained panel for juiciness, myofibrillar tenderness, overall tenderness, amount of connective tissue and flavor intensity. An eight-point scale was used for each palatability attribute, where 8 = extemely juicy, tender or intense flavor or no connective tissue, and 1 = extremely dry, tough or bland flavor or abundant connective tissue. Warner-Bratzler shear forces were obtained on six 1.27-cm cores after the steaks had been stored at room temperature for 2 hr as suggested by AMSA (1978) guidelines.

Sarcomere length for each group was measured by suspending finely pulverized muscle powder in a drop of .25 M sucrose solution on a slide. Ten sarcomeres from each of 20 myofibrils were measured. A Wild phase contrast microscope equipped with an eyepiece filar micrometer was used to estimate average sarcomere length. Moisture and fat content of the muscles were determined by AOAC (1965) procedures. Nonprotein hydroxyproline was extracted from plasma with five volumes of absolute alcohol (Bannister and Burns, 1970). Plasma nonprotein hydroxyproline content and total collagen in muscle samples were estimated by the spectrophotometric method described by Bergman and Loxley (1963). Solubility of the collagen was determined from the hydroxyproline content of the neutral salt soluble and acid soluble collagen. Neutral salt soluble collagen was obtained by extracting muscle samples with 10 volumes of .5 M buffered NaCl, pH 7.2 (Fielding, 1976). Acid soluble collagen was obtained by extraction with .5 M acetic acid (Bazin and Delaunay, 1976).

Data were analyzed by analysis of variance for significance of differences between treatments, and the Least Significant Difference was used to separate differences between means (Steel and Torrie, 1960).

TABLE 2. MEANS FOR CARCASS TRAITS OF CATTLE FROM THREE NUTRITIONAL REGIMENS

Carcass trait	Nutritional regimen		
	Group 1: grass	Group 2: fed	Group 3: grass and fed
Maturity[a]	A^{30}	A^{35}	A^{33}
Marbling[a]	$PD^{33\,b}$	$Sl^{66\,c}$	$Sm^{30\,d}$
Quality grade[a]	$Standard^{17\,b}$	$Good^{60\,c}$	$Choice^{07\,d}$
Hot carcass weight, kg	171.16^{b}	271.32^{c}	268.16^{c}
Adj. fat thickness, cm	$.21^{b}$	1.08^{c}	1.14^{c}
REA, cm^2	54.62^{b}	67.53^{c}	69.03^{c}
KPH fat, %	$.83^{b}$	1.83^{c}	2.30^{c}
Yield grade[a]	1.60^{b}	2.85^{c}	2.91^{c}

[a]Based on descriptions given in USDA (1975) beef grading standards; marbling score: PD = practically devoid, Sl = slight, Sm = small.

[b,c,d]Means in the same row with different superscripts differ ($P<.05$).

Results and Discussion

Carcasses from the three treatment groups did not differ in maturity score (table 2). Cattle fed a high energy diet (groups 2 and 3) had heavier carcass weights, higher marbling scores, quality grades and yield grade scores, larger ribeye areas; greater 12th rib fat thickness and higher percentages of kidney, pelvic and heart fat than cattle fed grass only (group 1; $P<.05$ in all cases).

The intramuscular collagen content of the LD muscle did not vary among the three groups, on either a fresh tissue basis or a moisture and fat-free basis (table 3). LD samples from group 2 had higher ($P<.05$) percentages of salt soluble collagen than those from group 1. LD samples from group 3 had higher ($P<.05$) percentages of acid soluble collagen and acid plus salt soluble collagen than did samples from groups 1 and 2. The results agree with those of

TABLE 3. MEANS FOR COLLAGEN CHARACTERISTICS OF *LONGISSIMUS* AND *BICEPS FEMORIS* MUSCLES FROM CATTLE ON THREE NUTRITIONAL REGIMENS

Item	Nutritional regimen		
	Group 1: grass	Group 2: fed	Group 3: grass and fed
	Longissimus		
Collagen content, mg/g			
Fresh tissue	3.88	4.26	4.28
Moisture and fat-free	17.62	18.22	18.41
% solubility			
Salt soluble	1.99^{a}	2.48^{b}	2.22^{ab}
Acid soluble	3.00^{a}	2.80^{a}	3.86^{b}
Salt + acid soluble	4.99^{a}	5.28^{a}	6.08^{b}
	Biceps femoris		
Collagen content, mg/g			
Fresh tissue	8.53^{ab}	9.12^{a}	7.74^{b}
Moisture and fat-free	45.70^{a}	44.71^{a}	36.46^{b}
% solubility			
Salt soluble	1.20^{ab}	1.29^{a}	1.05^{b}
Acid soluble	2.30	2.42	2.98
Salt + acid soluble	3.50	3.71	4.03

[a,b]Means in the same row with different superscripts differ ($P<.05$).

Corte (1977), who reported an increase in salt- and acid-soluble collagen with increasing plane of nutrition and increased length of feeding.

Salt-soluble collagen contains recently synthesized collagen, while the acid-soluble collagen fraction contains some of the younger collagen of the fibers, which are metabolically older than salt-soluble collagen (Bodwell and McClain, 1971). The increase in collagen solubility in LD muscle of grain-fed cattle was probably due to an increase in the rate of collagen biosynthesis or a decrease in the rate of collagen cross-link formation after the animals were placed on the higher nutritional plane.

Neither collagen solubility (salt-soluble, acid-soluble and salt- plus acid-soluble collagen) nor intramuscular collagen content (fresh tissue basis) of the BF muscle differed between grass-fed cattle (group 1) and cattle fed the high energy diet (groups 2 and 3), while some differences were found between groups 2 and 3 (table 3). The lower content of salt-soluble collagen and slightly higher content of acid-soluble collagen in the muscles of group 3 animals indicate that the collagen in group 3 was physiologically more mature than that in group 2. More of the synthesized collagen was aggregated into fibers and could be extracted only with acid solution.

Means for percentage salt plus acid-soluble collagen in the BF muscles were slightly greater after the animals had been fed a high energy diet for 120 days; however, the differences were not significant (table 3). The influence of nutrition on collagen solubility of BF muscle was less marked than the effect on LD muscle. In this study, BF samples had lower total salt- and acid-soluble collagen values than LD samples. Mean values ranged from 3.50 to 4.03% for the BF samples and from 4.99 to 6.08% for the LD samples (table 3).

When the nutritional plane was changed from pasture to high concentrate diet (group 3), a marked increase was observed in the plasma concentration of nonprotein hydroxyproline (NPH, degraded products of collagen, which include free hydroxyproline and hydroxyproline-containing peptides; table 4). The peak was reached after approximately 6 to 9 weeks on feed. After that, there was a rapid decline in plasma NPH content.

Body weight gain increased rapidly after animals were placed on the higher nutritional plane. Rate of body weight gain also peaked after approximately 6 weeks on feed, and then declined rapidly (table 4).

Hydroxyproline excretion has been used as a measure of collagen turnover. Kivirikko (1970) reported that an increase in the excretion of hydroxyproline was associated with the synthesis of new collagen during growth. Since newly synthesized collagen is more susceptible to collagenase attack, more free hydroxyproline and hydroxyproline-containing peptides will be released from collagenous tissue, enter the bloodstream and finally be excreted in the urine. Similar results have been reported by other researchers. Laurent *et al.* (1978) showed that collagen synthesis is stimulated during stretch-induced growth. They reported a marked increase in collagen synthesis during the first week of the hypertrophy in muscle of adult fowl. Kivirikko (1970) reported that the excretion of hydroxyproline in humans is high

TABLE 4. EFFECT OF HIGH ENERGY DIET ON THE PLASMA NONPROTEIN HYDROXYPROLINE CONCENTRATION AND LIVE WEIGHT OF GROUP 3 CATTLE

Weeks on high energy diet	Plasma nonprotein hydroxyproline, μg/ml							Animal weight, kg
	Individual animals							
	1	2	3	4	5	6	Mean	Mean
0	5.58	3.42	3.34	4.32	3.47	4.60	4.12^{bc}	341.3^{e}
3	5.67	3.41	3.47	4.21	4.11	4.16	4.17^{bc}	364.8^{d}
6	8.01	3.77	4.57	4.76	4.55	5.34	5.17^{a}	410.7^{c}
9	4.84	4.67	4.44	3.82	4.13	4.63	4.42^{ab}	419.1^{bc}
12	4.13	3.26	3.26	3.67	3.55	3.74	3.60^{bc}	436.4^{ab}
15								455.0^{a}
18	3.10	3.25	3.59	3.64	3.06	2.98	3.27^{c}	452.2^{a}

^{a,b,c,d,e}Means in the same column with different superscripts differ ($P<.05$).

during the growth period. During the maturation process, more and more cross-links are formed, and the collagen becomes increasingly resistant to solubilization and collagenase attack (Kivirikko, 1970). This may explain the decline in plasma NPH concentration after the animals in this study had been on a high energy diet for 6 weeks. Also, increases in weight after the sixth week may have been due primarily to fat deposition rather than muscle deposition.

No differences in sarcomere length were found among LD muscles from the three treatment groups (table 5). LD steaks from the three groups did not differ in taste panel ratings for overall tenderness, myofibrillar tenderness, flavor intensity and juiciness (table 5). Steaks from grass-fed cattle were generally rated as moderately tender. According to the taste panels, LD samples from group 3 cattle had less ($P<.05$) detectable connective tissue than samples from grass-fed (group 1) cattle. This finding agrees with collagen solubility data, which showed that LD samples from group 3 had more total salt plus acid-soluble collagen than samples from the grass-fed group (table 3). Shear force and cooking loss values did not differ among LD steaks from the three treatment groups (table 5).

BF steaks from group 1 cattle were rated as slightly to moderately tender and were given higher juiciness scores than steaks from cattle in groups 2 and 3 (table 5). As with the LD muscles, the feeding of the high energy diet for 120 days did not improve BF taste panel or shear tenderness. BF samples from group 2 steers had the lowest taste panel tenderness scores and highest shear force values. Samples from this group also had the most organoleptically detectable connective tissue and a slightly shorter average sarcomere length.

Kropf *et al.* (1975) reported that cattle fed before slaughter generally produced more tender meat than did grass-finished cattle. They found that grass-finished cattle produced an unacceptable product. However, in our study, the taste panel generally rated LD steaks from grass-fed cattle (group 1) as moderately tender and BF steaks as slightly to moderately tender. The apparent lack of improvement in tenderness as a result of nutritional regimen was probably

TABLE 5. MEAN SENSORY PANEL RATINGS[a], WARNER-BRATZLER SHEAR FORCE VALUES, COOKING LOSS AND SARCOMERE LENGTHS OF *LONGISSIMUS* AND *BICEPS FEMORIS* MUSCLES FROM CATTLE ON THREE NUTRITIONAL REGIMENS

Trait	Nutritional regimen		
	Group 1: grass	Group 2: fed	Group 3: grass and fed
	Longissimus		
Overall tenderness	5.9	6.1	6.6
Myofibrillar tenderness	6.0	6.0	6.5
Connective tissue amount	6.3[b]	6.6[bc]	7.2[c]
Flavor intensity	6.1	6.3	6.4
Juiciness	6.5	6.0	6.3
Shear force, kg	3.2	3.2	2.7
Cooking loss, %	24.8	28.3	28.2
Sarcomere length, μm	1.79	1.85	1.80
	Biceps femoris		
Overall tenderness	5.6[b]	5.1[c]	5.7[b]
Myofibrillar tenderness	6.6[b]	6.2[c]	6.4[b]
Connective tissue amount	5.0[bc]	4.5[c]	5.3[b]
Flavor intensity	6.3	6.3	6.3
Juiciness	6.7[b]	5.8[c]	5.6[c]
Shear force, kg	4.6[b]	5.6[c]	4.9[bc]
Cooking loss, %	24.7	29.0	27.7
Sarcomere length, μm	1.84[b]	1.77[c]	1.80[bc]

[a]Means based on eight-point rating scale, where 8 = extremely tender, intense flavor or juicy or no connective tissue and 1 = extremely tough, bland flavor or dry or abundant connective tissue.

[b,c]Means in the same row with different superscripts differ ($P<.05$).

due to the relative acceptability of grass-finished cattle. Factors such as pasture and feeding conditions, chilling rate, sampling time, fat covering and age of the animal may also account for the variation in results.

This study showed that feeding cattle high energy diets increased USDA quality and yield grades, caused rapid animal growth and increased collagen solubility of the LD muscle and the plasma NPH level, but did not consistently improve taste panel and shear force characteristics when compared to grass feeding.

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